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Diet of the Dattatreya night frog *Nyctibatrachus dattatreyaensis* from the central Western Ghats, India

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ABSTRACT – The Dattatreya night frog *Nyctibatrachus dattatreyaensis*, found in the Chandra Drona Parvatha massif, is a stream-dwelling, evolutionarily distinct and globally Endangered species threatened by increasing habitat loss and alteration. We examined the stomach contents of 104 individuals, from ten different streams, of which 42 had prey in their stomachs. The prey items were in 12 orders across 4 classes, mainly dipterans, hymenopterans and lepidopterans. The frog exhibits a passive foraging mode, has a moderate trophic niche breadth (B_{st} = 0.43), and may have a preference for agile prey. Apart from this, there were plant materials, sand grains and plastic debris found in the stomach contents, with 0.82 mm³ of plastic debris found in eight individuals across three streams. The presence of plastic debris indicates the impact of anthropogenic activities leading to a form of habitat degradation. The data presented indicates the need for immediate and efficient conservation strategies to be put in place for this understudied species.

INTRODUCTION

Feeding ecology plays a pivotal role in the overall biology of a species, impacting its survival, reproductive strategies, population dynamics, habitat preferences and social patterns (Hohmann et al., 2006). Anurans may be active or passive foragers (Duellman & Lizana, 1994) but these foraging strategies are not mutually exclusive and even within a species may vary depending on the individual and the habitat (Crnobrnja-Isailović et al., 2012). Additionally, anurans are classified as specialist, intermediate or generalist predators, according to their prey preferences and the diversity of available prey in their habitats (Toft, 1981). Understanding the feeding ecology of a species provides insights into its foraging mode and associated physiological processes (Taigen & Pough, 1983; Navas et al., 2008), as well as interactions with sympatric species, and the impact of invasive species (Sabagh et al., 2010; Mohanty & Measey, 2018). Furthermore, the outcomes of dietary studies can aid in designing ex-situ conservation initiatives for threatened species (Michaels et al., 2014; Jayson et al., 2018). They are also essential for assessing ecosystem health and understanding the impacts of environmental factors like habitat fragmentation and alteration due to human activities (Anderson et al., 1999; Hocking & Babbitt, 2014). Nevertheless, there is limited information available on the feeding ecology of post-metamorphic anurans from the Indian subcontinent, with just a few studies on species endemic to the Western Ghats, such as *Rhacophorus pseudomalabaricus* (Kanagavel et al., 2017) and *Indirana leithii* (Modak et al., 2018).

The genus *Nyctibatrachus* Boulenger, 1882 represents a fascinating and diverse assemblage of nocturnal amphibian species, paleoendemic to the Western Ghats, a biodiversity hotspot (Abraham et al., 2022). It currently consists of 34 recognised extant species known for their cryptic nature and unique modes of reproduction (Kumar et al., 2022). *Nyctibatrachus*, with 84% of its species threatened, is the fourth most threatened genus in the Indomalayan biogeographic realm (Re:wild, Synchronicity Earth & IUCN SSC ASG, 2023). However, little is known of their ecology, including diet and feeding preferences.

Dattatreya's night frog *Nyctibatrachus dattatreyaensis* is an evolutionarily distinct and Globally Endangered (EDGE) species with a global priority for conservation (Gumbs et al., 2023). Discovered by Dinesh et al. (2008), this species is endemic to the central Western Ghats of India. It is a stream-dwelling, range-restricted species, currently known only from the Chandra Drona Parvatha massif, in the Chikmagalur district of Karnataka, at elevations between 865 and 1,515 m a.s.l.. Unfortunately, it is severely threatened by habitat destruction and fragmentation resulting from residential and commercial development and agriculture. Climate change and associated changes in the seasonal rain patterns will likely have a negative impact on their population and distribution (IUCN SSC, 2023). Despite this, the species remains significantly understudied to the extent that the only available literature pertains solely to its taxonomy. Given this premise, we report here attributes related to the feeding ecology of *N. dattatreyaensis*, which include its diet composition, foraging mode and niche breadth.

MATERIALS & METHODS

Study site

Streams of Chandra Drona Parvatha massif were randomly sampled to assess the presence and abundance of *N. dattatreyaensis*. From these, ten streams with well-established breeding populations were selected for this study (Figs. 1 & 2). The altitudes of the sampling sites ranged between 1,113

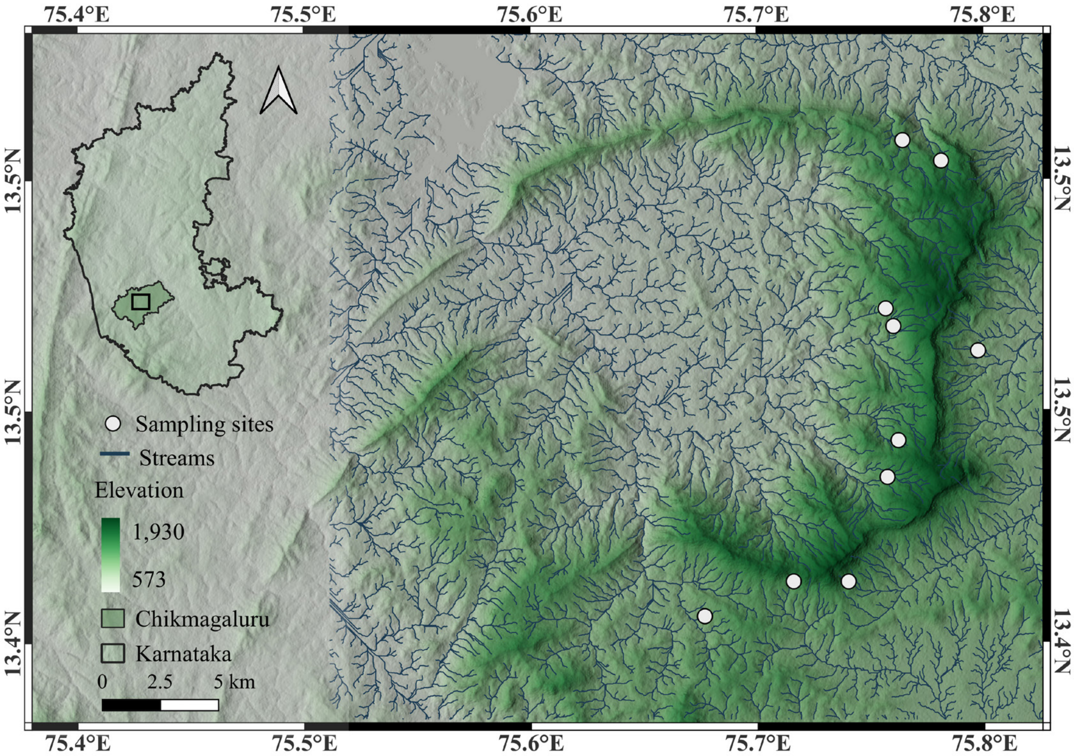

Figure 1. Map depicting Chandra Drona Parvatha massif and the sampling sites

and 1,420 m a.s.l.. The higher-elevation region of the massif has shola forests, where most of the streams originate. The massif also contains evergreen, semi-evergreen and moist deciduous forest types, and is fragmented with coffee estates, homestays and human settlements. Due to the cooler weather, picturesque mountains and waterfalls, the massif attracts numerous tourists throughout the year. The Bhadra Wildlife sanctuary borders and occupies a small portion of the massif.

Sampling

The frog population was sampled during the post-monsoon and winter seasons, between November 2022 and March 2023. The study area receives heavy rains during the monsoon season, which decreases the probability of detecting the species. A 100 m line transect was laid at each of the ten sampling site and surveyed from 20:00 to 23:00 h. Adult individuals were located using visual and acoustic encounter methods (Heyer et al., 1994). Individual frogs were captured gently using scoop nets and were handled using disposable vinyl nitrile gloves. Each stream section was sampled only once to prevent the recapture of individuals. Sex was determined by checking for the presence of femoral glands and nuptial pads which are secondary sexual characteristics of male *Nytcibatrachus* (Biju et al., 2011). In the case of females, pale, yellowish-coloured eggs were visible through the lateral side of the abdomen. We assigned adult individuals that lacked these characters to unknown sex. Snout-vent length (SVL) of all the individuals was measured using a digital vernier calliper with an accuracy of 0.1 mm.

Figure 2. Female *Nyctibatrachus dattatreyaensis* in its natural habitat

All individuals were weighed, and then their stomach contents were collected by flushing their stomachs. We followed the protocols of Solé et al. (2005) for stomach flushing and this conforms to the BHS Ethics Policy. To ensure minimal animal stress, the entire procedure on a single individual was completed within 10 minutes on-site. The frogs were then promptly returned to their original capture locations and monitored to ascertain if there were any adverse effects after stomach flushing. A sterilised, 14 Fr (4.6 mm diameter) silicone catheter was carefully inserted into the frog's stomach, and 10 ml of stream water was flushed through the catheter a maximum of three times using a syringe. Any stomach contents expelled from frogs during this procedure were collected in clean containers. The stomach contents were subsequently sieved and preserved in separate vials containing 70% ethanol. All the intact and partially digested prey items were assorted and identified under a stereomicroscope. The length and width of only the undamaged prey items were measured to the nearest millimetre using a graph sheet (Seshadri et al., 2021). The preserved prey items were identified to their lowest taxonomic level, usually to order, using literature (Tripplehorn & Johnson, 2005; Ramani et al., 2019).

Data analyses

To quantify the dietary intake of *N. dattatreyaensis*, we calculated the following parameters, Numeric percentage

$$N\% = \frac{N}{p} \times 100$$

where N is the number of prey items of a specific order/ class and p is the total number of prey items. Frequency of occurrence percentage

$$F\% = \frac{F}{n} \times 100$$

where F is the frequency of occurrence and n is the number of individuals it was extracted from. The volume of each prey item was calculated using the ellipsoid volumetric formula,

$$V\% = \frac{4}{3}\Pi \left(\frac{length}{2}\right)\left(\frac{width}{2}\right)^2$$

(Griffiths & Mylotte, 1987). For each prey category, we calculated the index of relative importance following Pinkas (1971), *IRI = (V% + N%) F%*, and then converted it to IRI%. Higher IRI% signifies higher relative importance of that prey category.

Statistical analyses

Shapiro-Wilk normality test indicated that the data do not follow a normal distribution, therefore non-parametric tests were selected for all the analyses. We used the Mann-Whitney test U to analyse the difference in size and weight between the male and female individuals. To assess the heterogeneity and evenness in diet, we used reciprocal Simpson's heterogeneity index 1/D

$$D = -\sum[ni(ni-1)]/([N(N-1)])$$

where N is the total number of prey items and *ni* is the number of prey items in the prey category *i* and Simpson's evenness index

$$E_{1/D} = \frac{\frac{1}{D}}{S}$$

where S is the number of species (Krebs, 1999). We calculated trophic niche breadth using Levin's measure of niche breadth (B) (Levins, 1968) and standardised trophic niche breadth (B_{st}) (Hurlbert, 1978; Krebs, 1999). The index ranges from 0 to 1; values closer to 0 indicate usage of narrow niche breadth (specialist diet), and values closer to 1 indicate usage of broader niche breadth (generalist diet). All the analyses were performed using R Statistical Software (R Core Team 2022).

RESULTS

We collected a total of 104 individuals of *N. dattatreyaensis* from across the sampling sites: 59 females, 37 males and 8 individuals whose sex could not be determined. Males had a mean SVL of 34.20 ± 3.35 mm and a weight of 6.49 ± 1.99 g. Females had a mean SVL of 34.57 ± 3.94 mm and a weight of 6.36 ± 2.10 g. There was no significant difference between males and females in either SVL (W = 225, p-value = 0.9207) and body weight (W = 233.5, p-value = 0.7532) (Fig. 3). At the same time, there was a strong positive correlation between the SVL and body weight (Fig. 4) with a correlation coefficient of 0.92 (Spearman's Rank Correlation).

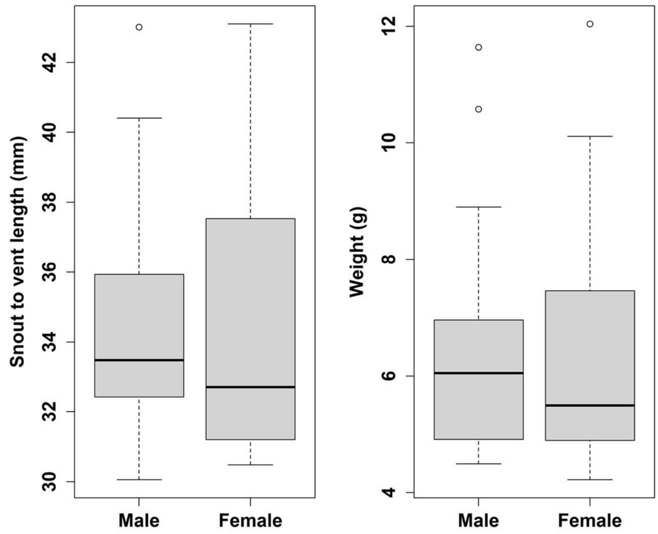

Figure 3. Comparison between female and male *Nyctibatrachus dattatreyaensis* in (left) SVL and (right) body weight

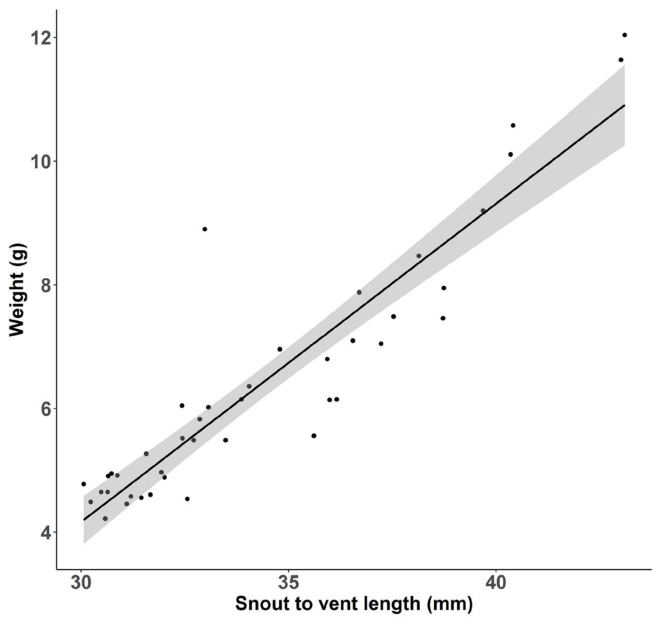

Figure 4. Relationship between body weight and SVL of adult *Nyctibatrachus dattatreyaensis*

After stomach flushing, we obtained prey items from 42 individuals (29 females and 13 males). We identified 67 prey items belonging to four classes: Oligochaeta, Insecta, Arachnida, and Crustacea. The prey items were identified to their respective orders, ten altogether (Table 1) except for two of the prey items, which remain unnamed. Apart from these, we also found plant materials and plastic debris in the

Table 1. Diet composition of *Nyctibatrachus dattatreyaensis* represented in numeric percentage (N%), percentage of frequency of occurrence (F%), volumetric percentage (V%), Index of relative importance (IRI) and its percentage representation (IRI%).

Class	Order	N%	F%	V%	IRI	IRI%
Oligochaeta	Unidentified	4.48	4.76	-	-	-
Insecta	Orthoptera	1.49	2.38	12.05	32.24	0.78
	Dermaptera	1.49	2.38	7.82	22.17	0.54
	Hymenoptera	17.91	16.67	6.44	405.78	9.82
	Coleoptera	10.45	7.14	1.51	85.38	2.07
	Lepidoptera	8.96	11.90	6.02	178.27	4.31
	Trichoptera	7.46	7.14	4.52	85.56	2.07
	Hemiptera	1.49	2.38	0.23	4.11	0.10
	Diptera	37.31	33.33	60.94	3274.98	79.25
Crustacea	Isopoda	1.49	2.38	0.17	3.97	0.10
Arachnida	Araneae	4.48	7.14	0.08	32.57	0.79
	Unknown	2.99	2.38	0.23	7.66	0.19

stomach contents. Plastic debris was found in eight individuals from three streams: two bisected by roads and one within a coffee estate. The total volume of the plastic debris was 0.82 mm³, with a mean volume of 0.07 ± 0.05 mm³. This excludes plastic debris with a width smaller than 0.1 mm such as lint microfibres and other microplastics. Dipterans (mosquitoes, crane flies) and hymenopterans were the most consumed among the prey items, with IRI 79.25% and 9.82%, respectively, followed by lepidopterans and coleopterans. The index of relative importance is calculated for all the prey categories, and the same can be found in Table 1.

The diet of *N. dattatreyaensis* is diverse with distinct prey items (Reciprocal Simpson's Heterogeneity Index (1/D): 4.960221), and the prey items preferred are moderately even (Simpson's Evenness Index E1/D: 0.41335). Based on the percentage utilisation of 12 food sources, the trophic niche breadth of the species is 5.76 according to Levin's measure of niche breadth (B) and 0.43 after standardisation (B_{st}). This indicates that the species has a moderate niche breadth and may favour certain types of prey over others.

DISCUSSION

Some anurans are specialist foragers, while others feed on diverse groups of prey items and exhibit preferences for certain specific taxa (López et al., 2007; Blanco-Torres et al., 2021). However, most anurans exhibit generalist feeding behaviour (Santos et al., 2004; Solé & Rödder, 2010). *Nyctibatrachus dattatreyaensis* is a generalist but would appear to have some preferences. We found 12 prey orders from four classes in their stomach contents, including undetermined prey items. Among them, dipterans were predominant in volume and frequency of occurrence (V = 550.85 mm^3, F = 14). Volumetrically, the largest single prey item they consumed belonged to dipterans (crane fly, V = 301.6 mm^3) and the smallest single prey items were hymenopterans (ant, V = 0.03 mm^3). Although they consume prey from 12 different orders with varying sizes, dipterans had the highest relative importance (IRI% = 79.25) followed by hymenopterans (IRI% = 9.82) and lepidopterans (IRI% = 4.31). The trophic niche breadth value of B_{st} = 0.43 indicates a relative predominance of some prey items (dipterans) over others in the composition of their diet. Whether there is any actual preference for specific prey can only be confirmed with a study on prey availability in their habitat (Woodhead et al., 2007).

Studies on other anurans have shown a correlation between the foraging mode, prey preference and metabolic characteristics of a species. The anurans that prefer sedentary prey tend to have high aerobic capacity and resting metabolism, apt for active foraging. In contrast, agile prey is selected by the anurans that have low aerobic capacity and resting metabolism that correlates with the opportunistic mode of foraging (Taigen & Pough, 1983; Twardochleb et al., 2020). The predominant prey type in the diet of *N. dattatreyaensis* in this study was agile dipterans. They also frequently consumed other highly mobile prey items, such as Lepidoptera, Coleoptera and Trichoptera, exhibiting a passive foraging mode (sit-and-wait foraging strategy) similar to *Fejervarya moodiei* (Mohanty & Anujan, 2022). Frogs that are active foragers are often known to have toxins to deter their predators while those that are passive foragers rely on camouflage to attack and consume their prey (Toft, 1981; Wells, 2010). We only observed *N. dattatreyaensis* sitting inactive, well camouflaged in the slower sections of the stream near vegetation, ambushing prey that moved in front of them; they are not known to have any toxins. These observations are consistent with the species being a passive forager.

Some anurans consume plant materials as a major part of their diet, intentionally and regularly, for example, *Boana albomarginata* (Tupy et al., 2021). But in most frogs, it is thought to be consumed accidentally (Rodrigues et al., 2023). Ingestion of sand grains may aid the mechanical digestion of insects such as beetles or could have been passively ingested from their microhabitat (Evans & Lampo, 1996). The plastic debris in the stomach content might have been ingested while consuming insects. Previous studies have documented the presence of microplastics or plastic debris in the stomach content of tadpoles (Kolenda et al., 2020) and toads (Döring et al., 2017) highlighting the impact of anthropogenic activities surrounding the freshwater streams inhabited by *N. dattatreyaensis*. Microplastics have been detected even in remote, high-altitude freshwater bodies, similar to the study area for *N. dattatreyaensis* (Iannella et al., 2019). When ingested and accumulated in the organs of tadpoles, microplastics lead to alterations in their external morphology, genetic mutations and cell damage (Araújo et al., 2020). They can be fatal, induce stress, and increased levels of microplastics in the water could impair the tadpole's ability to feed (Balestrieri et al., 2022). When coupled with chytridiomycosis, the bioaccumulation of microplastics amplified vulnerability among tadpoles (Bosch et al., 2021). Chytrid infection has been previously reported in species of *Nyctibatrachus* (Dahanukar et al., 2013). These findings highlight the importance of conservation efforts to protect freshwater ecosystems and the need to develop strategies tailored to conserve stream-dwelling anurans such as *N.*

dattatreyaensis. This study is the first to provide ecological information crucial for understanding the ecological role of this species and the health of its habitat. However, further research is needed to address how prey availability, seasonality, and ontogenic changes affect prey selection in this species.

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