The Impact of Location on Diet and Morphological Features of Frog (*Hoplobatrachus occipitalis*) in Ogun State, Nigeria

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ABSTRACT

Amphibians are a very diverse group of vertebrates, they inhabit varied habitat with most species living within terrestrial, fossorial, arboreal or freshwater ecosystem. This study investigated the impact of location on diet and morphological features of amphibians especially frog (*Hoplobatrachus occipitalis*).

Four amphibians each were collected from Hospital Area, Mechanic Village, Quarry Site and Residential Area across four Local Government Areas in Ogun State. Morphometric features such as Head Length (HL), Head Width (HW), Snout-Tympanum Length (STL), Mouth Angle-Snout Length (MSL), Nostril-Snout Length (NSL), Mouth Gap (MG), Trunk Length (TL), Snout Vent Length (SVL) Hind Limb Length (HLL), and Body Weight (BW) were determined using digital Vernier calipers.

Stomach contents of each sample were viewed under stereoscopic microscope. A guide to various foods of amphibians was used to identify each of the diets. Diet compositions were determined using percentage frequency of occurrence and numerical percentage of diet components. Total aerobic bacterial count (TABC) of the gut contents were determined using standard microbiological methods. Food and feeding habit were subjected to ecological diversity indices such as Margalet index, Shannon and Weiner index, and Pielou index.

Data obtained were analyzed using descriptive statistics, linear regression equation, Pearson's correlation co-efficient, and Analysis of Variance. Three species of amphibians from 3 families were identified in the study area namely, *Bufo bufo* (75.0%), *Hoplobatrachus occipitalis* (18.9%), and *Xenopus laevis* (6.1%). Mean distribution values for Hoplobatrachus occipitalis ranged from 2.00±0.2 to 9.00±0.1 across the study location. Across the collection site, mean distribution values for *H. occipitalis* ranged from 4.00±0.1 to 9.00±0.2. Morphometric parameters (cm) were better with *H. occipitalis* in Yewa North when compared with other locations There were significant ($p<0.05$) difference in the distribution, abundance and morphometric parameters of the species at the study sites. Feeding habit of *H. occipitalis* in the study area across locations showed 17 food items made up of 6 taxa; Coleoptera, Plecoptera, Hemiptera, Ephemeroptera, Crustacean and Anura, Percentage occurrences of isolated bacteria were: *Escherichia coli* (38.46%), *Salmonella sp.* (30.76%), *Pseudomonas sp.* (15.38%), *Proteus sp.* (7.69%), *Bacillus sp.* (7.69%). The study concluded that location influences the distribution, abundance morphometric parameters of amphibians most especially frogs in the study area. Good environmental management practices, conservation education and domestication efforts were part of suggested recommendations.

(Keywords: amphibian, frog, abundance, location, diet, habitat, morphometry, habitat ecology).

INTRODUCTION

Habitat ecology is a way of studying relationships between organisms and their environment that involves characterizing and explaining statistical patterns of abundance, distribution and diversity. In other words, it is the study of the mechanisms behind the distribution of species and the relationship between species and their environment on a large scale (Brown and Maurer 1989; Brown 1995).

Amphibians are a very diverse group of vertebrates; however, in general their feeding is opportunistic with food up to gape width being ingested. Amphibians such as frogs and toads only target moving prey and prefer elongated prey such as crickets or insect larvae that move across their field of vision. However, many aquatic amphibians will target food by scent and will consume inert food. The suitability and range of live feeds are assessed in the Amphibian Population Management Guidelines (Schad, 2007).

Anurans are the largest group of amphibians. Approximately 5,200 species are in this group (Frost 2006), and they reside on all of the major continents except Antarctica and on many oceanic islands (Pough, et al. 2004; Zug, et al. 2001). Compared with other tetrapods, anurans have short trunks (only 5-9 presacral vertebrae), broad, flat heads with extensive reduction of bone, and hind limbs that are substantially longer than the forelimbs in most species. With a few exceptions, adult amphibians are predators, feeding on virtually anything that moves that they can swallow. Their diet mostly consists of small preys that do not move too fast such as beetles, caterpillars, earthworms, and spiders. The sirens (*Siren spp.*) often ingest aquatic plant material with the invertebrates on which they feed (Gabbard, 2000). Amphibians usually swallow food whole but may chew it lightly first to subdue it. They typically have small hinged pedicellate teeth, a feature unique to amphibians.

According to the International Union for the Conservation of Nature (IUCN), amphibians may be the only major group currently at risk globally. IUCN assesses the status of species on a global scale and maintains a database of species that face a high risk of global extinction: the IUCN Red List of Threatened Species. The IUCN Red List, recent detailed worldwide assessment and subsequent updates show that nearly one-third of species (32.4%) are either globally extinct or threatened with extinction (Critically Endangered, Endangered and Vulnerable), representing 2,030 species (IUCN, 2011).

Populations of many species of frogs have declined dramatically in relatively undisturbed habitats at high altitudes and anthropized areas throughout the world (Blaustein and Wake, 1990, 1995; Collins and Storfer 2003; Pounds, et al., 2006; Reading, 2007). A number of causes are believed to be involved, including habitat destruction and modification, over-exploitation, pollution, introduced species, climate change, endocrine disrupting pollutants, destruction of the ozone layer (ultraviolet radiation has shown to be especially damaging to the skin, eyes, and eggs of amphibians), and diseases like chytridiomycosis. However, many of the causes of amphibian declines are still poorly understood, (Waddle, 2006; Hloch, 2010).

Currently, there are no established diet and morphological methods for most species of amphibians (especially frogs) and scanty information is available about ecology and physiology of many of them in Nigeria especially in the South- Western part of the country. Based on these and other ecological relevancies engendered this research with a view to assess impacts of location on abundance, distribution, morphological features and diets of amphibians especially frog (*Hoplobatrachus occipitalis*) in Ogun State, Nigeria

METHODOLOGY

Study Area

Ogun State was created in 1976 from the former Western State. The State is located in the South Western part of Nigeria. It covers a total of 1,640,076 square kilometers of land mass where over 60 percent are cultivable arable land, with an estimated population of about 3.391 million.

The State is heterogeneous comprising the Egba, Yewa, Egun, Awori, and Ijebu ethnic groups who speak different languages. The study area lies within Latitudes 6 41'N - 7 9'N and Longitudes 3 16'E - 3 41'E covering an aerial extent of about 915 km (Figure 1). The area has elevation ranging from 40 m in the South to 154 m in the North (Salami and Olorunfemi, 2014). Ogun State occurs within the humid tropical rain forest region of Nigeria characterized by two climatic seasons: the rainy season of about eight months (March to October) and the dry season of about four months (November to February).

Amphibian Collection

Samples were collected at different locations (i.e. Hospital areas, Mechanic villages, Quarry sites and Residential areas) and within the following Local Government Areas (Abeokuta North, Abeokuta South, Yewa North and Ijebu North) of Ogun State. Different species of amphibian were targeted using dip net method.

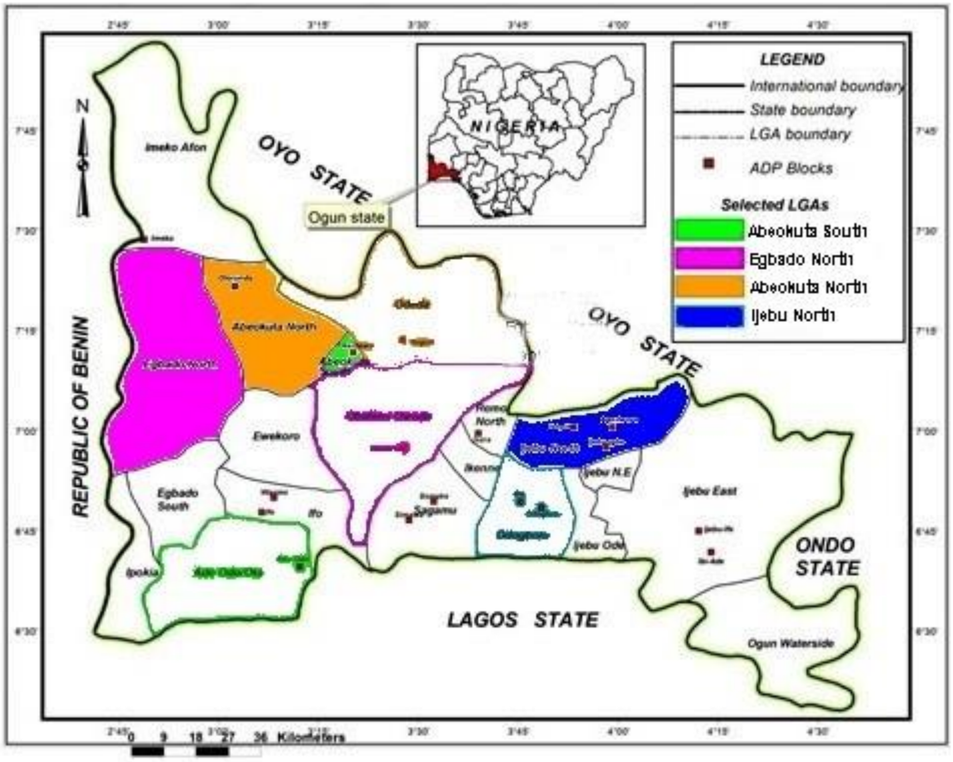

Figure 1: Map of Ogun State Showing the Study Area.

Four samples were collected from each sampling site making a total of 64 samples in 16 sampling sites for morphometric assessment.

Morphometric Assessment

Morphometric characters such as Head Length (HL), Head Width (HW), Snout-Tympanum Length (STL), Mouth Angle-Snout Length (MSL), Nostril-Snout Length (NSL), Mouth Gap (MG), Trunk Length (TL), Snout Vent Length (SVL) Hind Limb "Length (HLL), and Body Weight (BW) were determined according to (Islam, et al., 2008).

Head lengths were measure from back of mandible to tip of snout. Head widths were taken from left side back of mandible to right side back of mandible. Snout-tympanum lengths were measure from the tip of snout to front of tympanum. Mouth angle-snout length is the distance from the Tip of snout to end of mouth opening. Nostril- snout length is the distance from nostril to tip of snout. All measurement was taken with digital Vernier calipers to the nearest 0.1mm.

Diet Composition

The contents of each stomach were preserved in a labelling specimen bottle containing the morphological information using 10% formalin. Drops of the samples were placed on slide and viewed under stereoscopic microscope at X40 magnifying lens. A guide to various foods of amphibians were used in identifying each of the diets seen through the microscope. Analyses were carried out using frequency of occurrence and numerical methods as described by Hyslop (1980).

Percentage frequency of occurrence is given as:

$$F_i = \frac{N_i \times 100\%}{N} \quad \textbf{(1)}$$

where F_i is frequency of occurrence of the i^{th} food items in the sample, N is total number of stomachs with food in the sample, and N_i is number of stomachs in which the i^{th} item was found.

For many stomachs, it is difficult to identify the numbers in each food category because of mastication of the food. Occasionally, some food was observed to be crushed and others were at various stages of digestion.

Numerical percentage of diet components is given as:

$$N_i = \frac{F_i \times 100\%}{F} \quad \textbf{(2)}$$

Where N_i is numerical percentage of the i^{th} food items in the space, F_i is total number of i^{th} food items and F is total number of all food items.

Ecological Diversity Indices

Species Richness Index (d): The Species richness index (d) according to Margalef (1951) was used to evaluate the community structure. The equation below was applied and results were recorded to two decimal places.

$$d = \frac{S-1}{\ln N} \quad \textbf{(3)}$$

where: *d* is Species richness index, *S* is Number of species in a population and *N* is Total number of individuals in *S* species.

Shannon and Weiner Diversity Index (H): Shannon and Weiner (1949) diversity index (*H*) given by the equation:

$$H_s = -\sum p_i \ln p_i \quad \textbf{(4)}$$

Where H_s is Diversity Index, *i* is Counts denoting the i^{th} species ranging from 1 – n, p_i is proportion that the i^{th} species represents in terms of numbers of individuals with respect to the total number of individuals in the sampling space as whole.

Species Equitability (j)

Species Equitability or evenness (Pielou, 1969) was determined by the equation:

$$j = \frac{H_s}{\log_2 S} \quad \textbf{(5)}$$

where *j* is equitability index, H_s is Shannon and Weaver index, and *S* is Number of species in a population.

Correlation Coefficient Values (r)

The Pearson's coefficient of correlation (*r*) for the relationship between some physico-chemical parameters and biotic structure in the different stations were obtained using the formula:

$$r = \frac{n\sum XY - \sum X \sum Y}{\sqrt{n\left(\sum X^2 - \left(\sum X\right)^2\right)\left(n\sum Y^2 - \left(\sum Y\right)^2\right)}} \quad \textbf{(6)}$$

where *r* is Coefficient of correlation, X and Y = Variables under consideration.

Total Aerobic Bacterial Count

The frogs were sacrificed, and the guts were removed using a sterile surgical blade. The guts were then homogenized in 100 ml sterile Peptone Water (Lab M, UK) and then serial dilution was performed to sixth dilution factor. Then 1 ml of the 4th, 5th and 6th diluents were aseptically dispensed into sterile Petri dishes. Using the pour plate method, sterile Plate Count Agar (Lab M, UK) and Nutrient Agar (Lab M, UK) were poured aseptically into the inoculated plates.

The plates were then incubated at 37 ºC for 24 h after which the colonies were counted and sub-cultured to obtain pure colonies. This was carried out in duplicates. The pure colonies of bacterial isolates were sub-cultured on Nutrient Agar (Lab M, UK) and incubated at 37 ºC for 24 h. The pure isolates were maintained on Nutrient Agar (NA) slants and stored at 4ºC inside the refrigerator.

Storage of isolates, Characterization of bacterial isolates, Gram staining, Spore staining, Motility test, Catalase test, Coagulase test, Oxidase test, Urease test, Citrate test, Indole test, Starch hydrolysis test, Hydrogen sulfide (H_2S) production test, Methyl red test, Voges-Proskauer (VP) test, and test for Fermentation of sugars all followed Fawole and Oso (1998) and Cheesbrough (2006).

Statistical Analysis

Data collected for morphometric characters will be analyses using linear regression equation such as regression co-efficient, slope, intercept, Pearson's correlation co-efficient and co- variance. Food and feeding habit will be subjected to ecological diversity indices such as Margalet index, Shannon and Weiner index, Pielou index. Descriptive (mean, standard error, percentage) and inferential analysis (Duncan multiple range test, and t- test) will be used to test for significant differences between locations using Student Package for Social Sciences (SPSS) version 21.

RESULTS

Distribution and abundance of amphibian specie/location and collection site across the study area.

Bufu bufo was the most abundant amphibian specie encountered across the study locations. It had the distribution mean values of 22.00±0.5, 15.00±0.1, 32.00±0.1 and 30.00±0.2 for Abeokuta South, Abeokuta North, Yewa North and Ijebu North, respectively (Table 1), as well as the collection sites with the distribution mean values of 20.00±0.1, 27.00±0.2, 25.00±0.1 and 27.00±0.1 for Hospital Areas, Mechanic Villages, Quarry Sites and Residential Areas respectively (Table 2). Its dominance is probably attributed to its preferred habitats, breeding biology (explosive breeders) and its tolerance to harsh conditions.

Ezemonye and Tongo (2010) stated that *Bufo regularis* lives in dry savannah, bush land and grassland areas, but it can occupy moist humid savannah and tropical forest. The breeding biology of *Bufo regularis* also favored its abundance in these kinds of environments. Bufonidae is regarded as abundant species with large breeding assemblage and it breeds in temporary and semi-permanent water, pans, dams, and inundated grassland. The eggs of Bufonidae develop very quickly and it is estimated that a single female can lay up to 315 eggs (IUCN, 2013).

Hoplobatrachus occipitalis is one of the abundance amphibian specie across the study locations. At Abeokuta South, the distribution mean value was 9.00±0.1, Abeokuta North 8.00±0.1, Yewa North 2.00±0.2 and 2.00±0.2 at Ijebu North. It is known to be generalist feeders, abundant, widespread, and adaptable species and can tolerate a broad range of habitats.

Grosjean, et al. (2003) observed that *Hoplobatrachus chinensis*, *H. occipitalis*, and *H. tigerinus* are mostly found along streams and in temporary and permanent water. This species can also tolerate habitats (agricultural area or rice fields) exposed to severe pressure from human disturbance (IUCN, 2013). Dicroglossidae is also a generalist species that feeds on beetles, grasshoppers, ants and other invertebrates (Seale and Wassersug, 1979; Seale, 1982). All these preys might have accounted for the abundance of *H. occipitalis* in this study. *H. occipitalis* has been reported to be decreasing in population and its population status in most location/sampling zones is unknown (Rödel, et al., 2004).

The occurrence of *Xenopus laevis* noticed in this study area is an indication of invasive species, since no author has ever reported its existence and availability of this species in Nigeria. At Abeokuta South, the distribution mean value was 3.00±02, Abeokuta North 3.00±0.2, Yewa North 2.00±0.2 and at Ijebu North the distribution mean value was 0.00±0.0.

Table 1: Distribution and Abundance of Amphibian Species/Location across the Study Areas.

Species	Abeokuta South	Abeokuta North	Yewa North	Ijebu North	Percentage (%)
Bufo bufo (common toad)	22.00±0.5	15.00±0.1	32.00±0.1	30.00±0.2	77.3
Hoplobatrachus occipitalis (crowned bull frog)	9.00±0.1	8.00±0.1	2.00±0.2	2.00±0.2	16.4
Xenopus laevis (african clawed frog)	3.00±02	3.00±0.2	2.00±0.2	0.00±0.0	6.3

Table 2: Distribution and Abundance of Amphibian Species/Collection Site Across the Study Area.

Species	Hospital Area	Mechanic Village	Quarry Site	Residential Area	Percentage (%)
Bufo bufo (common toad)	20.00±0.1	27.00±0.2	25.00±0.1	27.00±0.1	75.0
Hoplobatrachus occipitalis (crowned bull frog)	9.00±0.2	4.00±0.2	4.00±0.1	8.00±0.2	18.9
Xenopus laevis (african clawed frog)	1.00±02	0.00±0.0	1.00±0.2	6.00±0.01	6.1

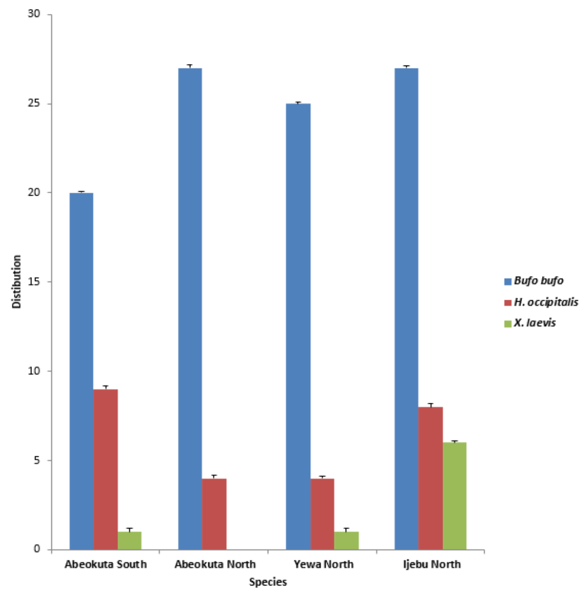

Figure 2: Distribution and Abundance of Amphibian Species/Location across the Study Areas.

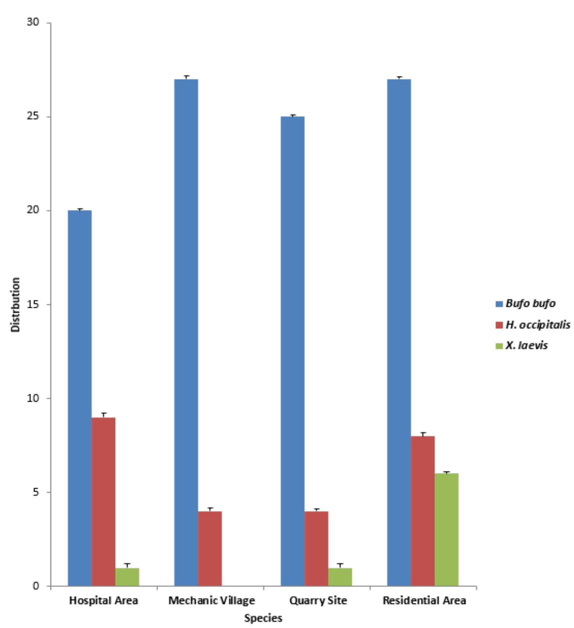

Figure 3: Distribution and Abundance of Amphibian Species/Collection Site across the Study Area.

Morphometric Parameters of *Hoplobatrachus occipitalis*

Morphometric features of *H. occipitalis* are illustrated in Table 3 across 4 locations. The respective mean values in Abeokuta South were 2.72±0.53, 2.52±0.53, 2.09±0.56, 2.61±0.52, 0.61±0.12, 2.70±0.51, 5.02±1.14, 7.74±1.16, 10.50±1.95 and 49.68±30.42 for Head Length, Head Width, Snout Tympanum Length, Mouth Angle Snout Length, Nostril Snout Length, Mouth Gap, Trunk Length, Snout Vent Length, Hind Limb Length, and Body Weight, respectively.

In Abeokuta North, Head length had mean value of 2.60±0.53, Head width 2.36±0.48, Snout Tympanum Length 1.92±0.53, Mouth Angle Snout Length 2.58±0.49, Nostril Snout Length 0.55±0.11, Mouth Gap 2.53±0.63, Trunk Length 4.42±0.93, Snout Vent Length 7.04±1.46, Hind Limb Length 9.99±1.72, and Body Weight 37.35±23.14. However, Yewa North recorded the following mean values for all the parameters 2.99±1.31, 2.56±1.01, 2.17±0.95, 2.86±1.03, 0.58±0.21, 2.37±0.67, 4.85±2.01, 7.85±3.32, 10.52±4.28, and 56.74±62.20, respectively.

Finally, Ijebu North observed respective mean values in Head Length (2.60±0.20), Head Width (1.71±0.97), Snout Tympanum Length (1.59±1.15), Mouth Angle Snout Length (2.05±1.03), Nostril Snout Length (0.54±0.05), Mouth Gap (1.93±0.64), Trunk Length (4.19±0.05), Snout Vent Length (6.55±0.15), Hind Limb Length (9.41±0.11), and Body Weight (27.70±3.96). Analysis of variances show that there was no significant difference ($p>0.05$) among the locations except in Yewa North (Hind Limb Length and Body Weight, respectively).

Table 3: Morphometric parameters of *Hoplobatrachus occipitalis.*

Parameters	Abeokuta S.	Abeokuta North	Yewa North	Ijebu North
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
HL(cm)	2.72±0.53[a]	2.60±0.53[a]	2.99±1.31[b]	2.36±0.20[a]
HW(cm)	2.52±0.53[a]	2.36±0.48[a]	2.56±1.01[a]	1.71±0.97[a]
STL(cm)	2.09±0.56[a]	1.92±0.52[a]	2.17±0.95[a]	1.59±1.15[a]
MSL(cm)	2.61±0.52[a]	2.58±0.44[a]	2.86±1.03[b]	2.05±1.03[a]
NSL(cm)	0.61±0.12[a]	0.55±0.11[a]	0.58±0.21[a]	0.54±0.05[a]
MG(cm)	2.70±0.51[a]	2.53±0.63[a]	2.37±0.67[a]	1.93±0.64[a]
TL(cm)	5.02±1.14[a]	4.42±0.93[a]	4.85±2.01[a]	4.19±0.05[a]
SVL(cm)	7.74±1.66[a]	7.04±1.46[a]	7.85±3.32[a]	6.55±0.15[a]
HLL(cm)	10.50±1.95[a]	9.99±1.72[a]	10.52±4.28[b]	9.41±0.11[a]
BW(g)	49.68±30.42[a]	37.35±23.14[a]	56.74±62.20[b]	27.70±3.96[a]

Mean values with the same superscript along the rows were not significantly ($p > 0.05$) different.
Mean values with the different superscripts along the columns were significantly ($p < 0.05$) different.

Regression Analysis for Selected Morphometric Variables of the Collected Samples

Regression analysis was performed for three morphometric variables viz., Head length (HL) with Snout vent length(SVL) and Hind limb length (HLL) with Snout vent length(SVL) and fitted regression equation $y = 1.005x + 0.437$ ($R^2 = 0.934$) and $y = 0.861x + 0.643$ ($R^2 = 0.868$) for *Hoplobatrachus occipital* species (Figure 4 and 5). This implies that an increase in HL will lead to increase in SVL. Similar pattern was observed in HL against HLL.

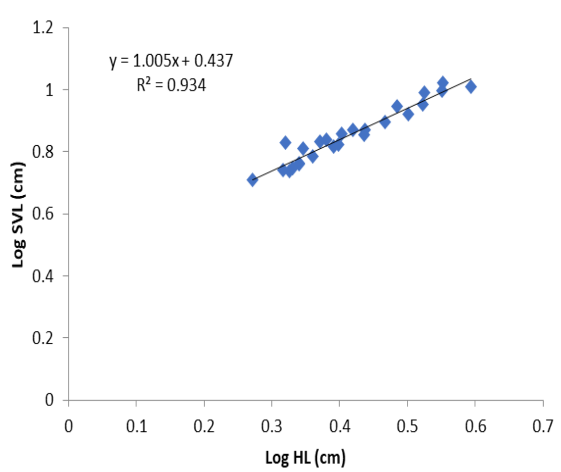

Figure 4: Regression Analysis Plots for Log HL against Log SVL for *Hoplobatrachus occipitalis* with Fitted Regression Equation.

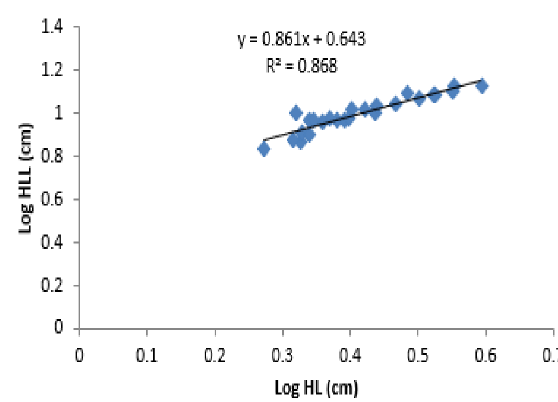

Figure 5: Regression Analysis Plots for Log HL against Log HLL for *Hoplobatrachus occipitalis* with Fitted Regression Equation.

Feeding Habit of *Hoplobatrachus occipitalis* in the Study Area across Location

Table 5 showed the result of 17 food items made up of 6 taxa namely, Coleoptera, Plecoptera, Hemiptera, Ephemeroptera, Crustacean, and Anura. Ephemeroptera had the highest prey items in Abeokuta South and the lowest dietary contents were in Hemiptera and Anura with 51.19% and 4.76, respectively. The most commonly observed taxon in Abeokuta North was Ephemeroptera with 40.63% while the least consumed prey items was indicated in Crustacean. However, Yewa North had two taxa of diet composition in Ephemeroptera and Plecoptera.

Table 5: Gut Content of *Hoplobatrachus occipitalis* in the Study Area across Locations.

Prey items/Taxon	Location							
	AbkS		Abk N		YN		IN	
	OF	%	OF	%	OF	%	OF	%
COLEOPTERA								
Dryopid sp	5		3		-		1	
Helophrus sp	3		1		-		2	
Laccophilus sp	2		2		-		-	
Bidessus sp	1	16.67	3	23.44	-		-	44.45
PLECOPTERA								
Nymphula sp	2		0		1		-	
Elophila sp	5		8		2		-	
Collembola sp	3		2		-		-	
Haliplidae sp	4	16.67	1	17.19	1	36.36	-	
HEMIPTERA								
Hesperocorixa sp	1		7		-		3	
Pelocoris sp	3	4.76	2	14.06	-		-	33.33
EPHEMEROPTERA								
Potamanthus sp	18		14		3			
Branchycerus sp	20		10		4		-	
Callibaetis sp	5	51.19	2	40.63	-	63.64		
CRUSTACEANS								
Diaptomus sp	3		1		-		-	
Cyclops sp	2	5.95	2	4.68	-		-	
ANURA								
Frog bone	1		-		-		1	
frog skin	3	4.76	-		-		1	22.22

OF- Frequency of occurrence, AbkS = Abeokuta South, AbkN = Abeokuta North, YN = Yewa North and IN = Ijebu North

Table 6: Total Aerobic Bacterial Count of the gut of *Hoplobatrachus occipitalis* collected across four LGAs.

S/N	Sampling location	Abeokuta South LGA ($x10^4$ CFU/g)	Abeokuta North LGA ($x10^4$ CFU/g)	Yewa North LGA ($x10^4$ CFU/g)	Ijebu North LGA ($x10^4$ CFU/g)
1	Hospital Area	12.0±1.1^{a}	20.0±0.6^{c}	18.0±0.6^{c}	11.0±0.6^{a}
2	Mechanic Village	13.0±0.6^{a}	11.0±0.6^{a}	7.0±0.6^{a}	9.0±0.6^{a}
3	Quarry Site	13.0±1.0^{a}	11.0±0.6^{a}	14.0±1.0^{b}	10.0±0.6^{a}
4	Residential Area	15.0±1.1^{a}	14.0±0.6^{b}	13.0±1.0^{b}	14.0±1.1^{b}

Values are means of triplicate readings ± Standard error

Finally, the dominance of Coleoptera was notices in Ijebu North with the least food items consumed in Anura with 44.45% and 22.22% respectively.

Total Aerobic Bacterial Count (TABC)

The results obtained from the plate counts were significantly different at $p < 0.05$. The Total Aerobic Bacterial Counts (TABC) obtained ranged from 0.7 – 2.1 x104 CFU/g. The highest TABC of 2.1 x104 CFU/g was obtained from Abeokuta North LGA at Hospital Area while the lowest TABC of 0.7 x104 CFU/g was obtained from Yewa North LGA at Mechanic Village (Table 6). Meanwhile, the average counts across all the four LGAs also revealed that Hospital Area had the highest counts while Mechanic Village had the least counts (Table 6).

Table 7: Microorganisms Isolated from the gut of *Hoplobatrachus occipitalis* Collected across Four LGAs.

Sampling location	Abeokuta South LGA	Abeokuta North LGA	Yewa North LGA	Ijebu North LGA
Hospital Area	*Escherichia coli*, *Salmonella arizona*, *Salmonella* sp., *Pseudomonas fluorescens*	*Escherichia coli*, *Salmonella arizona*, *Salmonella* sp., *Bacillus subtilis, Pseudomonas fluorescens*	*Escherichia coli*, *Salmonella arizona*, *Salmonella* sp., *Pseudomonas putida*	*Escherichia coli*, *Salmonella arizona*, *Salmonella* sp., *Pseudomonas putida, Proteus vulgaris*
Mechanic Village	*Escherichia coli*, *Salmonella arizona*, *Salmonella* sp., *Pseudomonas fluorescens*	*Escherichia coli*, *Salmonella arizona*, *Salmonella* sp., *Proteus vulgaris*	*Escherichia coli*, *Salmonella arizona*, *Salmonella* sp.,	*Escherichia coli*, *Salmonella arizona*, *Salmonella* sp., *Pseudomonas putida,*
Quarry Site	*Escherichia coli*, *Salmonella arizona*, *Salmonella* sp., *Proteus vulgaris, Pseudomonas putida*	*Escherichia coli*, *Salmonella arizona*, *Salmonella* sp., *Proteus vulgaris*	*Escherichia coli*, *Salmonella arizona*, *Salmonella* sp., *Pseudomonas putida*	*Escherichia coli*, *Salmonella arizona*, *Bacillus subtilis*, *Pseudomonas fluorescens*
Residential Area	*Escherichia coli*, *Salmonella arizona*, *Salmonella* sp., *Pseudomonas fluorescens, Bacillus subtilis*	*Escherichia coli*, *Salmonella arizona*, *Salmonella* sp., *Pseudomonas putida, Proteus vulgaris*	*Escherichia coli*, *Salmonella arizona*, *Salmonella* sp., *Pseudomonas fluorescens*	*Escherichia coli*, *Salmonella arizona*, *Salmonella* sp., *Pseudomonas putida, Proteus vulgaris*

Table 8: Biochemical Characterization of the Isolates.

LABEL	GRAM	SPORE	MOTILITY	GLUCOSE	LACTOSE	MANNITOL	MALTOSE	INDOLE	M.R	V.P	CITRATE	H_2S	SUCROSE	UREA	OXIDASE	COAGULASE	CATALASE	PROBABLE ORGANISM
A	GPB	-	+	+	+	+	+	+	+	-	-	-	NA	-	-	NA	+	*Escherichia coli*
B	GNB	-	+	+	-	-	-	-	--	-	+	+	-	-	-	NA	+	*Salmonella arizona*
C	GNB	-	+	+	-	-	-	-	+	-	+	+	-	-	-	NA	+	*Salmonella* sp.
D	GNB	-	+	+	-	+	+	-	+	-	+	+	-	+	+	NA	+	*Pseudomonas putida*
E	GNB	-	+	+	-	-	-	-	+	-	+	-	+	+	-	NA	+	*Proteus vulgaris*
F	GNB	-	+	+	-	+	+	-	+	+	+	+	+	+	+	NA	+	*Pseudomonas fluorescens*
G	GPB	+	+	+	+	+	+	-	-	+	+	-	+	-	-	NA	+	*Bacillus subtilis*

Key: - = Negative; + = Positive; GPB = Gram positive bacill; GPC = Gram positive cocci; NA = Not available

Characterization of the Isolates

Seven bacterial isolates were identified; one Gram positives and six Gram negatives. The bacterial genera and their incidence rate were as follows: *Escherichia coli* (38.46%), *Salmonella spp.* (30.76%), *Pseudomonas spp*. (15.38 %), *Proteus vulgaris* (7.69 %), and *Bacillus subtilis* (7.69 %).

Escherichia coli, *Salmonella Arizona*, and *Salmonella sp.* occur in all the sampling location across the four LGAs (Table 7).The biochemical characterization of the bacterial isolates is shown in Table 8.

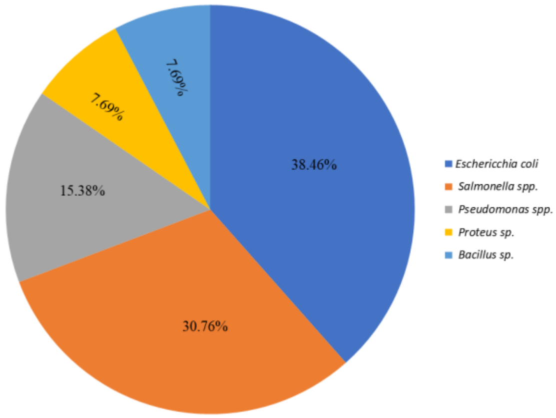

Figure 6: Incidence Rate of the Bacterial Isolates.

DISCUSSION

Morphological feature is unique being a rare data to be produced in terms of the species morphometric in the region and future studies in this regard would facilitate further understanding of the impact of immediate ecological conditions on the anuran morphology. Snout Vent Length (SVL) and Head Length (HL) are the two variables that mostly contributed for significant differences related to morphometric traits (Silva, et al., 2008, Bahuguna and Bhutia, 2010, Pichandi, et al., 2013). However, the result of this study presents variation in morphological traits due to spatial pattern among the location showing a substantial overlap in overall morphometric variables of the amphibians population. In anuran, adult body sizes depend on many factors, including time of metamorphosis, growth rate before and after maturity, age of maturity, and longevity (Rödel, et al., 2004).

Bufo bufo was the most abundant amphibian species encountered in Yewa North and Ijebu North, respectively. Its dominance is probably attributed to its preferred habitats, breeding biology (explosive breeders) and its tolerance to harsh conditions. The breeding biology of *Bufo regularis* also favored its abundance in this kind of environment. Bufonidae is regarded as abundant species with large breeding assemblage and it breeds in temporary and semi-permanent water, pans, dams and inundated grassland. The eggs of Bufonidae develop very quickly and it is estimated that a single female can lay up to 315 eggs (IUCN, 2013).

Other abundant amphibian species include *Hoplobatrachus occipitalis* which is known to be generalist feeders, abundant, wide spread, and adaptable species and can tolerate a broad range of habitats. This species can also tolerate habitats (agricultural area or rice fields) exposed to severe pressure from human disturbance (IUCN, 2013).

H. occipitalis has been reported to be decreasing in population and its population status in most location/sampling zones is unknown (Rödel, et al., 2004). The occurrence of *Xenopus laevis* noticed in this study area is an indication of invasive species, since any author has never reported its existence and availability of this species in Nigeria. However, *Xenopus muelleri* spends most of its time in water and is adapted to aquatic life (Spawls et al., 2006).

The highest abundance of amphibian species in the study area were probably attributed by the time of the study (during the rainy season), which favor the distribution of resources (food and breeding pools), moist and humid environment for most amphibian species.

Hoplobatrachus occipitalis were not widely distributed with the exception of Abeokuta North and South that had individuals' representatives in all habitats. Abeokuta North showed wide distribution in the study habitats. Though Hospital area and Residential area were noted to be most abundant amphibian species and this is possibly due to its preference in open savannah than other habitats (Gardener, et al., 2007). However, Razzetti and Msuya (2002) recorded high number of H. marmoratus in riverine, woodland, mbuga and cultivated soil habitats.

The low outcome of *H. occipitalis* recorded in Yewa North and Ijebu North might be as a result of ecological requirement for the survival of the species. The finding of this study concured with Spawls, et al. (2006), who reported that *H. marmoratus* and *H. guineensis* can occur together in same/different habitats. Le Roux (2010) also recorded both *H. marmoratus* and *H. guineensis* in Okavango Delta.

The species richness and diversity of amphibians differed among habitats. Abeokuta North supported higher amphibian species diversity and abundance probably due to the presence of different micro habitats such as pools, ponds, swamps, pans, tall and short grasses, herbs and open ground, which support different species ecological requirements (e.g., breeding sites). Biologically, most amphibians breed during early rains. The fact that this study was undertaken during early rainy season highlighted that amphibians were all over due to the presence of ponds (breeding sites) and abundant food as the main reasons of encountering their higher species richness in the grassland habitats. Brotherton et al. (2004) found that most amphibians migrate from wetland habitat to bogs, open ponds, roadsides, grassland and woodland for breeding, foraging and hibernation.

The low species diversity and abundance of amphibians in Yewa North and Abeokuta North were probably attributed to by lack of suitable microhabitats (e.g., ponds) as these habitats are made of rock outcrops. Though Yewa North and Abeokuta North are known to support unique fauna (Sinclair and Arcese, 1995), it is obvious that these fauna are not water-dependent.

Drewes (1997) recorded one species of tree frog (Hyperolius orkarkarri) that was exposed in full sunlight. Elsewhere in Africa, other species of tree frog such as *Hyperolius horstocki* of South Africa are known to rest in exposed position in sunlight (Wager, 1965; Drewes, 1997). However, many species were not encountered in this region for this study despite the active searching efforts made. The absence of water ponds around the sampling zones may likely affected food availability, feeding and breeding sites and hence low species richness and diversity of most water-dependent amphibians such as *Xenopus sp.*

Other researchers have also reported low species richness of amphibians in rock outcrops. Rakotondravony and Goodman (2011) recorded only four species of amphibians across five rock areas in Madagascar. Likewise, Karunarathna, et al. (2008) reported only two amphibian species in rock outcrops, being lowest species richness of amphibians compared to other habitats in the Nilgala Forest Area and its vicinity, Monaragala District in Sri Lanka. Karunarathna, et al. (2006) also pointed out that rock outcrops are xeric due to direct sun exposure and are not suitable habitats for amphibians.

A crucial part of the natural history of an animal is its diet, because not only does it reveal the source of the animal's energy for growth, maintenance and reproduction (Zug, et al., 2001), but it also indicates part of the ecological roles of the animal. Ates, et al. (2007) identified 17 food items in the guts of *H. occipitalis* species where the frequently occurring food items were Hymenoptera, Coleoptera, Plecoptera, Hemiptera, Ephemeroptera, Crustacean, and Anura.

The results of this study revealed diverse food items probably because the anurans were collected from large sampling sites and in four different savanna types. The wide variety of food items also suggests that anurans (*H. occipitalis*) are wide foraging generalists. Isaacs and Hoyos (2010) also observed a similar result in *R. marina* having a generalist diet with high proportion of hemipterans, coleopterans, and hymenopterans.

A similar study of Allingham and Harvey (2011) in Senegal but of different species of frogs showed that the most frequently taken prey items were Orthoptera (36 %), Hymenoptera (24 %) and Arachnida (10 %) suggesting that Senegal running Frog *Kassina senegalensis* is a wide foraging generalist. Fabricante and Nuñeza (2012) who studied the diet of *R. grandocula* and *R. magnus* in the same province where Agusan Marsh is located but in a higher altitude of Mt. Sambilikan, Diwata Range, Agusan del Sur also found these frogs as generalist feeders.

This study also showed a high bacteria count in the gut of *Hoplobatrachus occipitalis*. This is in line with Colombo, et al. (2015) who reported that adult frogs contain 106 bacteria per gram of intestinal contents. Similarly, Pryor (2008) reported higher counts of 7.7 x 109 and 9.4 x 109 CFU/g of colon and rectum of tadpole gut, respectively. Species of Bacillus and Pseudomonas have been indicated as common inhabitant of insects, fishes, amphibians, reptiles, birds, and the mammalian gut (Cantas, et al., 2011; Costello, et al., 2010).

Green and Muths (2005) isolated 14 bacterial genera, including *Echerichia coli*, *Bacillus spp*. and *Pseudomonas spp*. from the gut of adult amphibians. Banas, et al. (1988) reported Bacteroides and Clostridium (Anaerobic) as well as Bacillus and Pseudomonas (Aerobic) as major intestinal bacteria in hibernating and non-hibernating Leopard frog.

CONCLUSION

This study revealed that location influences the distribution and abundance of amphibians most especially frogs in the study area. However, the result of this study present variation in morphological traits due to spatial pattern among the locations showing a substantial overlap in overall morphometric variables of the amphibian population.

Hoplobatrachus occipitalis which is known to be generalist feeders were not widely distributed across the study area due to high predation pressure because some people feed on them, while some use it as there source of income by harvesting and selling it in the market.

The occurrence of *Xenopus laevis* noticed in this study area is an indication of invasive species, since any author has never reported its existence and availability of this species in Nigeria.

The species richness and diversity of amphibians differed among locations. Abeokuta North supported higher amphibian species diversity and abundance probably due to the presence of different micro habitats such as pools, ponds, swamps, pans, tall and short grasses, herbs and open ground, which support different species ecological requirements (e.g., breeding sites). All these micro habitats favored the occurrence of most amphibians. Seasonality also attributed to high species richness of amphibians in the grassland habitats.

The low species diversity and abundance of amphibians in Yewa North and Abeokuta North were probably attributed to by lack of suitable microhabitats (e.g., ponds) as these locations are made of rock outcrops.

RECOMMENDATIONS

A major concern arising from this research work is that amphibian species especially frogs are understudied in Nigeria and most especially in western zone. Most of the references presented here were from studies conducted outside Nigeria. A comprehensive research study (reproductive aspect and ecological variables) needs to be undertaken to understand anurans in Nigeria. All the sampling locations need to be protected and size variation of anurans also need to be monitored. The quality of water must be checked and disturbance (especially human impacts) with wide range of biodiversity of amphibians should be investigated.

The need for amphibian conservation awareness/education has to begin in harness couple with intensive domestication and multiplication practices to redeem the critical stage of amphibian biodiversity, especially frogs, in the ecosystem.

The use of uncensored fishing nets in aquatic environments has to be discouraged while monitoring agents has to be put in place to safeguard the lives of amphibians especially frogs in aquatic environments.

Industrial waste effluent directed to pools, ponds and water bodies has to be discouraged by government to save the lives of aquatic beings.
Laws should be promulgated that will prevent indiscriminate predatory instinct of man with accompany penalties.

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SUGGESTED CITATION

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