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Dietary assessment of five species of anuran tadpoles from northern Odisha, India

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Abstract: Anuran tadpoles are gregarious predators capable of differentiating food items among diverse types of prey via varied feeding and oral structures. Tadpoles were collected from different study sites in three districts of northern Odisha during three consecutive rainy seasons (from July–October of 2015–2017). After morphometric measurements (total length and body length), the stomach contents of 75 tadpoles belonging to five different anuran species (*Duttaphrynus melanostictus*, *Euphlyctis cyanophlyctis*, *Fejervarya orissaensis*, *Polypedates maculatus* and *Microhyla ornata*) belonging to four families namely Bufonidae, Dicroglossidae, Rhacophoridae and Microhylidae were examined. The food spectrum of tadpoles included mostly detritus, followed by phytoplankton (represented by 5 classes and 54 genera). Such studies contribute to the understanding of the natural diets of these anuran species that can assist in developing management strategies for them. Aquatic habitats must be conserved and maintained so that conservation of anurans can be ensured.

Keywords: Anuran, conservation, food, Odisha, predators, tadpoles.

Amphibians are significant components of many fresh water and terrestrial ecosystems. The larvae of frogs and toads (Order Anura) are grossly different from adults and have many developmental (Alford & Johnston 1989) and morphological (Altig & McDiarmid 1999) features not seen in other amphibian larvae. They exhibit biphasic life cycles which refers to the ability of these animals to sustain the first part of their lives in water and the second part on land. Many Indian anuran species co-breed and utilize variety of lentic and lotic water bodies ranging from ephemeral ponds, damp grounds, temporary puddles, permanent ponds, streams and rivers following the south-west monsoon rain (Saidapur 1989). Unpredictable temporal, spatial distributions and cyclic pattern of nutrient availability are common features of these habitats. Tadpoles in temporary ponds must grow quickly to complete metamorphosis before the pond gets dried. The metamorphosis duration depends on a number of variables such as drying, predation, competition, food availability and water temperature. The amount of food a tadpole consumes directly affects its growth (Kiffney & Richardson 2001) and the quality of food consumed affects the rate of growth (Kupferberg et al. 1994; Brown & Rosati 1997). Hence, tadpoles of different species that live together are subjected to both intra- and inter-specific competition for food, space and to predation pressure

There is a dearth of information on the tadpoles of India, especially from northern Odisha. Most of the

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studies on amphibians have been concentrated in the Western Ghats (biodiversity hotspot), and other areas remain understudied (Aravind & Gururaja 2011). Twenty-six species of frogs are found in Odisha and 21 species of anurans from Similipal Biosphere Reserve including representatives from the families like Bufonidae (three species), Dicroglossidae (eight species), Microhylidae (five species), Ranidae (one species) and Rhacophoridae (four species) (Dutta et al. 2009).

Understanding food and feeding strategies is central to tadpole biology. Amphibians are generally considered to be feeding opportunists with their diets reflecting the availability of food of appropriate size. Typically, tadpoles are characterized by an oral disc with keratinised jaw sheaths and equally keratinised labial "teeth" (also called keratodonts), which they use to rasp algae or bacterial films from underwater surfaces for consumption. Most tadpoles are primarily herbivorous (Duellman & Trueb 1986) consuming a wide variety of algal taxa as well as detritus, viruses, bacteria, protists, plant fragments, pollen grains, fungi, various kinds of small animals, anuran eggs, and other tadpoles (Kupferberg et al. 1994; Mahapatra et al. 2017a). Besides these general considerations, studies on natural diets of tadpoles, including systematic and comparative evaluation of the food habits of tadpoles are still rare (Alford 1999; Hoff et al. 1999). Knowledge of food and feeding behaviour of the tadpole is essential as early part of life history of amphibian is dependent on the availability of the food items in their natural habitat (Díaz-Paniagua 1985; Inger 1986). It was only over the past three decades that dietary information on anuran larvae has been published (Khare & Sahu 1984; Ao & Khare 1986; Sekar 1990; Saidapur 2001; Sinha et al. 2001; Khongwir et al. 2003). The aim of the present study was to investigate the feeding biology of the co-occurring tadpoles in their natural habitats of northern Odisha.

Materials and Methods

Study area

The study was conducted in three northern districts (Balasore, Mayurbhanj and Keonjhar) of Odisha, India. It forms a part of the Eastern Ghats hill ranges. The climate of the area is sub-tropical with a hot summer (March to May, 40–42 ^{0}C), rainy (June-October, actual average precipitation, 1283.4mm) and a chilling winter (November-February, 5–7 ^{0}C). The breeding of most of the anurans occur during the rainy season. The sampling sites were selected based on primary survey of these temporary ponds having multiple species of tadpoles.

Sampling

The tadpole assemblages were sampled from temporary water bodies during the rainy seasons (July–October) of 2015, 2016 and 2017 using dip net (mesh size 1mm). The larvae (N = 15 for each species) were preserved in 10% formaldehyde immediately after collection in the field in order to prevent complete digestion of ingested food particles. In the laboratory, individuals of stages 35–38 (Gosner 1960) were separated and subsequently preserved in 4% formaldehyde.

The gut of each tadpole was removed carefully; gut length was recorded with the help of a digital vernier caliper (Mitutoyo™ to the nearest 0.1mm). The first four centimetre 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 (Edmondson 1959; Smith 1994). Unidentified items, which formed a mass of organic material, were classified as detritus.

Results

Five species of anuran larvae namely *Duttaphrynus melanostictus, Polypedates maculatus, Fejervarya orissaensis, Euphlyctis cyanophlyctis* and *Microhyla ornata* were predominant co-occurring species in the study area and belonged to four families (Bufonidae, Dicroglossidae, Rhacophoridae and Microhylidae). They breed in most of the aquatic habitats (temporary ponds and ephemeral pools). All these tadpoles were exotrophic, lentic and representatives of Orton (1953) type IV except *M. ornata* type II.

Various types of food items were recorded from the gut contents of these co-occurring tadpoles. The trophic spectrum included mostly detritus, followed by phytoplankton represented by five classes and 54 genera and zooplanktons (Table 1). Most of the microalgae belonged to the class Bacillariophyceae followed by Chlorophyceae. Most of the zooplanktons belonged to *Amoeba, Hydra* and *Paramecium*.

Family: Bufonidae

***Duttaphrynus melanostictus* Schneider, 1799**

(Common Asian Toad)

General morphology of the tadpoles

(N = 15; Body Length: 8.22–8.66 mm; Total Length: 17.02–18.32 mm; Gut length: 55–67 mm)

The body is black in colour with many closely placed tiny melanophores on both inner and outer integuments (in life), roughly oval and elliptical in dorsal and lateral views, snout rounded. Eyes were large; located and oriented dorsolaterally. Spiracle sinistral. Vent tube was median and short. Oral disc was antero-ventral in location.

Gut contents

Phytoplanktons:

Cyanophyceae: *Merismopedia* sp., *Choococcus* sp., *Gloeotheca* sp., *Oscillatoria* sp.

Bacillariophyceae: *Naviculla* sp., *Pinularia* sp., *Fragillaria* sp., *Frustulia* sp., *Cymatopleura* sp. *Nitzschia* sp., *Synedra* sp., *Cymbella* sp., *Sellaphora* sp., *Actinella* sp., *Placoneis* sp., *Gomphonema* sp.

Chlorophyceae: *Oedogonium* sp., *Scehendesmus* sp., *Oocystis* sp., *Haematococcus* sp., *Cosmarium* sp., *Pediastrum* sp., *Tetrastrum* sp., *Closterium* sp., *Staurastrum* sp., *Euastrum* sp., *Ankistrodesmus* sp.

Euglenophyceae: *Phacus* sp., *Trachelomonas* sp.

Family: Dicroglossidae

1. *Euphlyctis cyanophlyctis* Schneider, 1799 (Indian skipper frog)

General morphology of the tadpoles

(N = 15; Body Length: 12.25-14.55 mm; Total Length: 45.95-47.2 mm; Gut length: 239.6-252.4 mm)

Body oval in both dorsal and lateral views. The snout was pointed in dorsal and rounded in lateral views. Eyes were large; located dorsolaterally. The nostrils were reniform. Spiracle sinistral. Oral disc was near ventral in location.

Gut contents

Phytoplanktons:

Cyanophyceae: *Merismopedia* sp., *Choococcus* sp., *Oscillatoria* sp., *Microcystis* sp.

Bacillariophyceae: *Amphipleura* sp., *Asterionella* sp., *Achnanthidium* sp., *Aulacoseira* sp., *Cocconeis* sp., *Craticula* sp., *Cyclotella* sp., *Cymbella* sp., *Diadesmis* sp., *Diatoma* sp., *Eunotia* sp., *Gomphonema* sp., *Gyrosigma* sp., *Naviculla* sp., *Nitzschia* sp., *Pinnularia* sp., *Tabellaria* sp.

Chlorophyceae: *Actinastrum* sp., *Ankistrodesmus* sp., *Ankyra* sp., *Closterium* sp., *Cosmarium* sp., *Oocystis* sp., *Scenedesmus* sp., *Staurastrum* sp., *Spirogyra* sp., *Ulothrix* sp., *Oedogonium* sp.

Euglenophyceae: *Phacus* sp., *Trachelomonas* sp.

Cryptophyceae: *Rhodomonas* sp.

Zooplankton: *Amoeba* sp., *Hydra* sp., *Paramecium* sp.

2. *Fejervarya orissaensis* Dutta, 1997 (Odisha Frog)

General morphology of the tadpoles

(N = 15; Body Length: 7.27–9.45 mm; Total Length: 21.67–26.7 mm; Gut length: 38.41–48.98 mm)

Body oval and elliptical in dorsal and lateral views. The snout was rounded in dorsal and lateral views. Eyes were large; located and oriented posterolaterally. The nostrils were spherical. Spiracle sinistral. Oral disc was near ventral in location.

Gut contents

Phytoplanktons:

Cyanophyceae: *Gloeotheca* sp., *Oscillatoria* sp., *Gomphospharia* sp.

Bacillariophyceae: *Naviculla* sp., *Pinnularia* sp., *Eunotia* sp., *Craticula* sp., *Nitzschia* sp., *Synedra* sp., *Fragillaria* sp., *Frustulia* sp., *Cymbella* sp., *Amphipleura* sp., *Diadesmis* sp., *Cocconeis* sp., *Cymatopleura* sp.

Chlorophyceae: *Closterium* sp., *Zygnema* sp., *Scenedesmus* sp., *Staurastrum* sp., *Chlamydomonas* sp., *Haematococcus* sp., *Cosmarium* sp., *Volvox* sp., *Ankistrodesmus* sp., *Oedogonium* sp., *Euastrum* sp., *Ankyra* sp.

Euglenophyceae: *Phacus* sp., *Trachelomonas* sp., *Euglena* sp.

Family: Rhacophoridae

***Polypedates maculatus* Gray, 1830 (Indian Tree Frog)**

General morphology of the tadpoles

(N = 15; Body Length: 13.68–17.87 mm; Total Length: 46.37–52.22 mm; Gut length: 184.34–211.54 mm)

Body oval and elliptical in dorsal and lateral views. Snout rounded. Eyes were large; located and oriented dorsolaterally. Nostrils spherical. Vent tube was dextral. Oral disc was anteroventaral in location.

Gut contents

Phytoplanktons:

Cyanophyceae: *Microcystis* sp., *Oscillatoria* sp., *Merismopedia* sp., *Choococcus* sp.

Bacillariophyceae: *Cyclotella* sp., *Fragillaria* sp., *Navicula* sp., *Nitzscia* sp., *Synedra* sp., *Cymbella* sp., *Pinnularia* sp., *Stauroneis* sp., *Amphipeura* sp., *Cocconeis* sp., *Craticula* sp., *Diadesmis* sp., *Frustulia* sp., *Gomphonema* sp.

Chlorophyceae: *Actinastrum* sp., *Ankistrodesmus* sp., *Cosmarium* sp., *Closterium* sp., *Oedogonium* sp., *Spirogyra* sp., *Chlamydomonas* sp., *Ulothrix* sp., *Scenedesmus* sp., *Oocystis* sp., *Pediastrum* sp., *Zygnema* sp., *Volvox* sp., *Pandorina* sp.

Euglenophyceae: *Phacus* sp., *Trachelomonas* sp., *Euglena* sp.

Family: Microhylidae

***Microhyla ornate* Dumeril and Bibron, 1841**

(Ornamented Pygmy Frog)

General morphology of the tadpoles

(N = 15; Body length: 8.41–10.96 mm; Total length: 26.90–31.47 mm; Gut length: 62.63–75.34 mm)

Dorsally the body shape was oval with a truncated anterior portion; laterally the body was ovoid and depressed on the dorsal side with an acutely rounded anterior and a broadly rounded posterior. Eyes were large, round and located and oriented laterally. Spiracle medial. Oral opening was at the anterior end of the body at the snout tip and visible dorsally and non-emarginated.

Gut contents

Phytoplanktons:

Cyanophyceae: *Merismopedia* sp., *Oscillatoria* sp., *Gloeotheca* sp., *Microcystis* sp.

Bacillariophyceae: *Naviculla* sp., *Pinnularia* sp., *Eunotia* sp., *Nitzschia* sp., *Frustulia* sp., *Cymbella* sp., *Cocconeis* sp.

Chlorophyceae: *Closterium* sp., *Scenedesmus* sp., *Staurastrum* sp., *Chlamydomonas* sp., *Haematococcus* sp., *Cosmarium* sp., *Ankistrodesmus* sp., *Oedogonium* sp., *Oocystis* sp., *Tetrastrum* sp.

Euglenophyceae: *Phacus* sp., *Trachelomonas* sp., *Euglena* sp.

Discussion

Anuran larvae are some of the least understood in terms of their trophic relations (Petranka & Kennedy 1999; Altig 2007). Most anurans breed in countless aquatic habitats, i.e., ephemeral ponds and puddles etc. of diverse nature that support the growth and abundance of different species of algae, diatoms and plankton. Though amphibians are leading a biphasic life, water is the basic need for their early larval development. Within the short period of time the tadpoles have to be metamorphosed by utilizing the ample source of nutrients in water and escaping from desiccation. Tadpoles may partition the available food resources. Duellman & Trueb (1986) commented that food partitioning among anuran tadpoles is caused by differences in the ability of the various species to ingest particles of varying sizes and also to the position they occupy in the water column, a consequence of morphological adaptations for the exploitation of specific microhabitats. Tadpoles of various species are often morphologically different and feed on different food items to reduce competition in single water bodies (Diaz-Paniagua 1985; Harrison 1987). Tadpoles feed at many sites throughout the water column (benthic, mid water, surface) and have characteristic morphologies and behaviour (McDiarmid & Altig 1999). Tadpoles of *F. orissaensis* and *E. cyanophlyctis* show characteristics of benthic water adaptation viz., dorsal eyes, weak tail fins and ventral mouth. On the other hand, *M. ornata* tadpoles are surface feeder and were always encountered on the surface with bulging lateral eyes, tail fins well developed, lower fin broader than upper one and antero-dorsal mouth. *D. melanostictus* tadpoles adopted to survive in shallow water and have thick black body, not so well-developed tail for swimming and weak tail musculature. *P. maculatus* show characteristics of nektonic habitat guild.

The result of the gut content analyses showed that apart from a large amount of detritus, the tadpole diet was largely based on microalgae as corroborated by several studies (Lajmanovich 2000; Rossa-Feres et al. 2004). We identified prey items from class Bacillariophyceae, Chlorophyceae, Euglenophyceae, Cyanophyceae and Cryptophyceae. 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 (Cummins & Klug 1979). Diet composition of all anuran tadpoles revealed members of class Bacillariophyceae to be the most important prey category, an observation similar to Sinha et al. (2001). The importance of Bacillariophyceae as a food source has also been reported for other anuran genera such as *Lithobates, Dendrosophus, Eupemphix* and *Scinax* (Hendricks 1973; Kupferberg 1997; Rossa-Feres et al. 2004). Bacillariophyceae can be richer in calories, mainly as a form of lipids and they are more easily accessible for consumption than filamentous algae (Kupferberg et al. 1994). Being a source of carbohydrates, chlorophytic algae also form another important food source (Bold & Wynne 1985). The zooplanktons as seen from tadpole diets were represented by *Paramecium* sp., *Hydra* sp. and *Amoeba* sp. in *E. cyanophlyctis* tadpoles, an observation similar to Mahapatra et al. (2017b). 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 successful completion of life cycle of amphibian tadpoles. Qualitative analyses of food spectrum of five species of anuran tadpoles (*B. melanostictus, Rhacophorus maximus, Amolops afghanus, Rana danieli* and *E. cyanophlyctis*) from Arunachal Pradesh, India by Sinha et al. (2001) recorded

Table 1. Phytoplankton species identified from the intestine of anuran tadpoles (DM: *Duttaphrynus melanostictus,* PM: *Polypedates maculatus,* FO: *Fejervarya orissaensis,* EC: *Euphlyctis cyanophlyctis* and MO: *Microhyla ornata*; + = Present, - = Absent).

Class	Genus	DM	EC	FO	PM	MO
Cyanophyceae	*Choococcus* sp.	+	+	-	+	-
	Gloeotheca sp.	+	-	+	-	+
	Microcystis sp.	-	+	-	+	+
	Merismopedia sp.	+	+	-	+	+
	Gomphospharia sp.	-	-	+	-	-
	Oscillatoria sp.	+	+	+	+	+
Bacillariophyceae	*Achnanthidium* sp.	-	+	-	-	-
	Actinella sp.	+	-	-	-	-
	Amphipleura sp.	-	+	+	+	-
	Asterionella sp.	-	+	-	-	-
	Aulacoseira sp.	-	+	-	-	-
	Cocconeis sp.	-	+	+	+	+
	Craticula sp.	-	+	+	+	-
	Cyclotella sp.	-	+	-	+	-
	Cymbella sp.	+	+	+	+	+
	Cymatopleura sp.	+	-	+	-	-
	Diadesmis sp.	-	+	+	+	-
	Diatoma sp.	-	+	-	-	-
	Eunotia sp.	-	+	+	-	+
	Fragillaria sp.	+	-	+	+	-
	Frustulia sp.	+	-	+	+	+
	Gomphonema sp.	+	+	-	+	-
	Gyrosigma sp.	-	+	-	-	-
	Naviculla sp.	+	+	+	+	+
	Nitzschia sp.	+	+	+	+	+
	Pinnularia sp.	+	+	+	+	+
	Placoneis sp.	+	-	-	-	-
	Sellaphora sp.	+	-	-	-	-
	Stauroneis sp.	-	-	-	+	-
	Synedra sp.	+	-	+	+	-
	Tabellaria sp.	-	+	-	-	-
Chlorophyceae	*Ankistrodesmus* sp.	+	+	+	+	+
	Actinastrum sp.	-	+	-	+	-
	Ankyra sp.	-	+	+	-	-
	Cosmarium sp.	+	+	+	+	+
	Closterium sp.	+	+	+	+	+
	Chlamydomonas sp.	-	-	+	+	+
	Euastrum sp.	+	-	+	-	-
	Haematococcus sp.	+	-	+	-	+
	Oedogonium sp.	+	-	+	+	+
	Oocystis sp	+	+	-	+	+
	Pandorina sp.	-	-	-	+	+
	Pediastrum sp.	+	-	-	+	+
	Scehendesmus sp.	+	+	+	+	+
	Spirogyra sp.	-	+	-	+	+
	Staurastrum sp.	+	-	+	-	+
	Tetrastrum sp.	+	-	-	-	+
	Ulothrix sp.	-	+	-	+	+
	Volvox sp.	-	-	+	+	+
	Zygnema sp.	-	-	+	+	-
Euglenophyceae	*Euglena* sp.	-	-	+	+	+
	Phacus sp.	+	+	+	+	+
	Trachelomonas sp.	+	+	+	+	+
Cryptophyceae	*Rhodomonas* sp.	-	+	-	-	-
Zooplankton	*Amoeba* sp.	-	+	-	-	-
	Hydra sp.	-	+	-	-	+
	Paramecium sp.	-	+	-	-	+

the presence of diatoms and Chlorophyta in all the five species which was also seen in the present study. Foraging behaviour is one of the most important components of reproductive fitness (Nishimura 1999). Therefore, the remarkable ability of most group-living organisms to distribute themselves precisely among feeding sites in proportion to habitat profitability is not surprising (Godin & Keenleyside 1984; Talbot & Kramer 1986). Tadpoles of anurans feed both on the phytoplankton community by means of filtration, and on a large variety of substrates (including algae, macrophytes & carrion) by rasping, scraping and chopping with their jaw sheaths and labial teeth (Seale & Wassersug 1979; Seale 1982).

Conclusion

In tropical aquatic ecosystems, the study of the natural diet of resident species is an important tool in understanding the biotic and abiotic interrelationships. Diet analysis of larvae provides valuable information on foraging pattern, nutritional requirements and trophic interaction in aquatic food webs which is critical for successful conservation and management. Further, such knowledge also indicates the susceptibility of the species in light of the current environmental alterations.

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