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DIETARY PREFERENCE OF EUPHLYCTIS CYANOPHLYCTIS TADPOLES IN DIFFERENT HABITATS IN AND AROUND SIMILIPAL BIOSPHERE RESERVE, ODISHA, INDIA

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Abstract

Amphibian tadpoles are the key consumers and play an important role in the food chain of aquatic ecosystems. Understanding the natural diet of tadpoles can help in developing management strategies for them. We characterized the diet of 170 Euphlyctis cyanophlyctis tadpoles collected from 34 sites during rainy seasons (July to October) of 2014 and 2015 in different temporary habitats in and around Similipal Biosphere Reserve, India. After morphometric measurements (total length, body length and body width), the complete intestine of each tadpole was analyzed for food items and quantified based on the numeric frequency (NF %) and frequency of occurrence (FO %). The food spectrum of tadpoles included mostly detritus followed by phytoplanktons (represented by 6 classes and 55 genera). The food items ingested were similar in all the habitats, suggesting that they are non-selective predators that lack an apparent dietary preference, and their diet is mostly dependent on the availability of food items. Knowledge of food habits and feeding behaviour of the tadpoles is essential, since the early part of the life history of amphibians is dependent on the availability of the food items in the natural habitat.

Keywords: Similipal Biosphere Reserve; Euphlyctis cyanophlyctis; Tadpole; Diet; Phytoplankton.

Introduction

Most of the anuran species have a biphasic life cycle with a larval stage called tadpole [1]. For tropical frogs, spatial and temporal dimensions have usually been considered to be the most important factors, particularly the choice of breeding sites [2]. Following the south-west monsoon, many Indian anuran species co-breed [3] and use a number of habitats ranging from lentic (e.g., ephemeral pools, temporary ponds, permanent lakes, water in rock cavities, tree trunks and inside the holes excavated by other animals) to lotic systems (e.g., rivers, creeks and streams) [4-6] for breeding. These aquatic habitats may vary in their hydro-period (e.g., temporary, permanent or ephemeral), structure (e.g., river width, pond area, pond depth, and canopy cover) and limnologic characteristics (e.g., pH, dissolved oxygen, conductivity, temperature, etc.) that, in turn, have a varied influence on tadpole assemblages [7, 8]. Amphibian tadpoles are the key consumers and play an important role in the food chain of aquatic ecosystems. Knowledge of food habits and feeding behaviour of the tadpole is essential,

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since the early part of the life history of amphibians is dependent on the availability of food in the natural habitat.

Anuran larvae are important components of many freshwater communities and have been described as "eating machines" [9]. They are known for the cosmopolitan nature of their diet. They have traditionally been regarded as microphagous, suspension-feeding herbivores and detritivores [10, 11]. However, observations of opportunistic oophagy, carnivory or necrophagy [12, 13, 14] have also been recorded. In herpetological research, the question of what tadpoles really eat is still largely unqualified and has been highlighted as one of the major questions [15]. Thus, there is an urgent need for detailed information on larval diets to understand whether food related factors limit wild populations and facilitate successful rearing of a species in captivity.

It was only in the last three decades that dietary information on anuran larvae has been available in India [16-21]. Information on the natural diets of *Euphlyctis cyanophlyctis* tadpoles in the eastern part of India is lacking. The objective of the present study was to verify the feeding spectrum of the Indian Skipper Frog (*E. cyanophlyctis*) tadpoles in three different habitats.

Materials and Methods

Study area

The study was conducted in the south-eastern transitional zone of Simlipal Biosphere Reserve [SBR] (Fig. 1) located in the Mayurbhanj district of Odisha state. It is in the eastern end of the Eastern Ghats and classified in the Chotanagpur biotic province of Mahanadian biogeographical region. In terms of biotic composition, Simlipal forests represent a link between the foot hills of Himalayas and the Eastern Ghats, as indicated from the biodiversity study. The herpetofauna of SBR comprises 21 species of frogs and 60 species of reptiles, including one species of crocodile, 6 species of turtles, 20 species of lizards and 33 species of snakes [22].



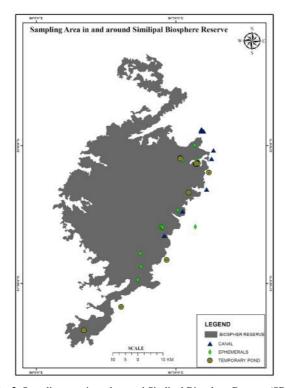
Fig. 1. Map of the study area

The climate of the area is sub-tropical with a hot summer (March to May, 40-42°C), rainy (June to October, actual average precipitation, 1283.4mm) and a chilling winter

(November to February, 5–7°C). The breeding of most of the anurans occurs during the rainy season. *Euphlyctis cyanophlyctis*, one of the predominant species in the area, occurs and breeds in most of the aquatic habitats (temporary ponds, ephemeral pools & canals) in the region.

Sampling

The tadpoles were collected from three different habitats: canals (n = 7), ephemeral water bodies (n = 15) and temporary ponds (n = 12; Fig. 2) during the rainy seasons (July to October) of 2014 and 2015 using dip net (mesh size 1mm). Since tadpoles were not available in permanent ponds, these habitats were excluded. The larvae (5 from each sampling point) were preserved in 10% formaldehyde immediately after field collection in order to prevent complete digestion of ingested food particles. In the laboratory, individuals of K.L. Gosner [23] stages 35–38 were separated and subsequently preserved in 4% formaldehyde. A total of 170 tadpoles [5x (7+15+12)] were used from all the three habitats for diet analyses.



 $\textbf{Fig. 2.} \ \textbf{Sampling} \ \textbf{area} \ \textbf{in} \ \textbf{and} \ \textbf{around} \ \textbf{Simlipal Biosphere} \ \textbf{Reserve} \ (\textbf{SBR})$

The gut of each tadpole was removed carefully; gut length was recorded with the help of a digital vernier caliper (MitutoyoTM) to the nearest 0.1mm. The first four centimeter of gut was used for diet analyses. The gut contents were flushed with distilled water, taken on a Sedgewick rafter chamber and analyzed under a compound microscope (Laboscope, CMS-2). Photographs of the gut contents were taken with the help of a Sony cyber shot camera (5.1 megapixels, DCSW5) attached to the microscope. The food items were identified up to the genus level and quantified following standard procedures [24, 25]. The items ingested by tadpoles were quantified based on the numeric frequency (NF% = total number of food items of a specific food group consumed/total number of items of all food groups consumed × 100) and frequency of occurrence (FO% = number of guts in which the specific food item was present/ total number of guts with these food items × 100). The importance index was obtained following *G.R. Colli*

et al., [26] (%NF plus %FO divided by two). Microhabitat of tadpoles was categorized based on the following characteristics: water temperature (°C), pH, depth (in feet), type of substrate (sand/mud/slit/gravel), plant cover on the bottom and on the surface (low, 0–25%; intermediate, 26–50%; high>50%). Diet diversity was calculated for each species by the Shannon-Wiener index (H'). Analyses were done using the software PAST (version 2.14). In order to determine the level of diet specialization, the niche breadth for the food items ingested was calculated through Levin's standardized index (B_A) [27]. This index allows measuring the amplitude or diversity of diet considering the quantitative distribution of each prey items. The data were analysed using one way analysis of variance (ANOVA), followed by Tukey's test to find out the level of significance among mean values. The data were presented as mean \pm SD.

Results

Euphlyctis cyanophlyctis tadpoles were found to live in comparable ecological conditions (Table 1). However, they were not present in all the habitats in a locality even with similar characteristics. Tadpole habitats in canals and temporary ponds were without or with very little aquatic vegetation cover and were mostly abundant at intermediate depth.

Characteristics	Canal	Ephemeral pool	Temporary pond
Shape	Elongated	irregular	Oval
Hydro-period	Permanent (slow	Temporary (dry within	Temporary (without water
	flowing water	1–2 months)	during the dry season)
	throughout the year)		
Canopy cover %	0.0	50.0	30.0
Max. Depth	2.0	0.7–1.5	4.2
Water temperature (°C)	31.0	33.0	33.0
pH	6.5	6.8	7.2
Bottom substrates	Sand, stones, mud	Mud, gravel	Gravel, stones, mud
Plant cover on the bottom	Low	high	Low
Plant cover on the surface	Low	low	Low

Table 1. Ecological characteristics of Euphlyctis cyanophlyctis tadpole microhabitats in the study area

The tadpoles occupied the shallow margin in ephemeral pools with adequate bottom vegetation (Table 1). The morphological characteristics like total length (mm), body length (mm), body width (mm) and gut length (cm) of tadpoles in ephemeral water bodies were significantly low compared to canals and temporary ponds (Fig. 3). The food spectrum of tadpoles included mostly detritus, followed by phytoplankton represented by 6 classes and 55 genera based on numeric frequency and importance index score (RII) of over 50 (Table 2). Most of the microalgae belonged to the class Bacillariophyceae followed by Chlorophyceae. Among Bacillariophyceae (25 genera), Achnanthidium, Pinnularia and Gomphoneies in canals; Navicula in ephemeral water bodies, Amphipleura and Navicula in temporary ponds, were important food items. Similarly, Ankistrodesmus, Coelastrum and Ulothrix in canals and Spirogyra in temporary ponds had maximum numeric frequency among Chlorophyceae (21 genera). Zooplanktons were mostly represented by the genera Amoeba and Mesocyclops in the gut of larvae collected from canals. However, Paramecium found in the gut of tadpoles collected from canal and ephemeral pools were absent in tadpoles collected from temporary ponds.

Based on the Shannon-Wiener function, tadpoles collected from temporary ponds had the highest prey diversity, followed by those from canals and ephemeral pools (Table 3). Tadpoles from the canal had the broadest dietary niche breadth, followed by temporary pond tadpoles and ephemeral pools.

Table 2. Dietary composition of Euphlyctis cyanophlyctis tadpoles from different habitats

Food items	Canal						meral pool				orary po	
	%NF	%FO	I	Mean±SD	%NF	%FO	I	Mean±SD	%NF	%FO	I	Mean±SD
Cyanophyceae												
Anabaena	0.47	53.3	26.9	0.8 ± 0.94	0.33	26.7	13.5	0.3 ± 0.62	0.60	53.3	26.9	1.1±1.3
Microcystis	10.46	100	55.2	17.7±4.4	2.24	80.0	41.1	2.3±1.71	4.50	80	42.3	8.1±5.5
Oscillatoria	0.75	66.7	33.7	1.3±1.10	1.58	66.7	34.1	1.6±1.8	1.53	80.0	40.8	2.7 ± 1.9
Bacillariophycea	e											
Achnanthes	1.06	86.7	43.9	1.8±1.15	0.79	40.0	20.4	0.8 ± 1.3	0.74	46.7	23.7	1.3±1.6
Actinella	0.24	40.0	20.1	0.4 ± 0.51	1.06	53.3	27.2	1.1±1.3	0.41	40.0	20.2	0.7 ± 1.1
Achnanthidium	2.01	100	51	3.4±1.88	2.04	80.0	41	2.1±1.4	2.05	66.7	34.3	3.7±3.2
Amphipleura	1.93	93.3	47.7	3.3±1.94	2.97	86.7	44.8	3.0±1.8	15.7	100.0	57.9	4.3±2.2
Amphora	-	-		5.5±1.74		-	-	5.021.0	0.41	26.7	13.6	0.7±1.3
Asterionella	1.10	60.0	30.6	1.9±1.96	2.04	66.7	34.3	2.1±2.1	1.53	86.7	44.1	2.7±1.9
							34.3	2.1±2.1	1.33	00.7	-	2.7±1.9
Aulacoseira	0.83	60.0	30.4	1.4±1.50	-	-	-	-	- 0.00	-		-
Cocconeis	1.49	86.7	44.1	2.5±1.85	3.23	86.7	44.9	3.3±2.1	0.82	73.3	37.1	1.5±1.3
Craticula	1.18	53.3	27.2	2.0 ± 2.4	1.19	53.3	27.2	1.2±1.5	1.01	60.0	30.5	1.8±1.8
Cyclotella	2.12	86.7	44.4	3.6±1.9	1.91	60.0	30.9	1.9 ± 2.1	1.34	86.7	44	2.4 ± 1.4
Cymbella	1.34	60.0	30.6	2.3 ± 2.1	1.12	53.3	27.2	1.1 ± 1.4	1.60	53.3	27.4	2.9 ± 3.2
Diadesmis	1.49	60.0	30.7	2.5 ± 2.3	1.65	66.7	34.2	1.7±1.6	1.68	80.0	40.8	3.0 ± 2.4
Diatoma	1.22	60.0	30.6	2.1 ± 2.1	1.45	53.3	27.4	1.5±1.7	2.53	73.3	37.9	4.5±3.2
Eunotia	1.65	66.7	34.2	2.8±2.3	1.72	66.7	34.2	1.7±1.6	1.30	66.7	34	2.3 ± 2.4
Fragilaria	1.89	73.3	37.5	3.2±2.5	1.25	60.0	30.6	1.3±1.3	1.30	80.0	40.6	2.3±1.5
Fragilariforma	1.18	73.3	37.2	2.0±1.5	0.73	60.0	30.6	0.7±0.7	1.27	60.0	30.6	2.3±2.4
Frustulia	1.02	73.3	37.1	1.7±1.8	1.12	66.7	33.9	1.1±1.2	0.82	66.7	33.7	1.5±1.4
			26.9		1.12	-	33.9	1.1±1.2		100.0		
Geminella	0.63	53.3		1.1±1.2					2.61		51.3	4.7±2.7
Gomphonema	1.97	86.7	44.3	3.3±2.2	-	-	-	-	1.53	46.7	24.1	2.7±3.8
Gyrosigma	1.02	66.7	33.8	1.7±1.6	-	-	-	-	1.23	53.3		2.2 ± 2.2
Gomphoneies	1.77	100	50.8	3.0±1.9	-	-	-	-	-	-	-	-
Navicula	4.41	86.7	45.5	7.5 ± 4.8	25.7	100	62.8	26±4.1	7.19	100.0	53.5	12.9±4.5
Brachysira	0.71	60.0	30.3	1.2±1.2	-	-	-	-	-	-	-	-
Pinnularia	3.86	93.3	48.5	6.5±3.7	0.92	46.7	23.8	0.9 ± 1.2	1.60	86.7	44.1	2.9 ± 1.8
Stauroneies	0.59	46.7	23.6	1.0 ± 1.3	-	-	-	_	0.45	40.0	20.2	0.8 ± 1.3
Cryptophyceae												
Rhodomonas	0.59	53.3	26.9	1.0±1.1	_	_	_	_	_	_	_	_
Dinophyceae	0.57	33.3	20.7	1.0±1.1	-	_	_	_	_	_	-	-
	2.16	60.0	21	27.20	1.12	72.2	27.2	1.1±0.9	1.20	72.2	27.2	22.10
Peridinium	2.16	00.0	31	3.7±3.8	1.12	73.3	37.2	1.1±0.9	1.30	73.3	37.3	2.3±1.9
Euglenophyceae		40.0	•••									
Euglena	0.79	40.0	20.4	1.3±2.2	0.53	46.7	23.6	0.5±0.6	0.71	53.3	27	1.3±1.4
Phacus	5.86	100	52.9	9.9±3.8	3.50	73.3	38.4	3.5 ± 3.0	8.0	100	54	14.3±2.7
Trachelomonas	1.10	60.0	30.5	1.9±2.1	0.92	53.3	27.1	0.9 ± 1.1	3.31	86.7	45	5.9±3.0
Strombomonas	1.65	80.0	40.8	2.8 ± 2.1	4.42	86.7	45.5	4.5±3.0	1.97	73.3	37.6	3.5 ± 2.9
Chlorophyceae												
Ankyra	_	-	_	_	_	_	-	_	1.01	60.0	30.5	1.8 ± 2.0
Ankistrodesmus	16.76	100	58.3	28.4±3.6	-	_	_	_	5.36	100.0	52.6	9.6±3.2
Anadyomena	0.98	60.0	30.49	1.7±2.1	_	_	_	_	-	-	-	-
	0.98	13.3	6.7	0.1±0.4	-	-	-	-	0.67	53.3	26.9	1.2±1.4
Chlamydomonas								-				
Coelastrum	12.08	100	56.0	20.5±3.2	1.50	-	40.0	16.13	2.61	86.7	44.6	4.7±3.4
Closterium	0.47	53.3	26.9	0.8±0.9	1.58	80.0	40.8	1.6±1.2	1.27	73.3	37.2	2.3±1.9
Cosmarium	1.38	80.0	40.7	2.3±1.6	0.26	26.7	13.4	0.3±0.5	1.01	60.0	30.5	1.8±2.0
Kirchneriella	1.14	60.0	30.6	1.9±2.1	1.32	66.7	34	1.3±1.4	1.19	66.7	33.9	2.1±1.9
Oedogonium	3.74	93.3	48.5	6.3±2.9	2.51	73.3	37.9	2.5 ± 2.0	2.01	86.7	44.3	3.6 ± 2.2
Oocystis	-	-	-	-	-	-	-	-	1.01	73.3	37.1	1.8±1.6
Pediastrum	2.12	73.3	37.7	3.6 ± 2.8	1.72	80.0	40.8	1.7±1.2	3.39	100.0	51.7	6.1±3.0
Scotiella	1.02	46.7	23.8	1.7±2.3	-	-	-	-	-	-	-	-
Scenedesmus	1.34	73.3	37.3	2.3±2.0	0.99	46.7	23.8	1.0±1.3	2.49	86.7	44.5	4.5±2.6
Spirogyra	3.93	100	52	6.7±2.2	3.10	53.3	28.2	3.1±3.2	28.3	100	64.1	50.7±4.1
Straurastrum	1.73	80.0	40.8	2.9±2.0	1.72	66.7	34.2	1.7±1.6	3.61	100.0	51.8	6.5±2.9
								1./±1.0				U.J±2.9
Tetradron	0.16	20.0	10	0.3±0.6	- 70	-	- 24	0.0.1.2	1 20	-	-	25.10
Tetrastrum	0.47	46.7	23.5	0.8±1.0	0.79	33.3	34	0.8±1.3	1.38	73.3	37.3	2.5±1.9
Ulothrix	13.34	100	56.6	22.6±2.6	2.31	53.3	27.8	2.3±2.5	1.64	66.7	34.1	2.9±2.3
Zygnema	1.34	66.7	34	2.3 ± 2.0	4.62	86.7	45.6	4.7 ± 2.9	4.95	100.0	52.4	8.9 ± 3.3
Chladophora	6.65	100	53.3	11.3±4.2	4.42	80.0	42.2	4.5±2.9	4.21	100.0	52.1	7.5 ± 2.4
Klebsormidium	-	-	-	-	0.33	33.3	16.8	0.3±0.5	-	-	-	-
Zooplankton												
Amoeba	0.67	46.7	23.6	1.1±1.5	_	_		_			_	_
Amoeba Arcella	1.85	66.7	34.2	3.1±2.9	-	-	-	-	0.45	40.0	20.2	0.8±1.1
								-	0.43	40.0	20.2	U.011.1
Mesocyclopssp.	0.20	26.7	13.4	0.3±0.6	-	-	-	-	- 0.00	-	-	-
Hydra	0.12	20.0	10	0.2±0.4		-	-	-	0.30	46.7	47	0.5 ± 0.6
Paramecium	0.16	26.7	13.4	0.3 ± 0.5	1.06	46.7	23.8	1.1±1.3	-	-	-	-
Detritus	46.30	100	73.1	78.5±9	84.9	100	92.4	85.8±4.3	33.6	100.0	66.8	60.1±3

(%) NF-Numeric frequency, %FO - Frequency of occurrence, I- Importance index.

Table 3. Diversity (H'= Shannon-Wiener) index and Breadth (B_A= standardized Levin's measure) of the diet of tadpoles in three different habitats

Habitat	H'	$\mathbf{B}_{\mathbf{A}}$	
Canal	3.098	0.17	
Ephemeral Pool	2.295	0.01	
Temporary Pond	3.115	0.06	

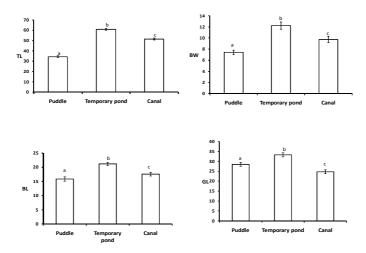


Fig. 3. Morphological characteristics (mm) of the tadpoles from different habitats.

TL-Total length, BW- Body width, BL-Body length, GL- Gut length.

Data are expressed as mean±SD. Bars having superscripts of different letter differ significantly from each other

Discussion

Amphibian larvae act as a link between aquatic and terrestrial ecosystems since the material consumed by the larvae in the aquatic habitat will be carried to the terrestrial environment by the adult [28]. Diet has a crucial role in the natural history of an animal, because not only does it reveal the source of the animal's energy for growth, maintenance, and/or reproduction [29, 30], but also it indicates part of the ecological roles of the animal. Dietary descriptions of a species from different localities are essential as it records temporal and spatial variations [31-33]. According to *R.A. Alford* [34], field studies of anuran larvae are still uncommon. Moreover, little information is available on the diets of anuran larvae.

Microhabitat selection is an important strategy of anuran larvae as it plays a key role in ensuring their survival and growth [3]. Many ecological factors such as predation pressure, food availability, competition, etc. can influence microhabitat use by tadpoles [34, 35]. *Euphlyctis cyanophlyctis* tadpoles are facultative suspension feeders and representative of *Orton* (1953) type IV [36]. These are bottom dwellers that scrape algae, macrophytes with the help of their ventrally situated heavy and keratinized teeth [36]. The result of the gut content analyses showed that apart from a large amount of detritus, the tadpole diet was based on microalgae, as corroborated by several studies [37, 38]. We identified prey items from class Bacillariophyceae, Chlorophyceae, Euglenophyceae, Cryptophyceae, Cyanophyceae and zooplanktons as the most important prey in order of their occurrence. Systematic and comparative evaluations of the food habits of tadpoles are uncommon because many ingested items pass damaged through the gut while other soft-bodied organisms and bacteria are not detected [13]. In this study, some of the

algae were found broken and without any organelles in the gut of the tadpoles, but it is unknown if the tadpoles digested these algae because they lack cellulase for digesting plant materials [13]. Diet composition of E. cyanophlyctis tadpoles in the three habitats revealed members of class Bacillariophyceae to be the most important prey category, an observation similar to B. Sinha et al., [20] and H.T. Lalremsanga et al., [36]. The importance of Bacillariophyceae as a food source has also been reported for other anuran genera such as Lithobates, Dendrosophus, Eupemphix and Scinax [38-40]. Bacillariophyceae can be richer in calories, mainly as a form of lipids and they are more easily accessible for consumption than filamentous algae [12]. Being a source of carbohydrates, chlorophytic algae also form another important food source [41]. Detritus, packed along the length of larval intestine, is mostly composed of degraded plant materials, which often bears little resemblance to the original plant tissue in terms of its structure and nutritional content. Much of the nutritional value of detritus may come from associated microbes than its particles per se [42]. Presence of detritus in all tadpole guts in the present study indicated that the larvae of E. cyanophlyctis from canals, ephemeral pools and temporary ponds had ingested substantial amounts of microbes. J.T. Heinen and J.A. Abdella [43] reported that tadpoles that ingest animal matter grew faster; yet they appear to require supplementary plant matter for optimal growth [3]. The zooplankton as seen from tadpole diets was represented by Paramecium in ephemeral pools; Arcella and Hydra in temporary ponds and Paramecium, Mesocyclops, Arcella and Hydra in canals. Despite similarity in diet composition, the relative abundance of each food item differed in different water bodies. Bacillariophyceae (43-46%) followed by Chlorophyceae (32-34%) and Euglenophyceae to a lesser extent (7-10%) were the preferred food items in all the three habitats. According to C. Diaz-Paniagua [44], different diets of tadpoles of a species in different habitats indicate that tadpoles can be flexible by changing their feeding habits as the availability of food items changes. H.T. Lalremsanga et al., [36] reported 27 genera and 5 classes of microalgae from the gut of these tadpoles from Meghalaya. However, we identified 55 genera belonging to 7 classes of microalgae from the gut of these tadpoles. Wide range of food items preferred by E. Cyanophlyctis tadpoles in different habitats proves them to be generalist predators that lack an apparent dietary preference and their diet most likely dependent on the prey availability. In spite of this, tadpoles from ephemeral pools had a lower prey diversity index compared to larvae from canals and temporary ponds. S. Sengupta et al., [45] suggested that tadpoles maintain the omnivore concept and ingest microbes via various food sources to support their growth and development. The wide spectrum of food choices indicated that they are highly adapted to various habitats: stream, ponds, lake, and lotic connected shallow standing pools [36].

Conclusions

The growth of tadpoles was highest in temporary ponds followed by canals. Anthropogenic inputs (washing of dishes and clothes, recreational use) in temporary ponds increases eutrophication leading to the observed algal abundance and higher food provision. On the contrary, ephemeral pools were free from human use and hence least food availability. The nearby agricultural lands can also contribute to eutrophication of surface water [46].

Considering the worldwide decline of amphibian populations and rapid degradation of habitats, it is essential to study the natural diet of the species. This reflects not only the availability of food in the environment but also the choice of the most appropriate feeding items to fulfil its nutritional necessities to metamorphosis. The analysis of diet also helps us to understand the relationship between biotic and abiotic components not only in aquatic environment but also in water ecotone.

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