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Correspondence

Habitat and food preference of tadpoles in the lower Basistha River, Northeast India

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Knowledge of the morphology and habits of tadpoles is highly relevant for the understanding of ecological requirements and the natural history of anuran species. Yet, tadpole morphology used to receive little attention in spite of the known importance of larval characters for resolving taxonomic uncertainty and evaluating phylogenetic relationships (Wassersug 1984, Haas 2003). Tadpoles show great plasticity in body shape (Inger 1985), development times (Bardley & Beebee 1998), diet, and habitat utilization (Tyning 1990), and these differences are primarily a strategy of avoiding overlaps in resource use (Schoener 1974, Toft 1985). According to Inger (1966), the major factors that ultimately limit the distribution of tropical amphibians are ecological aspects such as temperature, total rainfall, vegetation and competition, as well as geographical and geological factors.

There is a general shortage of information on the tadpoles of India, especially those of northeast India. It was only over the last three decades that dietary information on anuran larvae from this region has been published (Khare & Sahu 1984, Ao & Khare 1986, Sekar 1990, Saidapur 2001, Sinha et al. 2001, Khongwir et al. 2003). However, reports on the ecology of stream-dwelling amphibian communities in northeast India are still wanting. The present study was designed to study the habitat and resource utilization by tadpole communities in the lower Basistha river of the Garbhanga Reserve forest during the monsoon season.

The Garbhanga Reserve Forest [RF] (26°07' to 26°09' N and 91°33' to 91°55' E, 70–638 m above sea level, surface area 114.65 km², Assam, India) has mixed deciduous and semi-evergreen types of forest with a riparian zone all along its perennial watercourses. The principal river of the RF is the Basistha, which, with its many small tributaries, cuts through a major part of the reserve and finally drains into the Bahini river (Das et al. 2002). The lower portion of the river (3.5 km), which was about one to three metres wide with a depth ranging from six centimetres in fast-flowing parts to 120 cm in the deepest pools, was sampled for tadpoles during June to July in 2009 and 2010. The bottom consisted of rocks, gravel and sand, covered with leaf litter in the calm pools.

Tadpoles were scooped with dip nets, euthanised in MS-222, preserved in buffered 10% formalin, and deposited in the Museum of the Arya Vidyapeeth College (AVCM). Detailed collection data were recorded for each specimen. Species identification of the larvae was based on descriptions provided in earlier literature (Sahu 1994, Inthara et al. 2005) and also by rearing larvae in captivity to ascertain/confirm their identities.

Thirty tadpoles of each species at Gosner (1960) stages 36 were subjected to gut content analyses. The entire alimentary canal was removed from the individuals and the gut content identified following Needam & Needam (1966). For each feeding item, the numeric frequency (NF% = total number of items of a specific food group consumed / total number of items of all food groups consumed × 100) and the frequency of occurrence (FO% = number of guts in which the specific food item was present / total number of guts with these food items × 100) were calculated. Food groups in this study were blue green algae, green algae, diatoms, rotifers, crustacean, desmids and plant debris.

Furthermore, each sampled section of the river was inspected exhaustively in an attempt to record all tadpoles present (at least one hour was spent sampling in each section). For each individual tadpole found, time and features of the microhabitat occupied, including water temperature (°C), pH, cover (exposed or covered), depth (in m), type of

Table 1. Microhabitat types and different ecological conditions of habitat of five species of tadpoles collected from study site.

Species	Depth (m)	Flow (m/min)	Water temp. (°C)	pH	Stream bottom	covered/ exposed	Aquatic vegetation	Atmospheric temp. (°C)	Stream habitat
C. alticola	0.3556–0.5334	2.37	27	6.9	Sand	Covered	Yes	31	Riffle
L. smithi	0.1778- 03048	0.197	29	6.4	Rocky	Exposed	Yes	33	Riffle
H. leptoglossa	0.1524–0.2032	0	27	6.5	Muddy	Covered	No	33.5	Pool
A. assamensis	0	10.2	26	6.7	Rocky	partially covered	No	32	Torrent
R. bipunctatus	0.6096–0.762	0	30	6.6	Sand	Exposed	No	33	Pool of intermittent stream
L. smithi	1.219–1.83	0.212	28	7.4	Sand	Covered	leaf litter	33	Slow flowing

substrate (sand/silt or rocks), aquatic vegetation (presence or absence), current (rapid, slow, pool), and atmospheric temperature were recorded. Stream habitats were further classified as either torrents, riffles, open pools, seepage or pool of intermittent streams. Niche analyses [software SPECDIV (Ludwig & Reynolds 1988)] were performed using different food and habitat resource states, where niche width was expressed as the Shannon diversity index, $H' = \sum p_i \ln p_i$. General Overlap (GO) = e^E, where $E = \sum^s_i \sum^r_j [n_{ij} (\ln c_j - \ln p_{ij})] / T$. Statistics: V = -2T ln GO. The hypothesis tested in General Overlap (GO) was for a complete overlap of species. In the statistics, if the value of V exceeded the critical value for chi-square at p = 0.05, then the null hypothesis was rejected.

The Jaccard index, C_j, value was estimated for quantifying the differences between dietary and habitat resources, $C_j = j / (a + b - j)$, wherein j = number of joint resource states between two species; a = number of resource states in species A; b = number of resource states in species B.

The indices were then arranged in a matrix and subjected to hierarchical cluster analysis, using BDPRO (McAleece et al., 1997) to test species associations based on microhabitat features.

In the present study, the tadpoles of *Leptobrachium smithi* Matsui, Nabhitabhata & Panha, 1999 [Megophryidae], *Hylarana leptoglossa* (Cope, 1868) [Ranidae], *Clinotarsus alticola* (Boulenger, 1882) [Ranidae], *Amolops assamensis* Sengupta, Hussain, Choudhury, Gogoi, Ahmed & Choudhury, 2008 [Ranidae], and *Rhacophorus bipunctatus* Ahl, 1927 [Rhacophoridae] were recorded from the lower course of the Basistha river and adjacent pools connected to the stream. All these tadpoles were exotrophic, benthic, lotic and representative of Orton (1953) type IV. The species were found living in different ecological conditions (Tab. 1). Except *H. leptoglossa* and *R. bipunctatus*, which were found in the lotic parts of the watercourse, all were rheophilic (lentic). Amongst the lentic species, *A. assamensis* was found in the torrent zone, whereas *L. smithii* occurred in the least flowing zone. The tadpoles were also found to segregate their shared habitat in terms of depth of occurrence (Tab. 1).

The tadpoles of *L. smithi* foraged amongst aquatic vegetation and found retreats either in between aquatic vegetation or between the pebbles of the streambed. Gosner 25 stage (Gosner 1960) tadpoles occured throughout the year and had probably observed a long period of dormancy (including overwintering). The earlier stages (Gosner 22 & 23) were found in side pools of riffles in slightly acidic water. *H. leptoglossa* tadpoles were found to forage amongst leaf litter on the muddy bottom of temporary rain-fed pools of various sizes, where the water was slightly acidic. Tadpoles of *C. alticola* inhabited, in gregarious association, the vegetation near the bank of the flowing stream (pH 6.9) that was shaded by the tree canopy. *Amolops assamensis* tadpoles were unique in having poison glands and a large gastromyzophorous adhesive disk (abdominal sucker), which enabled them to attach themselves to the surface of vertical rock and boulder faces in the swift-moving parts of the river they inhabited. They foraged by climbing the rocks against the flow, and released their hold and escaped by dropping into the turbulent water below when feeling threatened or approached by predators. Tadpoles of *R. bipunctatus* were found in scattered stream-pools without aquatic vegetation, where, due to shallow water, temperatures were comparatively elevated.

Various types of food items were recorded from the gut contents of the tadpoles of various species. The larval anurans mostly consumed phytoplankton, and green algae and diatoms were the most dominant groups in their diets. The different food items consumed are detailed in Table 2. Plant debris was consumed by all species and this group accounted for hundred percent in occurrence frequency. *L. smithi* had consumed mostly diatoms, while *A. assamensis* fed on both diatoms and green algae equally. Diatoms were also the major food group of *R. bipunctatus* and *C. alticola*, while in *H. leptoglossa*, green algae was found to be the dominant food group (Tab. 2). The analysis of occurrence frequencies of food items (Tab. 3) revealed that amongst phytoplankton, diatoms were most frequently consumed by all species except *A. assamensis*, in which blue green and green algae were the most frequently ingested food groups. This was probably due to their living in torrents.

Sinha et al. (2001) reported an absence of blue green algae in the diets of stream-dwelling larval anuran species, namely *A. afghanus*, *H. danieli* (presently a synonom

Table 2. Numeric frequency of different groups of food items of larval amphibians inhabiting the Basistha river.

Species	Blue green algae	Green Algae	Diatoms	Rotifers	Protozoans	Crustaceans	Desmids
L. smithi	3.23	4.83	41.93	8.06	3.23	4.83	1.61
R. bipunctatus	5.08	18.64	33.9	11.86	1.69	3.39	3.39
C. alticola	8.93	7.14	46.43	7.14	3.57	0	0
H. leptoglossa	4.82	39.76	16.87	6.02	3.61	1.21	10.84
A. assamensis	19.23	23.08	23.08	13.46	1.92	1.92	0.96

Table 3. Occurrence frequency of different groups of food items of larval amphibians found in the Basistha river.

Species	Blue green algae	Green algae	Diatoms	Rotiferans	Protozoans	Crustaceans	Desmids	Plant debris
L. smithi	40	60	100	60	40	40	20	100
R. bipunctatus	60	40	100	80	20	40	20	100
C. alticola	80	40	100	80	40	0	0	100
H. leptoglossa	60	100	100	60	60	20	100	100
A. assamensis	100	100	60	20	60	20	20	100

of *Hylarana garoensis)* and *Rhacophorus maximus*. However, in the present study, all tadpoles were found to consume blue green algae, and in *A. assamensis*, it was one of the major dietary groups. A study on the food of the tadpoles of *C. alticola* previously conducted by Sahu & Khare (1988) in Meghalaya state of northeastern India revealed that tadpoles were herbivorous during the early stages of their life and turned into carnivores during the later post-metamorphic stages. Heinen & Abdella (2005) reported that tadpoles that ingested animal matter grew faster; yet they appear to require supplementary plant matter for optimal growth (Saidapur 2001). In our study, the absence of crustaceans and desmids in the diet of *C. alticola* tadpoles indicated that rotifers and protozoans probably compensate the absence of crustaceans and desmids.

Plant debris provides higher nutritional value from associated microbes than its particles per se (Cummins & Klug 1979) and fungal biomass may account for 18–23% of the mass of leaf detritus in the headwaters of streams (Methvin & Suberkropp 2003). In this study, the presence of plant debris in all tadpole guts indicated that the tadpoles of the stream had ingested substantial amounts of microbes. Moreover, *A. assamensis* tadpoles were found to be epilithon-eaters, scraping biomasses from moist stones. Protozoans were also observed to be one of the constituents of the diets of all larval anurans in the present study. Altig et al. (2007) suggested that a tadpole diet of phytoplankton might exploit a substrate as a harvestable carrier for associated food resources such as microbes and protozoans. The concept of herbivory in tadpoles probably stems from the abundance of algal-based items in the gut and the length of the gut. Naitoh et al. (1999) speculated that the long guts of tadpoles served to compensate for its weak or no peristaltic movement. This study suggests that tadpoles maintain the concept of omnivory and ingest microbes via various food carriers and so support their growth and development.

Table 4. Niche widths of, and niche overlaps between different species of a larval amphibian community in the Basistha river in the Garbhanga R.F.

Niche breadth (H')	
Species	H'
Leptobrachium smithi	2.429
Rhacophorus bipunctatus	2.706
Clinotrarsus alticola	2.584
Hylarana leptoglossa	2.727
Amolops assamensis	2.693

General Overlap					
No	GO	G_{min}	G_{adj}	v	Df
5	0.504	0.207	0.375	375.308	212

Wherein GO = General overlap, G_{min} = Minimum General overlap, and G_{adj} = Adjusted General overlap.

Based on the dietary diversity, the trophic niche widths of the five species of tadpoles investigated and their trophic niche overlaps are evaluated and presented in Table 4. The general overlap between the five species was GO = 0.504. The overlap was not complete, because the value of V = 375.308 is far greater than the critical chi-square value (at 212 df, $p < 0.01$; the relative intensity of overlap between the species was tested against a complete overlap).

The Jaccard indices of different pairs of species that estimate the differences among resources are presented in

Table 5. Jaccard indices (Cj) matrix showing the differences between larval anuran species in terms of dietary and habitat resources in the Basistha river.

	N. alticola	*H. leptoglossa*	*A. assamensis*	*R. bipunctatus*	*L. smithi*
C. alticola	1	0.22	0.38	0.24	0.39
H. leptoglossa	*	1	0.33	0.6	0.38
A. assamensis	*	*	1	0.32	0.38
R. bipunctatus	*	*	*	1	0.38
L. smithi	*	*	*	*	1

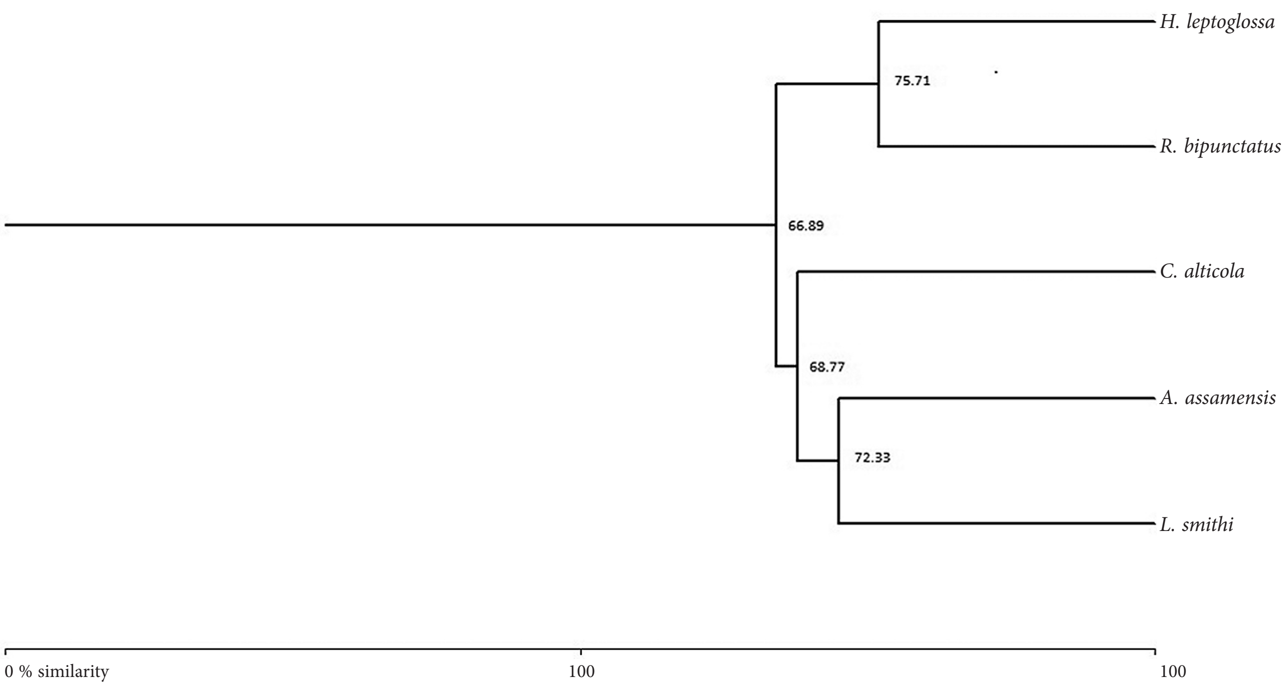

Figure 1. Bray-Curtis analysis (single link) showing "resource relationships" between five larval anuran species.

Table 5. Cluster analysis reveals three groups of tadpoles. "*R. bipunctatus* and *H. leptoglossa*", "*L. smithi* and *A. assamensis*" and "*Clinotarsus alticola*" (Fig. 1). Several ecological factors govern the selection of microhabitat by tadpoles and they occur in microhabitats where food can easily be obtained (Horat & Semlitsh 1994, Inger et al. 1986). The visible gut contents of the sympatric species in the study reflect different sites of activity, microhabitat and feeding preferences.

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