Environtropica, December 2023, Vol. 18, 046 - 054 ISSN 1597-815X

Evaluation of the Efficacy and Effects of Stomach Flushing for Dietary Analysis on Five Frog Species found at Obafemi Awolowo University, Nigeria.

AJIBOLA M. E. and ✉OMOSHEHIN T. O.
Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria
✉Corresponding Author: taiwoomoshehin@yahoo.com Tel: +2348060276196

Accepted on November 11, 2023.

Abstract

Dietary studies in anurans have commonly been done by sacrificing the animals and excising their stomach. In view of the declining populations of amphibians, studies which affect the biodiversity of organisms are being carried out with care. The stomach flushing technique was carried out on five frog species; *Hoplobatrachus occipitalis, Xenopus laevis, Ptychadena aequiplicata, Rana galamensis* and *Hylarana albolabris* with a view to determining its efficacy in retrieving ingested food items and the effect of the procedure on the species. Specimens were collected weekly for 32 weeks and stomach flushing was carried out not later than two hours after capture and returned to their capture sites. Recovery of 1,534 prey items from the stomachs of 264 specimens was successful for diet analysis and complete for most species after three replicates of the procedure with an average prey recovery rate of 87% and 98% in the first and second flushing sessions. This demonstrates the potential of the procedure in dietary studies of these species. Only four fatalities were observed. The mortality rate of 1.52% was accounted for mainly by *H. albolabris* with 1.14% while 0.38% was represented by *X. laevis*. Mortality was not observed for *H. occipitalis*, *P. aequiplicata* and *H galamensis*.

Keywords: Stomach Flushing, Diet, Amphibian, Anura, Ecology, Conservation, Efficiency.

Introduction

Amphibians (frog, toad etc.) play diverse roles in natural ecosystems, and their decline may cause other species to become threatened or may undermine aspects of ecosystem function (Matthews *et al.,* 2002; Whiles *et al.,* 2006). They are important predators of many insects and agricultural pests and are therefore valuable for natural biological pest control (Oza, 1990). Frogs in particular are an important prey for diverse animal species and their tadpoles which are usually filter feeders, contribute to stabilize water quality in ponds and streams (Mohneke *et al.*, 2010). The tadpoles of frogs are usually herbivorous but species such as *Hoplobatrachus occipitalis* are carnivorous and feed on small aquatic animals such as the tadpoles of other species of frogs as well as the larval stages of mosquitos, making them an important part of the food chain. Adults consume a wide variety of organisms which include both invertebrates and vertebrates. A drop in population of frogs as a predator, due to overexploitation of the species, or any other reason could lead to increases in prey populations (Mohneke *et al.*, 2010).

In view of the declining populations of amphibians, STUDIES which affect the biodiversity of organisms are being carried out with care (Solé *et al*., 2005). Dietary studies in anurans have commonly been done by sacrificing the animals and excising their stomach e.g. Toft (1981), Vences *et al*. (1999), Silva *et al*. (2009), Bwong and Measey (2010), Tohé *et al*. (2013).

Excision of frog stomach for diet analysis is no longer conventional, especially in situations where the species is threatened, endangered, economically valuable, or comes from a low density population. Other methods for obtaining stomach contents from vertebrates include stomach suction, stomach flushing, use of gastroscopes, tubes, forceps, and administration of emetics (Kamler and Pope, 2001). It is important to make improvements on the existing procedures to allow for comprehensive data collection and yet avoid unnecessary loss of specimens. Alternative methods of obtaining stomach contents have been developed, of which the stomach flushing technique is particularly simple and effective (Solé *et al*., 2005). The stomach flushing technique has been used extensively for the dietary studies of live vertebrates. Initially applied to salamanders (Fraser, 1976), it was employed by Legler (1977) who studied the feeding habits of live-captured wild freshwater turtles. The technique was also successfully used on lizards and frogs (Legler and Sullivan, 1979), crocodile (Fitzgerald, 1989; Rice, 2004), turtles (Caputo and Vogt, 2008), bird (Martin and Hockey, 1993), fish (Waters *et al*., 2004) frogs and toads (Solé *et al*., 2005; Mahan and Johnson, 2007), terrestrial salamanders (Crovetto *et al*., 2012) and, compared with dissection (Wu *et al.*, 2007; Balint *et al*., 2010).

Hoplobatrachus occipitalis is one of the most widely distributed anuran species of Afro-tropical zoogeographic realm (Jirku and Modry, 2006). *H. occipitalis* is a common and widespread West African frog occurring in both savanna and disturbed forest habitats. It is known to be consumed by humans in several countries e.g. Benin, Burkina Faso, Ghana, Guinea, Ivory Coast, Nigeria (Hirschfeld and Rödel, 2011. Due to its large size, *H. occipitalis* is the most commercialized species (Onadeko *et al.*, 2011). Onadeko and Rodel (2009) reported the presence of *H. occipitalis* from most locations in South-western Nigeria, even in disturbed environments.

Xenopus laevis is commonly studied as a model organism for developmental biology, cell biology, toxicology, and neuroscience (Wallingford *et al.,* 2010; Harland and Grainger, 2011). Frogs in the genus *Xenopus* are ubiquitous in sub-Saharan Africa, they are commonly found in anthropogenically disturbed habitats (Bwong and Measey, 2010). These carnivores mostly consume aquatic invertebrates, but also include small vertebrates, including other *X. laevis*, in their diet (McCoid and Fritts, 1980; Tinsley *et al*., 1996; Lafferty and Page, 1997; Measey, 1998a; Channing, 2001; Lobos and Jaksic, 2005). They are also capable of capturing terrestrial prey (Measey, 1998b).

Rana galamensis belongs to the family Ranidae, which has widest distribution of any frog family, and the Class Amphibian (Muhammad and Ajiboye, 2010). They are found in savannah region of West Africa, South Africa and East Africa (Rodel *et al.*, 2004). In Nigeria, they are found in many states such as Lagos, Ogun, Oyo, Kwara, Osun, Ondo, Ekiti, Kaduna and Benin City (Walker, 1967). Its natural habitats are dry savanna, moist savanna, subtropical or tropical rivers, shrub-dominated wetlands, swamps, freshwater lakes, intermittent freshwater lakes, freshwater marshes, intermittent freshwater marshes, rural gardens, urban areas, water storage areas, ponds, and canals and ditches (Rodel *et al*., 2004).

Hylarana albolabris also known as *Amnirana albolabris* is a species of frog in the Ranidae Family found in its natural habitats which are subtropical or tropical moist lowland forests, subtropical or tropical moist montane forests, rivers, swamps, freshwater marshes, intermittent freshwater marshes, rural gardens, heavily degraded former forests, and ponds (Amiet *et al*., 2004).

All the frogs utilized in this study are classified by the International Union for the Conservation of Nature (IUCN) Red List into the Least Concern category. The current population trend of *X. laevis* is on the increase, *H. occipitalis* and *P. aequiplicata* is stable while that for *H. albolabris* and *H. galamensis* is unknown (IUCN, 2014).

Though the stomach flushing procedure has been carried out on many frog species, there is paucity of information on the effect of the technique on populations of the selected species in Nigeria, hence this study. The objectives here are to determine the efficiency of stomach flushing in the extrication of ingested food items and to determine if the procedure has detrimental effects on selected species of frogs.

Materials and Methods

Specimens were collected weekly within Obafemi Awolowo University, Ile- Ife, Osun State, Nigeria from April to November, 2020 between the hours of 23:00 and 02:00 GMT with the aid of a torch light and dip-net. Collection was occasionally carried out earlier on rainy days. Specimens were sampled from four locations namely; Biological Sciences Area (07°31′3″N, 004°31′3″E), Health Sciences Area (07°31′1″N, 004°31′31″E), Oxidation Pond Area (07°30′26″N, 004°30′41″E) and White House Area (07°31′7″N, 004°31′12″E) (Figure 1). No sampling site was visited more than once in thirty days to avoid stomach flushing the same specimen more than once a month. The captured specimens were brought to the laboratory in separate captivity cages, sorted and identified using standard identification keys by Rödel and Spieler (2000). Care was taken to tag each cage with its location so as to be able to return them to their precise habitat in order to prevent possible dispersion of pathogens. Gonadal development and presence of secondary sexual traits e.g. vocal sacs, swollen thumbs and throat colouration (Silva *et al*., 2009) and size were used to ascertain the maturity of the frogs.

Stomach flushing was carried out immediately after capture or not later than two hours after, in accordance with Caldwell (1996) and Secor and Faulkner (2002) so as to avoid rapid digestion of the prey in the stomach of the captured specimens.

Stomach flushing procedure was carried out by inserting and gently pushing a narrow flexible tube which had been lubricated by coating with mineral oil into the esophagus of the captured specimen until an appreciable resistance was met. This is usually when the tube has reached the wall of the stomach. The tube was then pulled back slightly so as to prevent damaging the wall of the stomach and to allow for the ingested items to be dislodged by the pressure of the flushing water. which was continuously pumped to facilitate the evacuation of the stomach contents as described by Sole *et al*. (2005). The internal and external diameter of the gastric tube were 3 mm and 4 mm respectively. This flexible tube was attached to a 60 ml syringe which was refilled after each flushing session until no more stomach content was observed in the regurgitated water. The water used for flushing was obtained from the water body close to where the frogs were captured. For specimens which there was no water close to their capture site, untreated water was used. There was no use of anesthesia because the limbs of the frogs were held tight by a second person while the other participant performed the flushing procedure. This procedure can however be carried out by an individual if the frog specimens are anesthetized. Apart from being inconvenient and taking longer to perform, the use of anesthesia makes it more difficult to determine the immediate effect of the flushing procedure on the frogs and may have deleterious effects on the frogs.

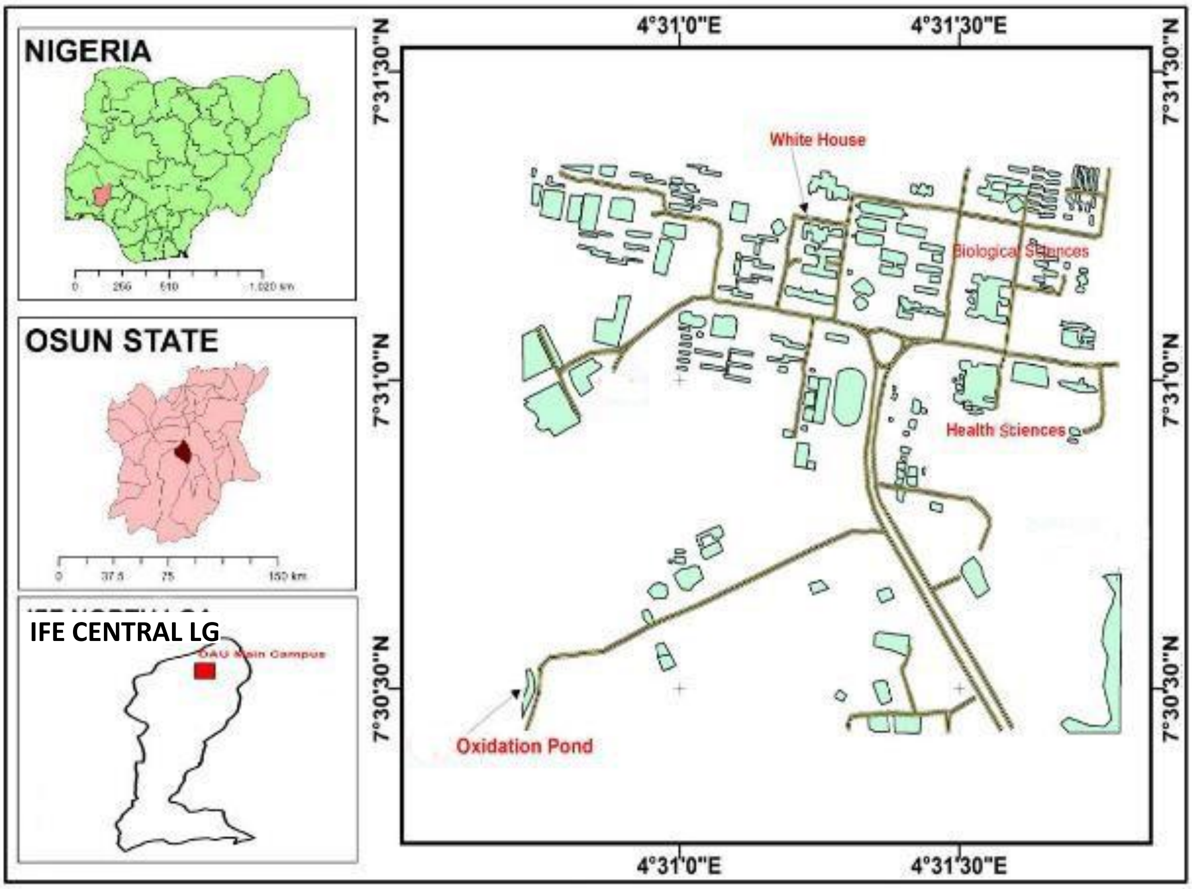

Figure 1: Map Showing Sampling Locations in Obafemi Awolowo University, Ile-Ife, Nigeria.

Stomach flushed frogs were maintained in the laboratory and fed with live termites. They were released back to their habitats after recovery from the flushing procedure was apparent.

The stomach flushing procedure was essentially stopped when the water regurgitated was clear and not laden with any food item. The regurgitated water became less laden with food after repetitions such that after the third one, most of the food items had been successfully evacuated. Thus restriction to three replicates can provide sufficient information. This restriction reduced the handling time and the stress to the specimens.

After each stomach flushing session, the abdominal region of the specimens was palpated to determine if there are remains that were not successfully dislodged. Whenever this was the case, a further flushing session was carried out. In a few cases where repeated stomach flushing failed to evacuate stomach contents, specimens were subjected to dissection but where no palpable evidence of stomach remains was found, frogs for dissection were selected randomly to confirm the efficacy of the procedure. Dissected specimens were preserved in formalin and stored in the laboratory as voucher specimens.

Food items collected by stomach flushing were preserved in 4% formalin prior to being analyzed in the laboratory under a binocular microscope to identify to the lowest taxonomic unit possible using standard keys.

Powder free latex gloves were used in handling the frogs so as to forestall zoonotic infection and prevent a reaction with the epithelial secretions of the frogs.

Results

Five species of frogs were identified in the 264 sampled specimens comprising; *Hoplobatrachus occipitalis* (36%), *Xenopus laevis* (26%), *Ptychadena aequiplicata* (16%), *Hylarana galamensis* (14%) *and Hylarana albolabris* (8%). Only 4 fatalities (1.62%) were recorded represented by 1.14% in *H. albolabris* and 0.38% in *X. laevis*. Mortality was not observed for *H. occipitalis*, *P. aequiplicata* and *H. galamensis* (Table 1).

A total of 1,534 prey items were recovered from the specimens processed with the highest number of 782 recovered from *H. occipitalis* and the lowest 88 food items recovered from *H. albolabris* (Table 2). Prey items recovered by flushing included prey items from Phylum Annelida (order Oligochaeta), Phylum Arthropoda {Orders Blattodea, Coleoptera, Diptera, Hemiptera, Hymenoptera (families Apidae and Formicidae), Isoptera, Lepidoptera, Odonata, plant matter and Phylum Mollusca (Class Gastropoda), Sub-phylum Vertebrata (Order Anura).
H. occipitalis showed the quickest recovery from the flushing process with complete recovery occurring within five minutes of being stomach flushed. *H. galamensis* specimens were also observed to fully recover within 8 minutes of being stomach flushed. All frogs were observed to have completely recovered within 15 minutes of being stomach flushed based on the resumption of their normal activities with the exception of four specimens which eventually accounted for the mortalities recorded. These specimens showed sluggishness after the procedure and died within 30 hours of being stomach flushed.

Table 1. Average Size of processed frogs showing number of processed specimens.

Species	Mean Size (cm)	Flushed	Dissected	Mortality
Hoplobatrachus occipitalis	10.27 ± 0.106	95	9	Nil
Xenopus laevis	6.18 ± 0.190	69	7	1
Ptychadena aequiplicata	6.13 ± 1.01	41	5	Nil
Hylarana galamensis	6.27 ± 1.203	38	5	Nil
Hylarana albolabris	4.85 ± 2.35	21	4	3

Table 2. Number of prey items recovered per flushing session.

Species/ Flushing session	First Flush	Second Flush	Third Flush	Fourth Flush	Total
Hoplobatrachus occipitalis	602	133	39	8	782
Xenopus laevis	215	24	3	-	242
Ptychadena aequiplicata	128	8	2	-	138
Hylarana galamensis	239	34	11	-	284
Hylarana albolabris	83	05	-	-	88
Total	1267	204	55	8	1534

Table 3. Percentage of prey items recovered during flushing sessions.

Species/ Flushing session	First Flush	Second Flush	Third Flush	Fourth Flush
Hoplobatrachus occipitalis	77	17	5	1
Xenopus laevis	89	10	1	-
Ptychadena aequiplicata	93	6	1	-
Hylarana galamensis	84	12	4	-
Hylarana albolabris	94	6	-	-

Discussion

Usually, the stomach flushing procedure was able to evacuate the stomach of ingested food items and palpation of the abdominal region didn't indicate the presence of food items. However, some frogs did not regurgitate any food item, due to their stomachs being empty. Seven of the twelve frogs which had empty stomachs were of the species *X. laevis*; this species is known to survive starvation conditions for at least 12 months and can rapidly regain lost weight when food is once again available (Tinsley *et al*., 1996).

The use of the syringe for flushing allow for proper regulation of the force at which the water was being pumped into the stomach of the specimens, using less force for smaller specimens so as to avoid the probable cause of death described by Pietruszka (1981) and Powell and Russell (1984). Although the procedure may cause some discomfort to specimens as reported by Fitzgerald (1989) who observed minor irritation of the oesophagus and cardiac sphincter of *Caiman crocodilus*, the effects are usually very short lived and do not affect the specimen's ability to carry out its activities afterwards.

The recovery of food items by the procedure varies depending on species. Wu *et al*. (2007) in the study of four anuran species *Rana nigromaculata*, *Rana plancyi*, *Rana limnochari* and *Bufo bufo gargarizans* recorded the flushing procedure to be about 93%, 92%, 96% and 68% efficient, respectively, in retrieving ingested food.

No mortality was observed for *H. occipitalis* and *H. galamensis*. These species also showed the quickest recovery, this may not be unconnected with their size. *H. albolabris*, the smallest of the sampled frog species had the highest mortality, with 12 % of the specimens (1.14 % of all processed frogs) not surviving the procedure. This relatively high mortality rate could not be directly associated with the stomach flushing procedure as necropsy carried out did not reveal any life threatening damage caused by the stomach flushing procedure. The deceased *X. laevis* specimen also didn't show any apparent damage to the gut wall or trachea as a result of the procedure, it was assumed that the mortalities may be related to the trauma associated with procedure or other unrelated reasons. Pietruszka (1981) and Powell and Russell (1984) alluded the deaths which resulted from gut flushing to excessive pumping pressure which caused rupture of the gut, or directly caused by the inserted tube. This was prevented by using syringe to pump the water as this could easily be regulated. It is possible that the success and recovery rate of the stomach flushing procedure is proportional to the size of the frog specimen. The mortality rate from stomach flushing has been reported to be over 5% in some cases as reported by Pietruszka (1981) and Powell and Russell (1984). They reported that the specimens which succumbed were all small lizards. Size may not be the factor responsible for the recovery of species from the procedure as Sole *et al*. (2005) recorded about 86% mortality for *Hylodes meridionalis* with snout-vent length which ranged between 35 – 50 mm, yet no mortality for *Hyla minuta* with snout-vent length which ranged between 21 – 28 mm, *Physalaemus cuvieri* (24 – 32 mm), *Physalaemus lisei* (21 – 33 mm) and *Elachistocleis bicolor* (20 – 45 mm).

Food items such as ingested frogs and snails which were relatively large were difficult to obtain. This may have been because the food items were very compact and required extra effort in order to dislodge the ingested food items, palpation of the abdominal region helped to loosen the food items making them easier to obtain. The stomach flushing procedure was however not successful in retrieving large compact bolus such as ingested gastropods. Obtaining large prey items may depend on the gape size as well as the type of food item. During swallowing, gravity and peristalsis made it easier for such food items to be swallowed. This difficulty of retrieving relatively large prey item was reported by Caputo and Vogt (2008) as they were not able to flush out presence of seeds greater than 1 cm. Though the flushing procedure does not always guarantee absolute evacuation of food items from the gut as dissection does, recovered prey items which usually reach over 95% can still provide sufficient insight into the feeding habits of the specimens being studied, especially when combined with other non-lethal methods such as field observation and faecal analysis. It is therefore not justifiable to kill specimens on the account of the negligible percentage of remnant prey items.

The results obtained by the stomach flushing procedure show that it is very efficacious in obtaining ingested food items from the guts of anurans, recovering as high as 94% in the first flush. Continuous repetition of the procedure after three sessions aside from yielding little or no results, may increase the stress level of the anurans. It is therefore needless to sacrifice anurans for the purpose of studying their diets.

References

Amiet J. L., Rödel M. O., Howell K., Lötters S. (2004) *Amnirana albolabris*. 2006 IUCN Red List of Threatened Species.

Balint N., Indrei C., Ianc R. and Ursuţ A. (2010) On The Diet of the *Pelophylax ridibundus* (Anura, Ranidae) in Ţicleni, Romania. *South Western Journal of Horticulture, Biology and Environment* 1 (1): 57 – 66.

Bwong B. A., Measey, G. J. (2010) Diet composition of *Xenopus borealis* in Taita Hills: effects of habitat and predator size. *African Journal of Ecology* 48: 299–303.

Caldwell J. P. (1996) The Evolution of Myrmecophagy and its Correlates in Poison Frogs (Family Dendrobatidae). *Journal of Zoology* 240: 75-101.

Caputo F. P. and Vogt R. C. (2008) Stomach Flushing Vs. Fecal Analysis: The Example of *Phrynops rufipes* (Testudines: Chelidae). *Copeia* 2: 301-305.

Channing A. (2001) Amphibians of Central and Southern Africa. Cornell University Press, Ithaca. 470.

Crovetto F., Romano A., Salvidio S. (2012) Comparison of two non-lethal methods for dietary studies in terrestrial salamanders. *Wildlife Research* 39: 266–270.

Fitzgerald L. A. (1989) An evaluation of stomach flushing techniques for crocodilians. *Journal of Herpetology* 23: 170-172.

Fraser D. F. (1976) Coexistence of salamanders in the genus *Plethodon*, a variation of the Santa Rosalia theme. *Ecology* 57: 238-251.

Harland R. M. and Grainger R. M. (2011). Xenopus research: metamorphosed by genetics and genomics. *Trends in Genetics* 27 (12) 507-515.

Hirschfeld M. and Rödel M. O. (2011) The diet of the African Tiger Frog, *Hoplobatrachus occipitalis*, in Northern Benin. *Salamandra* 47 (3): 125-132.

IUCN (2014). The IUCN Red List of Threatened Species. Version 2014. https:/www.iucnredlist.org.

Jirku M. and Modry D. (2006) *Eimeria terraepokotorum* n. sp. (Apicomplexa: Eimeriidae) from *Hoplobatrachus occipitalis* (Anura: Ranidae) from Kenya. Acta Protozoology 45: 443 – 447.

Kamler J. F. and Pope K. L. (2001) Nonlethal Methods of Examining Fish Stomach Contents. *Reviews in Fisheries Science* 9(1): 1–11.

Lafferty K. D. and Page C. J. (1997) Predation on the endangered tidewater goby, *Eucyclogobius newberryi*, by the introduced African clawed frog, *Xenopus laevis*, with notes on the frog's parasites. *Copeia* (3): 589-592.

Legler J. and Sullivan L. (1979) The application of stomach flushing to lizards and anurans. *Herpetologica* 35: 107-110.

Legler J. M. (1977) Stomach flushing: a technique for chelonian dietary studies. *Herpetologica* 33: 281–284.

Lobos G. and Jaksic, F. M. (2005) The ongoing invasion of African clawed frogs (*Xenopus laevis*) in Chile: Causes of concern. *Biodiversity and Conservation* 14(2): 429-439.

Mahan R. D. and Johnson J. R. (2007) Diet of the Gray Treefrog (*Hyla versicolor*) in Relation to Foraging Site Location. *Journal of Herpetology*, 41(1): 16–23.

Martin A. P. and Hockey P. A. (1993) The effectiveness of stomach flushing in assessing wader diet. *Wader Study Group Bulletin* 67: 79-80.

Matthews K. R., Knapp R. A. and Pope K. L. (2002) Garter snake distributions in high elevation aquatic ecosystems: is there a link with declining amphibian populations and non-native trout introductions. *Journal of Herpetology* 36: 16–22.

McCoid M. J. and Fritts T. H. (1980) Notes on the diet of a feral population of *Xenopus laevis* (Pipidae) in California. *The Southwestern Naturalist* 25: 272-275.

Measey G. J. (1998a) Diet of feral *Xenopus laevis* (Daudin) in South Wales, U.K. *Journal of Zoology* 246(3): 287-298.

Measey G. J. (1998b) Terrestrial prey capture in *Xenopus laevis*. *Copeia* (3): 787-791.

Mohneke M., Onadeko A. B., Hirschfeld M. and Rodel M. O. (2010) Dried or Fried: Amphibians in Local and Regional Food Markets in West Africa. *TRAFFIC Bulletin* 22: 117-128.

Muhammad N. O.and Ajiboye B. O. (2010) Nutrient composition of *Rana galamensis. African Journal of Food Science and Technology* 1(1): 27-30.

Onadeko A. B. and Rodel M. O. (2009) Anuran surveys in south western Nigeria. *Salamandra* 45: 1-14.

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: 67-76.

Oza G. M. (1990) Ecological effects of the frog’s legs trade. *The Environmentalist* 10: 39- 41.

Pietruszka R. D. (1981): An evaluation of stomach flushing for desert lizard diet analysis. Southwest. *Nature* 26: 101-105.

Powell G. L. and Russell A. P. (1984) The diet of the eastern short-horned lizard (*Phrvnosoma douglassi brevirostre*) in Alberta and its relationship to sexual size dimorphism. *Canadian Journal of Zoology* 62: 428-440.

Rice A. N. (2004) Diet condition of American alligators, *Alligator mississipiensis* in three central Florida lakes. A thesis presented to the Graduate School of the University of Florida in partial fulfilment of the requirement for the degree of Master of Science. 88 pp.

Rödel M. O. and Spieler M. (2000) Trilingual keys to the savannah – Anurans of the Comoé National Park, Ivory Coast. *Stuttgarter Beiträge ZurNaturkunde, Serie A Biologie* 62 (31): 1-31

Rodel M. O., Poynton J. C., Largen M., Howell K. and Lotters S. (2004). *Amnirana galamensis*. 2006 IUCN Red List of Threatened species.

Secor S. M., Faulkner A. C. (2002) Effects of meal size, meal type, body temperature, and body size on the specific dynamic action of the marine toad, *Bufo marinus*. *Physiological and Biochemical Zoology* 75: 557 – 571.

Silva E. T., Evelyze P. D., Renato N. F. and Oswaldo P. R. (2009) Diet of the Invasive Frog *Lithobates catesbeianus* (Shaw, 1802) (Anura: Ranidae) in Viçosa, Minas Gerais State, Brazil. South *American Journal of Herpetology* 4 (3): 286-294.

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.

Tinsley R. C., Loumont C. and Kobel H. R. (1996) Geographical distribution and ecology. Pp. 35-59. In: R. C. Tinsley and H. R. Kobel (editors). The Biology of Xenopus. Clarendon Press for The Zoological Society of London, Oxford. 440 pp.

Toft C. A. (1981) Feeding ecology of Panama litter anurans: Patterns of diet and foraging mode. *Journal of Herpetology* 15(2): 133-144.

Tohé B., Kouamé N. G., Assemian N. E., Gourène G. and Rödel M. O. (2013) Dietary Strategies of the Giant Swamp Frog *Hoplobatrachus occipitalis* in degraded areas of Banco National Park (Ivory Coast). *International Journal of Scientific Research and Reviews* 3(2): 34 – 46.

Vences M., Glaw F. and Zapp C. (1999) Stomach Content Analyses in Malagasy Frogs of the Genera *Tomopterna*, *Aglyptodactylus*, *Boophis*, and *Mantidactylus*. *Herpetozoa* 11(3/4): 109 -116

Walker B. (1967) An elusive frog, *Rana galamensis*. *Nigeria field* 32: 22 – 26.

Wallingford J., Liu K. and Zheng, Y. (2010) *Current Biology* v. 20: 263-264.

Waters D. S., Kwak T. J., Arnott J. B. and Pine W. E. (2004) Evaluation of Stomach Tubes and Gastric Lavage for Sampling Diets from Blue Catfish and Flathead Catfish. *North American Journal of Fisheries Management* 24: 258–261.

Whiles M. R., Lips K. R., Pringle C. M., Kilham S. S., Bixby R. J., Brenes R., Connelly S., Colon-Gaud J. C., Hunte-Brown M. and Huryn A. D. (2006) The effects of amphibian population declines on the structure and function of Neotropical stream ecosystems. *Frontiers in Ecology and the Environment* 4: 27–34.

Wu Z. J, Li Y. M. and Wang Y. P. (2007) A comparison of stomach flush and stomach dissection in diet analysis of four frog species. *Acta Zoologica Sinica* 53: 364–372.