THE GUT CONTENT OF *HOPLOBATRACHUS OCCIPITALIS* (ANURA: DICROGLOSSIDAE) PROVIDES AN INKLING OF ITS AGE-MODULATED VORACITY, PREY DIVERSITY AND CHOICES

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ABSTRACT

Notably known to consume small animals, anurans' diets are sometimes affected by their age. This study examined the diet composition of Hoplobatrachus occipitalis, its prey diversity and preferred taxon using snout-vent lengths (SVLs) as a metric for age. With a non-destructive stomach-flushing technique, the gut contents of each actively captured Hoplobatrachus frogs were introduced into properly labelled vials and their prey items fixed in 70% alcohol for microscopic visualization and identification. Before releasing captured frogs, their SVLs [grouped as small (27 – 56 mm, n = 18), medium (57 – 88 mm, n = 35), and large (89 – 120 mm, n = 6)] were determined. A total of 392 preys belonging to 14 Orders, seven Classes and four Phyla of animals, a twig and pebble were found. The small and medium frogs significantly (p<0.05) preyed on more Hymenopterans (ants) than any taxon, while the large frogs showed no prey preference. The prey taxa among the small frogs were significantly fewer and less diverse with more dominant taxa than those found among the medium frogs, but not the large ones. Non-parametric estimates showed over 80% prey inventory completeness (a metric for sampling efforts); in conformity with taxa-accumulation curves, which approached their asymptotes for small and medium frogs, unlike the large ones, which had 53%. In sum, age-specific differences were seen in the prey contents, diversity and preference of H. occipitalis. Ontogenetic changes, among other plausible implications, may impose nutritional demands that modulate the predator's choices and voracity.

Keywords: Diet composition, Ontogenetic changes, Food selection, Anurans, Predatory frogs

INTRODUCTION

Hoplobatrachus occipitalis Günther, 1858 (Anura: Dicroglossidae), commonly known as the crowned bullfrog, is a widely distributed native species of least conservation concerns, and a local delicacy of commercial value, in some parts of sub-Saharan Africa (Mohneke *et al*., 2010; IUCN, 2014). Its feeding habit, behaviour, endoparasites, exposure to toxicants, and ecosystem services as a predator are well known (Aisien *et al*., 2009; Ezemonye and Enuneku, 2011; Ogoanah and Uchedike, 2011; Ajibola *et al.,* 2016). *H. occipitalis* is an opportunistic generalist predator whose gut contents are much more inclined toward myrmecophagy – i.e., ant-eating (Solé *et al.,* 2005; Ogoanah and Uchedike, 2011; Ajibola *et al*., 2016; Luría-Manzano and Ramírez-Bautista, 2019). At full development, it attains a large body size, which fairly explains its nutraceutical use by humans (Mohneke *et al.* 2010; Onadeko *et al*., 2011). For rearing in captivity (e.g., to meet commercial demands), adopting insects as frogs' meals – which is akin to applied entomophagy (Aigbodion *et al.* 2012) would be apt. Nonetheless, the use of *H. occipitalis* in local delicacies has health risks with pathogenic concerns, though manageably mild if processed appropriately (Ogoanah and Enomongale*,* 2017).

Frogs feed on small animals (both invertebrates and vertebrates) relative to their body size and infrequently on plant matters. Whilst the former are hunted, the latter are believed to be accidental acquisitions (Kovács *et al.,* 2007; Solé *et al.,* 2009; Ogoanah and Uchedike, 2011); nonetheless, plants' fragments in frogs' diets vary among species (Ogoanah and Uchedike, 2011; Luría-Manzano and Ramírez-Bautista, 2019). Anurans' prey-selection tactic is a passive sit-and-wait hunting strategy that involves capturing invertebrates (e.g., arthropods and molluscs) and rarely vertebrates (e.g., mammals, birds, fishes and amphibians) (Ogoanah and Uchedike, 2011; Moreno-Barbosa and Hoyos-Hoyos, 2014; Luría-Manzano and Ramírez-Bautista, 2019). Ingested plant matters are arguably digestion-aiding roughage and endoparasite-dislodging tools for anurans (Anderson *et al.,* 1999). Collectively, the food sources of anurans include both terrestrial and aquatic preys, with slight cannibalism.

Additionally, anurans' diets may be influenced by multiple factors such as habitat, seasonality and frog age. Evidence of age-driven changes in frogs' prey contents abound in literature, e.g., a proportional age-linked decline in dependency on collembolans and mites, but a heightened dependency on ants across age cohorts of *Schoutedenella xenodactyloides* (Anura: Ranoidea) (Blackburn and Moreau, 2006). Prey types influenced dietary composition of predatory anurans, whose sizes in turns moderately affect prey selection among semi-arid anurans with restricted trophic niche that worsen with age (Luría-Manzano and Ramírez-Bautista, 2019), but not always (Moreno-Barbosa and Hoyos-Hoyos, 2014). Ontogenetic changes in food selection seemed not to be universally evident among anurans; and such knowledge might be useful to commercial frog breeders, and to conservationists, especially for vulnerable habitats as less diverse prey species could affect predation, reduce food resources, and presumably increase inter- and intra-specific competitions. The cascading effects of ontogenetic nutritional changes in a poorly diverse habitat on anurans' fitness and resistance to parasites and diseases could plunge their demography down an extirpation vortex locally.

The knowledge of *H. occipitalis* nutritional ecology in human-modified ecosystems and its age-related choice of diet are elementary. Whether *H. occipitalis* switches food types as it ages; at what stage, if affirmed, and its food diversity remain vague to many, hence the need to examine its gut's contents by age group. Stomach-flushing technique – a non-destructive means of sampling anurans' guts was adopted (Ajibola *et al.,* 2016). The groups were determined using snout-vent lengths (millimetre) as a yardstick for age cohorts, with a focus on its prey abundance, diversity and choices, to establish the presence or absence of an ontogenetic shift in *H. occipitalis* diets. Generally, the absence of ontogenetic differences in diet among co-occurring conspecific predators would favour increased intraspecific competition for a limited number of preys due to lack of resource partitioning. Nonetheless, the key predictions were that resources are partitioned along ontogenetic frontiers and that as the frogs grow bigger and older, so will their voracity increase with hunting experience and age-related tissue requirements.

MATERIALS AND METHODS

Study Site: Sampling for adult frogs (*H. occipitalis*) was conducted within three predefined areas viz.: Faculty of Life Sciences (6° 23′ 54.93″ N, 5° 36′ 55.47″ E), Faculty of Physical Sciences (6° 23′ 56.87″ N, 5° 36′ 56.49″ E) and Basement (6° 23′ 48.58″ N, 5° 36′ 54.00″ E), all within the University of Benin, Edo State, Nigeria. The samples were collected at night with the aid of a hand-held lamp (torchlight) plus a technical staff as a field assistant. The sampled sites were characterised by puddles and potholes along the footpaths surrounded by patches of forested spots, bushes and marshes of grasses that are sparsely dotted by temporary pools of water as dictated by rainfall – from August till the end of October 2019. These sites were selected on account of specimen availability, field-laboratory proximity, site accessibility and sampler's safety.

Frog Collection and Stomach-flushing Protocol: Using visual and acoustic cues, *H. occipitalis* were caught through active searching between 20.00 and 23:00 hours for every sampling night. After collections, the frogs' Snout-Vent Lengths (SVLs) were measured; the basis for which the gut contents were grouped. The guts were emptied immediately using gastric lavage (Leclerc and Curtois, 1993; Solé *et al.*, 2005), and to minimise enzymatic digestion of ingested food items, the contents were instantly stored in well-labelled vials containing 70% alcohol.

Previously sampled sites were resampled once every two weeks to minimise flushing those with empty bowel arising from the gastric lavage as they were not tagged before reintroducing them to their original locations after sampling. Gastric lavage – involves the use of a spatula to gently open the mouths of frogs to allow the insertion of a (2 mm) soft, flexible tube attached to a (10 ml) syringe filled with water. The water was obtained from the frogs' biotope. The tube was inserted into the frog's oesophagus to deliver water into the frog's stomach to facilitate flushing out its stomach content, while the frogs were held vertically head-down. This allows the force of the injection and gravity to pull out the gut content through the mouth into a Petri dish. Usually, this technique works well with all stomach contents flushed on the first try, although it was repeated until only water was visible. If no content was found upon the first flushing attempt, the lavage was repeated twice and unsuccessful flushing was discontinued, after a second attempt.

All extracted prey items were preserved in 70% alcohol and subjected to further separation and/or sorting, followed by identification to order level. The preys were sorted in Insect Ecology and Diversity Unit within Entomology Laboratory at the University of Benin, where proper collation with their respective host frogs – organised into three classes using their body sizes (SVL: snout-vent length, mm) –was conducted. The three body sizes were: i) 25 – 56 mm, ii) 57 – 88 mm, and iii) 89 – 120 mm. The SVL was measured using a flexible metric tape from the snout through the dorsal body plate to the anal terminal (the vent).

Prey Diversity and Sampling Effort: The prey diversity indices were computed using Shannon-Wiener (H), Dominance (D) and Evenness in PAST. Using rarefaction curves, the array of preys found in the gut of *H. occipitalis* were used to predict the number of preys (to the taxonomic level of order) associated with the frog. These estimators provide a conservative value of the likelihood of obtaining an additional prey that had not been previously sampled, should additional sampling efforts be made

Statistical Analyses: Besides histogram with a normal curve, Q-Q plots and boxplots that were used in ascertaining the parametric-test assumptions of homogeneity of variance, normality and outliers graphically, the data were further validated statistically using Shapiro-Wilk's and Levene's tests. Data for the age-specific food preference did not satisfy the parametric assumptions with alpha at a 5% significance value. Hence, the non-parametric Kruskal-Wallis test (α = 0.05) was adopted. With significant p-values, except otherwise stated, post hoc tests were conducted using the Bonferroni correction factor to strongly control family-wise errors. The key prediction, amongst others, was that *H. occipitalis* evenly utilises its prey regardless of its ontogenetic stage (i.e., developmental stage) upon attaining adulthood. Data were analysed in R version 4.1.1 - "Kick Things" (R Core Team, 2021). Chao 2 and Jackknife 1 values were computed as predictors of sampling (or predatory) efforts demonstrating the likelihood of sampling an additional prey (in a new order) associated with *H. occipitalis* gut contents. Species accumulation (exact and fitted) curves were computed using 'specaccum (vegan)' in R (Oksanen *et al.,* 2022). The rarefaction curves associate taxa richness to a community of predators of similar SVLs via several accumulator methods. Here the data were randomly rearranged without substitution (Gotelli and Colwell, 2001) to create the rarefaction curves.

RESULTS

Prey Composition: From a total of 59 adults of *H. occipitalis* that were sampled for three months, a total of 394 prey items (inclusive of a piece of twig and a pebble) were recovered from their stomachs. Of these preys, 143 preys were found in small adults, 213 in medium-sized adults, and 36 in large ones, which comprised 36.3, 54.1 and 9.1% of the total preys that were captured by the frogs, respectively (Table 1). The proportion of inanimate objects found in the medium size frogs was 0.5%. In all, the prey/foods were drawn from two kingdoms viz.: Animalia and Plantae. The gut contents associated with the taxonomic group Animalia were spread across four phyla (Annelida, Arthropoda, Chordata and Mollusca). Whilst some phyla had only a representative (Class: Order) viz.: Annelida (Clitellata: Ophisthophora), Chordata (Amphibia: Anura) and Mollusca (Gastropoda: Stylommatophora), the Phylum Arthropoda was represented by four classes (with orders in parentheses) namely: Arachnida (Araneae), Diplopoda (Polydesmida), Insecta (Collembola, Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Odonata, Orthoptera and one unknown insect – (digested beyond recognition to its taxonomic order albeit with three visible pairs of legs), and Malacostraca (Isopoda) (Table 1).

In all, two-thirds (74.9%) of the entire prey found in the gut of *H. occipitalis* (n = 59) were arthropods, constituting the most preferred taxon amongst the observed phyla. In fact, among the small-size frogs, arthropods made up 58% of 143 preys, closely followed by Chordata (38.5%) and others (Annelida 2.1% and Mollusca 1.4%). For the medium-sized *H. occipitalis*, though the most preferred taxon was also Arthropoda (85.1% of 215 preys), it was comparatively higher than the proportion encountered among the small-sized frogs (58%). This was then followed by Mollusca (8.4%), Annelida (5.6%), and a piece of twig and a pebble (0.5% each). The large *H. occipitalis* had a comparatively high proportion of arthropods (80.6%), followed by Mollusca (13.9%) and Chordata (5.6%) (Table 1).

For the small-sized *H. occipitalis*, the Kruskal-Wallis independent-sample test for equality of median conducted on the individual count returned a statistically (H_{13} = 34.5, $p<0.001$) unequal distribution among the taxa (orders of prey). Post hoc assay using Bonferroni correction revealed that this category preferred animals in the order Hymenoptera to those in other taxa; except Orthoptera, Coleoptera, Anura and Araneae, which were not significantly different ($p>0.05$) from their preference for Hymenopterans. The preys belonging to the orders Diptera, Lepidoptera, Hemiptera, Collembola, Isopoda and Stylommatophora were the least consumed; alongside those in the orders Ophisthophora, Odonata and Polydesmida. In proportions, Anura (tadpoles) represented 38.5% of the total prey counts observed among small frogs, followed by Hymenoptera (18.9%) and Orthoptera (11.2%).

The medium-sized *H. occipitalis* (with 57 – 88 mm SVLs) had significantly different (H_{17} = 126.9, $p<0.001$) prey contents among taxa. The *post hoc* test showed that the predator had a strong preference ($p<0.001$) for prey in the order of Hymenoptera to other taxa. Though ranked closely, *H. occipitalis* nutritional dependency on Hymenopterans at this level of development was significantly higher ($p<0.001$) than their dependency on Coleopterans. Consumption of Coleopterans was not statistically higher ($p>0.05$) than others – which constituted the least consumed taxa (viz.: Isopoda, Orthoptera, Diplopoda, Stylommatophora, Lepidoptera, Araneae, Diptera, and Hemiptera). In proportions, Hymenoptera contributed the greatest number of individuals (23.7%), followed by Coleoptera (15.8%) and Isopoda (12.1%).

Table 1: Taxonomic categories of the dietary (prey) composition of *Hoplobatrachus occipitalis* across three heterogeneous body sizes delimited by snout-vent (s-v) lengths

Taxonomic category of preys found in the gut of *H. occipitalis*				**Count per taxon (% of total)**			**Total proportion (%)**
Kingdom	**Phylum**	**Class**	**Order**	**Small (n_1)**	**Medium (n_2)**	**Large (n_3)**	
Animalia	Annelida	Clitellata	Ophisthophora	3(2.1)	12(5.6)	-	3.8
	Arthropoda	Arachnida	Araneae	6(4.2)	10(4.7)	1(2.8)	4.3
		Diplopoda	Polydesmida	1(0.7)	11(5.1)	**8(22.2)**	5.1
		Insecta	Collembola	2(1.4)	2(0.9)	-	1.0
			Coleoptera	6(4.2)	34(15.8)	1(2.8)	10.4
			Diptera	5(3.5)	8(3.7)	6(16.7)	4.8
			Hemiptera	3(2.1)	4(1.9)	1(2.8)	2.0
			Hymenoptera (largely ants)	27(18.9)	**51(23.7)**	4(11.1)	20.8
			Lepidoptera (moths)	5(3.5)	22(10.2)	3(8.3)	7.6
			Odonata (nymphs)	2(1.4)	1(0.5)	-	0.8
			Orthoptera	16(11.2)	12(5.6)	1(2.8)	7.4
		Insecta	Unknown	-	2(0.9)	1(2.8)	0.8
		Malacostraca	Isopoda	10(7.0)	26(12.1)	3(8.3)	9.9
	Chordata	Amphibia	Anura (adult)	-	-	1(2.8)	0.3
			Anura (tadpole)	**55(38.5)**	-	1(2.8)	14.2
	Mollusca	Gastropoda	Stylommatophora	2(1.4)	18(8.4)	5(13.9)	6.3
Plantae			A piece of twig	-	1(0.5)	-	0.3
N/A*			Stone	-	1(0.5)	-	0.3

***Note**: Asterisk (*) represents an inanimate object; 'n' represents the number of sampled H. occipitalis and the corresponding subscripts (1, 2 and 3) represent the small, medium and large frogs: (n_1 = 18; n_2 =35; n_3 =6). The results are the number of prey taxa per cohort and their respective proportion of the total prey per cohort, which are presented in parenthesis, while the values in bold prints emphasize the most abundant taxa within a given cohort (N = 59 frogs)*

Lastly, the oldest cohort of *H. occipitalis* evenly fed on its preys (viz.: Dipterans, Diplopodans, Stylommatophorans, Hymenopterans, Lepidopterans, Isopodans, an Araneaen, Anurans (1 tadpole and an adult), a Coleopteran, a Hemipteran, and an Orthopteran) as no taxon was significantly preferred to others (H_{15} = 21.1 p = 0.135). Nonetheless, the most featured taxon was Diplopoda (22.2%) followed by Diptera (16.7%), and the Mollusca (Stylommatophora 13.9%) (Table 1).

Prey Diversity: Generally, the gamma diversity of encountered prey taxa (orders) was 16, with an alpha (α) diversity of 14 among the small and medium frogs, while the large frog had an α diversity of 13. The β diversity (i.e., unique taxa) between the small versus medium frogs, medium versus large frogs, and small versus the large frogs were 1, 3, and 3 respectively. The least average number of taxa (order) consumed as prey by the small frogs was 2.72 ± 0.10, ranging from 1 to 7 taxa. The average taxa count was significantly lower (H_2 = 9.61; p = 0.008) than those associated with the medium frogs (4.51 ± 0.07) that ranged from 1 to 13 taxa, but did not differ from those encountered by the large frogs (4.17 ± 0.40), which ranged from 2 to 9 taxa (Figure 1a). Taken together, the small frogs consume relatively fewer taxa than the medium size conspecific frogs, which explore more prey than both the small and the large frogs. In other words, the medium-size frogs are better exploiters of prey resources than the other cohorts. Dominance (D) indices of their gut contents were not statistically even (H_2 = 7.67, p = 0.023), as the D index of the prey taxa in the small frogs (0.56 ± 0.02; ranging from as low as 0.18 to 1) was significantly higher than those encountered by the medium frogs (0.37 ± 0.01; 0.14 - 1) as opposed to large size (0.36 ± 0.03; 0.14 - 0.56) frogs (Figure 1b); which indicate that the small frogs had more dominant taxa than the medium and large frogs, but no taxon had equal representation (where D = 0) as prey in any cohort. Nonetheless, the observed ranges did show that in both the small and the medium frogs, some individuals did have a dominant taxon in their gut content; hence D was 1 (i.e., the upper limits of dominance).

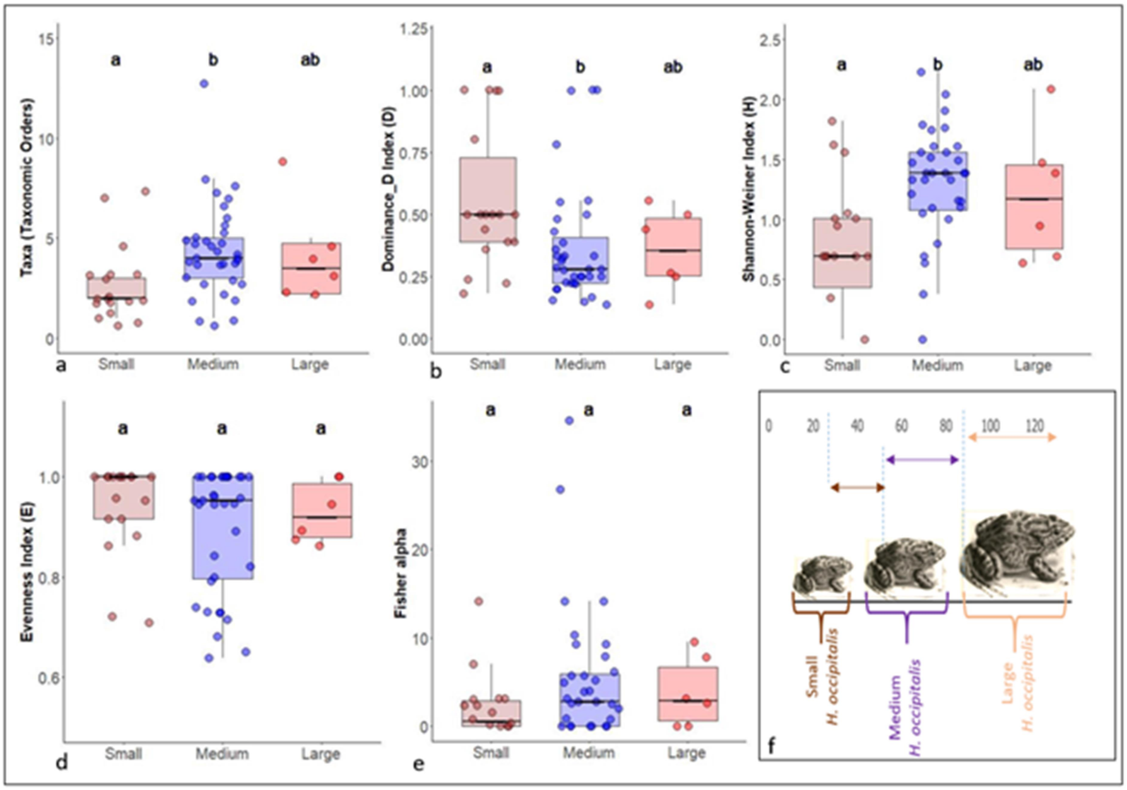

Figure 1(a-f): Box-Jitter plots of variability in the diversity of prey (pooled into similar taxonomic level (order) extracted from the gut of three age groups of *Hoplobatrachus occipitalis*. ***Note:*** *Figure (1a) represents the taxa count of prey found in the guts of different age groups of* H. occipitalis*. (1b) denotes the Dominance Index (D), (1c) Shannon-Wiener Index (H) and (1d) Evenness Index (E) or Shannon Equitability Index of preys obtained from the guts of* H. occipitalis. *While (1e) presents the Fisher's alpha index, (1f) depicts the three age categories of* H. occipitalis *alongside their range of snout-vent lengths. The colour codes across figures consistently represent the age factor. While the brown signifies the small frog, followed by the blue representing the medium frog, the red represents the large ones. Boxplots with similar letters above them were not statistically different (*p>0.05*) from each other as tested using the non-parametric Kruskal-Wallis ranked test and controlled for family-wise error using Bonferroni Correction. The intra-box points are data within the Inter-Quartile Range (IQR), while the crossbar within a box represents median. The whiskers above and below the boxes represent 1.5 times the IQR of the data sets as presented. Overlapping data points are darker than the single points*

Unlike those cohorts, the large frogs showed a certain preference for 'diet mixing' or a 'cocktail of prey' as no individual had its gut content laden with one prey taxon. Albeit polyphagous, some small and medium frogs accommodated monophagy, but the large ones did not. In Figure 1c, the Shannon-Wiener (H) indices showed that the small frogs had less diverse prey as meal (H-index = 0.75 ± 0.03; range: 0 – 1.82), than the medium frogs did with a significantly ($p<0.05$) higher H index of 1.24 ± 0.02 (with a range of 0 – 2.23). Nonetheless, the prey diversity of the large frog with H-index of 1.20 ± 0.08 (0.64 – 2.08) was not significantly ($p>0.05$) more diverse than those of the preys found among the small frogs.

Unlike the above indices, no statistically significant variance ($p>0.05$) was detected for the occurrence of prey in the frogs' guts when Evenness (E) index – a conceptual inverse of dominance – and Fisher's ɑ (alpha) (Evenness: $H_2 = 1.74$; p = 0.418; Fisher's alpha $H_2 = 2.30$, p = 0.316; Figure 1d and 1e) were considered (Figure 1f). Minimal disparity in taxa representation was observed as β-diversity indices between any paired cohorts of *H. occipitalis* was low depicting a few unique taxa between paired cohorts (Table 2).

Table 2: Whittaker β-diversity indices between frogs' cohorts

Whittaker *β*-diversity	Small	Medium	Large
Small	0	0.07	0.19
Medium	0.07	0	0.19
Large	0.19	0.19	0

Inventory Completeness: Estimates of the two most-reliable non-parametric predictors did not only show that the surveyed *H. occipitalis* had a high proportion of its available preys, but that more opportunities of preying on more taxa abound; seen differently, that further sampling of *H. occipitalis* could yield more prey individuals as well as a few more taxa. Specifically, Chao 2 and Jackknife 2 returned an estimated prey richness of 14.71 (≡ 95.2%), and 16.99 (82.4%) as opposed to the observed (14) taxa for the small frogs; to average at 89% inventory completeness. The medium size frogs had 87% average prey-inventory completeness, given their taxa estimates of 16.97 (Chao 2), and 19.91 (second-order Jackknife) against the 16 observed taxa. The prey estimates for the large conspecific frogs were 24.6 (Chao 2) indicating 53% inventory completeness relative to the thirteen observed prey taxa, and 24.5 (53%) for Jackknife 2; both averaging at 53%.

Taxa Rarefaction: The asymptotic 'predatory' maximum of animal taxa that the small frogs could attain was 20.39, while half its maximum richness was attained at 5.67, and the rate of increase in richness (i.e., slope) was 2.49. The medium frogs may reach their asymptotic maximum at 16.17 with half its maximum richness attained at 2.91 at the rate (slope) of 2.79. Across the three cohorts, though the small and medium frogs were fast approaching their plateaus (Figure 2), their respective fitted models showed the odds of having more taxa should more sampling be done; though less so for the small frogs and medium frogs, unlike the large ones. Sufficient sampling effort is attained when the rarefaction curve reaches its asymptote or plateau, which is numerically higher among the small frogs than the medium ones.

DISCUSSION

Herein examined were the prey composition and variability in taxa richness, evenness and dominance following ontogenetic shifts in a predator's body size – a metric for age and hunting experience (Table 3). The findings from 59 conspecific frogs support the occurrence of ontogenetic changes in prey utilization by *H. occipitalis*, but the age groups had several prey taxa in common. Each cohort of frogs had its preferred taxon with ants occurring twice – among the small and medium frogs; some preys were notably consumed more than others. For small frogs, the tadpoles were the most consumed prey followed by ants and grasshoppers. Nonetheless, the same preference was elusive among individuals in the medium category, which preyed mostly on ants, followed by beetles and isopods (Crustacea). Unlike the others, the large frogs had a completely different set of taxa for the three most-abundant prey viz.: millipede, flies and snails in descending order of abundance. Although several prey taxa, e.g., ants, occurred across the categories of frogs, they were numerically different. Ants are a key resource for frogs, as they constitute much more of the prey items (Shuman-Goodier *et al.*, 2019), but this is not always the case (Strüssmann *et al.*, 1984). Although ontogenetic differences in taxa utilizations have been noticed here, whether they are spatiotemporally fixed remains unknown, but not expected given the possible differences in species distribution across different terrains.

The small frogs were presumably the young and amateurish 'predators' and had fewer per capita taxa (richness) but with higher dominance than others. Dominance ranges from 0 to 1 (Hammer, 2018) and a high dominance value (of 1) implies a single taxon dominated the prey community. In this case, high dominance could have arisen when individuals of the same taxon were repeatedly fed on within the same vicinity, e.g., through a passive (sit-n'-wait) predatory strategy or at most an active hunt within an extremely limited hunting arena.

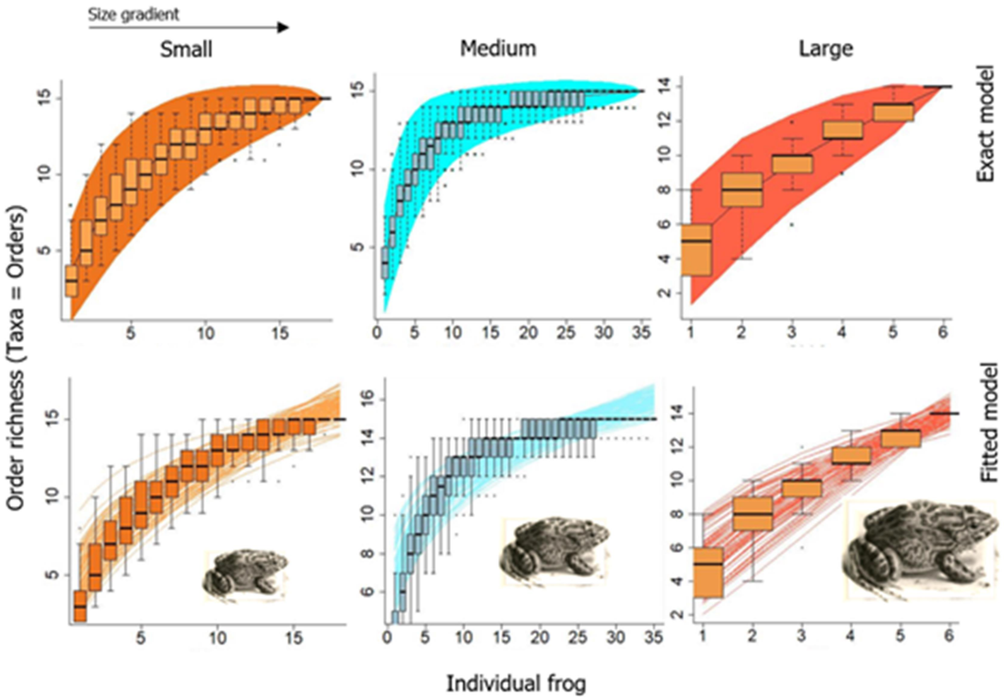

Figure 2: Taxa accumulation curves of predatory efforts of *Hoplobatrachus occipitalis* as categorised by snout-vent lengths. ***Note:*** *The exact curves were above, while their corresponding fitted models were below. The small frogs had 25-56 mm snout-vent length, the medium frogs had 57-88 mm SVL, while large ones had 89-120 mm SVL*

Table 3: Summary of the taxa preyed on by different ontogenetic stages of *Hoplobatrachus occipitalis* and their indices

Cohorts	Preys' taxonomic groups (Order) of found in *H. occipitalis**	Indices
Small	Anura > Hymenoptera > Orthoptera > Isopoda > Araneae / Coleoptera > Diptera > Lepidoptera > Ophisthophora / Hemiptera > Collembola / Odonata / Stylommatophora > Polydesmida	Low richness, high dominance, low diversity
Medium	Hymenoptera > Coleoptera > Isopoda > Lepidoptera > Stylommatophora > Orthoptera > Ophisthophora > Polydesmida > Araneae > Diptera > Hemiptera > ?unknown insect order > Collembola > A piece of twig / Stone / Odonata	High richness, low dominance, high diversity
Large	Polydesmida > Diptera > Stylommatophora > Hymenoptera > Isopoda/ Lepidoptera > Anura / Coleoptera / Orthoptera / Araneae / Hemiptera	Low richness, moderate dominance and moderately diverse

**Note: the use of slash indicates the taxa with similar numerical abundance (cf. Table 1 for details)*

Presumably, only a naïve predator would under-explore its hunting arena and repeatedly hunt prey into constituting a dominant food in its repertoire as it seeks satiation. Thus, one could hypothesize that an actively mobile (less passive) *H. occipitalis* would encounter more diverse prey taxa with low dominance than a passive one. How this hypothesis holds for other models would be intriguing and worthy of further investigation. The medium frogs, though young, were regarded as experienced predators that hunted higher per capita taxa, with much lower taxa dominances in favour of diverse foods (see Shannon-Wiener indices). As theorized above, the medium size frogs would be consequently less passive in hunting, covering a wider area than the small frogs. Numerous hunting bouts by the medium frogs might have contributed to their successful hunts in acquiring nutrients and sundry chemicals required for reproduction as they invest in egg production and defence, e.g., against predators. Some frogs sequester precursor chemicals for their defences from prey such as mites and ants

(Saporito *et al*., 2012). With increased body size and demands (for tissue maintenance and reproduction), so will their foraging range, intensity, and predatory risks increase. Several diet-linked alkaloids that originated from arthropods, e.g., myriapods, coleopterans, hymenopterans and arachnids are useful to frogs (Saporito *et al.,* 2012). Also, the direct consumption of a diverse array of arthropods, mainly insects, freely renders pest-control service in favour of improved crop yield with minimal pest-induced damage (Teng *et al*., 2016), alongside reduced dependencies on synthetic chemicals for pest control.

In agroecosystems, e.g., rice farms, the arthropod community plays diverse and notable make-or-mar roles in crop productivity (Igbinosa *et al.* 2007), but the use of generalist predators in such systems could substantially minimise pests (Teng *et al*., 2016; Zou *et al*., 2017). Also, the overlapping prey resources of generalist predators, regardless of their ontogenetic preference, as seen in the present study with low β-diversity, might increase predation via intraspecific competition when preys are scarce. Given the array of crop-associated arthropods, e.g., in rice fields (Igbinosa *et al.,* 2007) or any other suitable agricultural systems, the *in-situ* establishments of breeding sites for frogs will promote an ecosystem-driven pest management (Hirschfeld and Rödel, 2011; Hocking and Babbitt*,* 2014; Feit *et al*., 2015; Khatiwada *et al*., 2016; Holzer *et al.,* 2017; Lindell *et al.,* 2018), that could slash reliance on synthetic chemicals. Thus, it will reduce the cost of food production and the number of toxicants left in the wake of chemical control and their deleterious environmental effects. Generalist predators are not target-specific as they consume both problematic arthropods and beneficial ones, but the overall impact reduces the most abundant species, which often outnumber the beneficial ones and constitute pests. Though non-selective predators (Ogoanah and Uchedike, 2011; Ajibola *et al.* 2016; Ogoanah and Enomongale, 2017), frogs' ecosystem services as natural control agents found relevance against rice pests with yield improvements in Nepal using indigenous frogs, and in China with *Hoplobatrachus rugulosus* Wiegmann, 1834 (Anura: Dicroglossidae) – a non-native East Asian bullfrog, etc. (Khatiwada *et al*., 2016; Teng *et al*., 2016; Zou *et al*., 2017). While the introduction of bullfrogs, *H. rugulosus*, to trial plots remarkably reduced leafhopper infestation by half and leaf folder infestation by approximately 24% with commensurable improvements in yield (Teng *et al*., 2016), a much higher rate of predation in which *Fejervarya limnocharis* Gravenhorst, 1829 (Anura: Dicroglossidae) reduced 75% of the infestation of brown plant hopper, *Nilaparvata lugens* Stål, 1854 (Hemiptera: Delphacidae), was possible (Zou *et al*., 2017).

H. occipitalis is predictably capable of consuming more animal taxa than the observations indicated when evaluated using the rarefaction curves and non-parametric estimators. While the rarefaction curves of the small and medium frogs rapidly approached their respective asymptotes (or maxima), the large frogs lagged by several folds. The observed lag may be seen as a shortcoming for the sampling efforts in that category, but it should be borne in mind that it only reflects the predatory efforts of the large frogs, which were relatively low.

Worthy of further explorations arising from the lessons garnered in this study are numerous. Given the low predatory potentials of the large cohorts, the performance of *H. occipitalis* in less diverse and/or arthropod-poor habitats, where less will be available to the frogs, would be an interesting aspect to further explore. Also, if *H. occipitalis* is sensitive to reduced prey-range, then they might become less fit, less defended and more immunocompromised than well-fed conspecifics, living in highly diverse habitats – a perspective worth testing for conservation reasons and to advert demographic implosion in the face of disease outbreaks or any stressor. Evidence of sequestrations of prey-associated chemicals acting as precursors in amphibians' defence repertoire are well known (Saporito *et al.,* 2012), but how such chemicals if ever sequestered by *H. occipitalis* would shape their prey-selection criteria remains to be appraised. Lastly, given the similar pest control successes

(Khatiwada *et al.*, 2016; Teng *et al.*, 2016; Zou *et al.*, 2017), another intriguing aspect for further research is how *H. occipitalis* would play vital roles in pest management within their natural range, if artificially enhanced in farms via man-made ponds for their breeding (Greenlees *et al.*, 2006; Feit *et al.*, 2015); in that all its ontogenetic stages are predators with widely varied preferences.

Conclusion: Conclusively, it would suffice to say that age-specific differences abound in the prey contents, diversity and preference of *H. occipitalis* with plausible modulation from hosts' nutritional requirements. Ontogenetic shift appeared in *H. occipitalis* prey utilization. And as opposed to heightened intraspecific competition for limited prey in the absence of ontogenetic differences in diets, the findings rather support the existence of resource partitioning among the age cohorts of *H. occipitalis*. Thus, the findings add credence to the view that frogs' resources are partitioned along ontogenetic frontiers as delineated using snout-vent lengths.

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REFERENCES

AIGBODION, F. I., EGBON, I. N. and ERUKAKPOMREN, E. (2012). A preliminary study on the entomophagous response of *Gallus gallus domesticus* (Galliformes: Phasianidae) to adult *Periplaneta americana* (Blattaria: Blattidae). *International Journal of Tropical Insect Science*, 32(3): 123 – 125.

AISIEN, M. S. O., OGOANNAH, S. O. and IMASUEN, A. A. (2009). Helminth parasites of amphibians from a rainforest reserve in southwestern Nigeria. *African Zoology*, 44(1): 1 – 7.

AJIBOLA, M. E., OMOSHEHIN, T. O. and OLOSUNDE, S. O. (2016). Evaluation of food and feeding habits of *Hoplobatrachus occipitalis* (Günther, 1858) from four locations on Obafemi Awolowo campus, Ile-Ife. *Ife Journal of Science*, 18(1): 85 – 94.

ANDERSON, A. M., HAUKOS, D. A. and ANDERSON, J. T. (1999). Diet composition of three anurans from the Playa wetlands of Northwest Texas. *Copeia*, 1999(2): 515 – 520.

BLACKBURN, D. C. and MOREAU, C. S. (2006). Ontogenetic diet change in the arthroleptid frog *Schoutedenella xenodactyloides*. *Journal of Herpetology*, 40(3): 388 – 394.

EZEMONYE, L. I. N. and ENUNEKU, A. A. (2011). Biochemical alterations in *Hoplobatrachus occipitalis* exposed to sub lethal concentrations of cadmium. *Turkish Journal of Fisheries and Aquatic Sciences*, 11(3): 485 – 489.

FEIT, B., DEMPSTER, T., GIBB, H. and LETNIC, M. (2015). Invasive cane toads' predatory impact on dung beetles is mediated by reservoir type at artificial water points. *Ecosystems*, 18(5): 826 – 838.

GOTELLI, N. J. and COLWELL, R. K. (2001). Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters*, 4(4): 379 – 391.

GREENLEES, M. J., BROWN, G. P., WEBB, J. K., PHILLIPS, B. L. and SHINE, R. (2006). Effects of an invasive anuran [the cane toad (*Bufo marinus*)] on the invertebrate fauna of a tropical Australian floodplain. *Animal Conservation*, 9(4): 431 – 438.

HAMMER, Ø. (2018). *Paleontological Statistics Version 3.20, Reference Manual*. Natural History Museum, University of Oslo, Oslo, Norway. https://edisciplinas.usp.br/pluginfile.php/4407502/ modresource /content/1/past3manual.pdf

HIRSCHFELD, M. and RÖDEL, M. O. (2011). The diet of the African tiger frog, *Hoplobatrachus occipitalis*, in northern Benin. *Salamandra*, 47(3): 125 – 132.

HOCKING, D. J. and BABBITT, K. J. (2014). Amphibian contributions to ecosystem services. *Herpetological Conservation and Biology,* 9(1): 1 – 17.

HOLZER, K. A., BAYERS, R. P., NGUYEN, T. T. and LAWLER, S. P. (2017). Habitat value of cities and rice paddies for amphibians in rapidly urbanizing Vietnam. *Journal of Urban Ecology*, 3(1): 1 – 12.

IGBINOSA, B. I., OIGIANGBE, N. O. and EGBON, N. I. (2007). Insect pests of rain-fed upland rice and their natural enemies in Ekpoma, Edo State, Nigeria. *International Journal of Tropical Insect Science*, 27(2): 70 – 77.

IUCN (2014). *IUCN Red List of Threatened Species.* International Union for the Conservation of Nature. https://www.iucnredlist.org/species/58299/18361413 Accessed August 22, 2022.

KHATIWADA, J. R., GHIMIRE, S., KHATIWADA, S. P., PAUDEL, B., BISCHOF, R., JIANG, J. and HAUGAASEN, T. (2016). Frogs as potential biological control agents in the rice fields of Chitwan, Nepal. *Agriculture, Ecosystems and Environment*, 230: 307 – 314.

KOVÁCS, É. H., SAS, I., COVACIU-MARCOV, S. D., HARTEL, T., CUPSA, D. and GROZA, M. (2007). Seasonal variation in the diet of a population of *Hyla arborea* from Romania. *Amphibia-Reptilia*, 28(4): 485 – 491.

LECLERC, J. and COURTOIS, D. (1993). A simple stomach flushing method for ranid frogs. *Herpetological Review*, 24(4): 142 – 143.

LINDELL, C., EATON, R. A., HOWARD, P. H., ROELS, S. M. and SHAVE, M. E. (2018). Enhancing agricultural landscapes to increase crop pest reduction by vertebrates. *Agriculture, Ecosystems and Environment*, 257: 1 – 11.

LURÍA-MANZANO, R. and RAMÍREZ-BAUTISTA, A. (2019). Ontogenetic variation in the diet of the anuran community from a semi-arid environment in the southeastern Chihuahuan Desert. *PeerJ*, 7: e7908. https://doi.org/10.7717/peerj.7908

MOHNEKE, M., ONADEKO, A. B., HIRSCHFELD, M. and RÖDEL, M. O. (2010). Dried or fried: amphibians in local and regional food markets in West Africa. *Traffic Bulletin*, 22(3): 117 – 128.

MORENO-BARBOSA, S. E. and HOYOS-HOYOS, J. M. (2014). Ontogeny of the diet in anurans (Amphibia) collected at La Vieja River Basin in the Deparmento of Quindio (Colombia). *Caldasia*, 36(2): 365 – 372.

OGOANAH, O. S. and UCHEDIKE, E. (2011). Diet and feeding behavior of the edible frog *Hoplobatrachus occipitalis* (Amphibia: Anura). *African Scientist*, 12(4): 209 – 213.

OGOANAH, S. O. and ENOMONGALE, M. E. (2017). Prey availability or electivity in the diet of some amphibians from Olinlin-Uzea, Esan North East, Edo State, Nigeria. *Nigerian Journal of Environmental Sciences and Technology*, 1(1): 69 – 74.

OKSANEN, J., SIMPSON, G. L., BLANCHET, F. G., KINDT, R., LEGENDRE, P., MINCHIN, P. R., O'HARA, R. B., SOLYMOS, P., HENRY, M., STEVENS, H., SZOECS, E., WAGNER, H., BARBOUR, M., BEDWARD, M., BOLKER, B., BORCARD, D., CARVALHO, G., CHIRICO, M., DE CACERES, M., DURAND, S., EVANGELISTA, H. B. A., FITZJOHN, R., FRIENDLY, M., FURNEAUX, B., HANNIGAN, G., HILL, M. O., LAHTI, L., MCGLINN D., OUELLETTE M., CUNHA, E. R., SMITH, T., STIER, A., TER BRAAK, C. J. F. and WEEDON, J. (2022). *Vegan: Community Ecology Package.* R Package Version 2.6 – 2. https://CRAN.Rproject.org/package=vegan

ONADEKO, A. B., EGONMWAN, R. I. and SALIU, J. K. (2011). Edible amphibian species: local knowledge of their consumption in southwest Nigeria and their nutritional value. *West African Journal of Applied Ecology*, 19(1): 67 – 76.

R CORE TEAM (2021). *R: A Language and Environment for Statistical Computing [R Version 4.1.1 "Kick Things"].* R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

SAPORITO, R. A., DONNELLY, M. A., SPANDE, T. F. and GARRAFFO, H. M. (2012). A review of chemical ecology in poison frogs. *Chemoecology*, 22(3): 159 – 168.

SHUMAN-GOODIER, M. E., DIAZ, M. I., ALMAZAN, M. L., SINGLETON, G. R., HADI, B. A. and PROPPER, C. R. (2019). Ecosystem hero and villain: native frog consumes rice pests, while the invasive cane toad feasts on beneficial arthropods. *Agriculture, Ecosystems and Environment*, 279: 100 – 108.

SOLÉ, M., BECKMANN, O., PELZ, B., KWET, A. and ENGELS, W. (2005). Stomach-flushing for diet analysis in anurans: an improved protocol evaluated in a case study in Araucaria forests, southern Brazil. *Studies on Neotropical Fauna and Environment*, 40(1): 23 – 28.

SOLÉ, M., DIAS, I. R., RODRIGUES, E. A., MARCIANO-JR, E., BRANCO, S. M., CAVALCANTE, K. P. and RÖDDER, D. (2009). Diet of *Leptodactylus ocellatus* (Anura: Leptodactylidae) from a cacao plantation in southern Bahia, Brazil. *Herpetology Notes*, 2: 9 – 15.

STRÜSSMANN, C., DO VALE, M. B. R., MENEGHINI, M. H. and MAGNUSSON, W. E. (1984). Diet and foraging mode of *Bufo marinus* and *Leptodactylus ocellatus*. *Journal of Herpetology*, 18(2): 138 – 146.

TENG, Q., HU, X. F., LUO, F., CHENG, C., GE, X., YANG, M. and LIU, L. (2016). Influences of introducing frogs in the paddy fields on soil properties and rice growth. *Journal of Soils and Sediments*, 16(1): 51 – 61.

ZOU, Y., KRAKER, J. D., BIANCHI, F. J., VAN TELGEN, M. D., XIAO, H. and WERF, W. (2017). Video monitoring of brown planthopper in rice shows importance of frogs for biological pest control. *Scientific Reports*, 7: 42210. https://doi.org/10.1038%2Fsrep42210