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Trophic Ecology of African Dwarf Crocodiles (*Osteolaemus* spp.) in Perennial and Ephemeral Aquatic Habitats

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Abstract.—**Crocodilians occupy diverse aquatic and riparian habitats, and through their movements and ontogenetic niche shifts, link the flow of energy and nutrients between aquatic and terrestrial food webs. We analyzed the trophic ecology of African dwarf crocodiles *Osteolaemus tetraspis* and *O. osborni* at one site for each species in Cameroon by analyzing carbon and nitrogen stable isotope ratios. We hypothesized that *Osteolaemus* species inhabiting a perennial river would be primarily associated with aquatic food webs, whereas those inhabiting a small ephemeral stream would assimilate significant fractions of terrestrial-derived material into their biomass. We also hypothesized that *Osteolaemus* species would undergo ontogenetic trophic shifts, including changes in vertical trophic position (TP). We found that crocodiles in both systems assimilated material from both terrestrial and aquatic-based food chains, and therefore provide a trophic link between habitats. In the perennial river, aquatic gastropods, fish, and amphibians were estimated to have higher maximum feasible contributions to crocodile biomass than terrestrial invertebrates. Prey contributions to crocodile biomass in the ephemeral stream could not be estimated because model results suggested that at least one important prey had not been sampled. Even though isotopic overlap was high between juveniles and adults, and between sexes, ontogenetic shifts in TP were apparent in both *Osteolaemus* populations.**

Mobile amphibious predators, such as crocodilians, link the flow of nutrients and energy between food webs of heterogeneous aquatic and riparian wetland habitats (Fittkau, 1973; Subalusky et al., 2009; Rosenblatt and Heithaus, 2011). Crocodilians inhabit diverse wetland and fluvial ecosystems, and therefore the degree to which they transport nutrients and energy may differ accordingly (Quevedo et al., 2009; Matich et al., 2011; Schriever and Williams, 2013). For example, species inhabiting perennial rivers are generally associated with riverine food webs, whereas species inhabiting small ephemeral streams and pools may be more strongly associated with either terrestrial or aquatic food webs (Jackson et al., 1974; Magnusson et al., 1987; Magnusson and Lima, 1991; Wallace and Leslie, 2008).

Ontogenetic niche shifts can increase food web complexity because life stages of species might occupy distinct trophic positions (TPs) (Polis, 1984; Polis et al., 1989; Post, 2003; Baxter et al., 2005). Most crocodilians undergo ontogenetic shifts in habitat and diet (Magnusson et al., 1987; Fitzgerald, 1988; Tucker et al., 1996; Subalusky et al., 2009). As they grow and mature, crocodilians typically transition from nearshore or smaller aquatic habitats to deeper and larger aquatic habitats. During such a transition, significant movements and foraging may occur in terrestrial environments. Smaller forest-dwelling species, such as African dwarf crocodiles and dwarf caimans, are thought to occupy forests to a greater extent than other crocodilian species. Diets of these forest-dwelling species are initially dominated by arthropods and shift to include larger vertebrate prey as they age (Cott, 1961; Fitzgerald, 1988; Tucker et al., 1996; Wallace and Leslie, 2008). Ontogenetic niche shifts have not been described for the poorly studied species of African dwarf crocodiles in the genus *Osteolaemus* (*O. tetraspis* and *O. osborni*). Total length of these dwarf crocodiles only increases by two orders of magnitude from hatchling to adult; by comparison, many crocodilians increase by four to five orders of magnitude.

Using analysis of stable isotopes of carbon and nitrogen, we investigated spatial and ontogenetic variation in the trophic ecology of African dwarf crocodiles *O. tetraspis* and *O. osborni*. These species occur in a variety of aquatic habitats in rain forests of West and Central Africa (Kofron, 1992; Thorbjarnarson and Eaton, 2004; Shirley et al., 2009; Smolensky, 2014). Analyses of stomach contents of *Osteolaemus* species reveal a generalized diet of terrestrial, amphibious, and aquatic vertebrate and invertebrate fauna (Schmidt, 1923; Luiselli et al., 1999; Riley and Huchzermeyer, 2000; Pauwels et al., 2007). Insects, crabs, and other aquatic and terrestrial arthropods were the predominant prey, but their proportional dietary contributions varied by location. Stable isotope analysis complements analysis of stomach contents and has been used to make inferences about crocodilian trophic ecology (Rosenblatt and Heithaus, 2011; Wheatley et al., 2011; Radloff et al., 2012; Caut, 2013).

We compared the trophic ecology of two species of African dwarf crocodiles inhabiting a large river (*O. osborni*) and small ephemeral stream (*O. tetraspis*) in Cameroon through analyses of stable isotope ratios of carbon and nitrogen in keratin and collagen tissues. We hypothesized that *O. osborni* from the Dja River consistently feed on prey from aquatic habitats. In contrast, *O. tetraspis* from an ephemeral stream should undergo stronger seasonal dietary shifts, potentially consuming more terrestrial prey during the dry season. We further hypothesized that these crocodiles undergo ontogenetic dietary shifts, including an increase in vertical TP with increase in body size.

Materials and Methods

Study Regions.—We sampled crocodiles and common elements of local aquatic and terrestrial riparian food webs at two sites located in lowland Congo-Guinean rain forest of Cameroon (Fig. 1). The first site was the Dja Rivera right-bank tributary of the Congo River, (2°05′–3°25′N, 12°43′–14°50′E, WGS84). The hydro-

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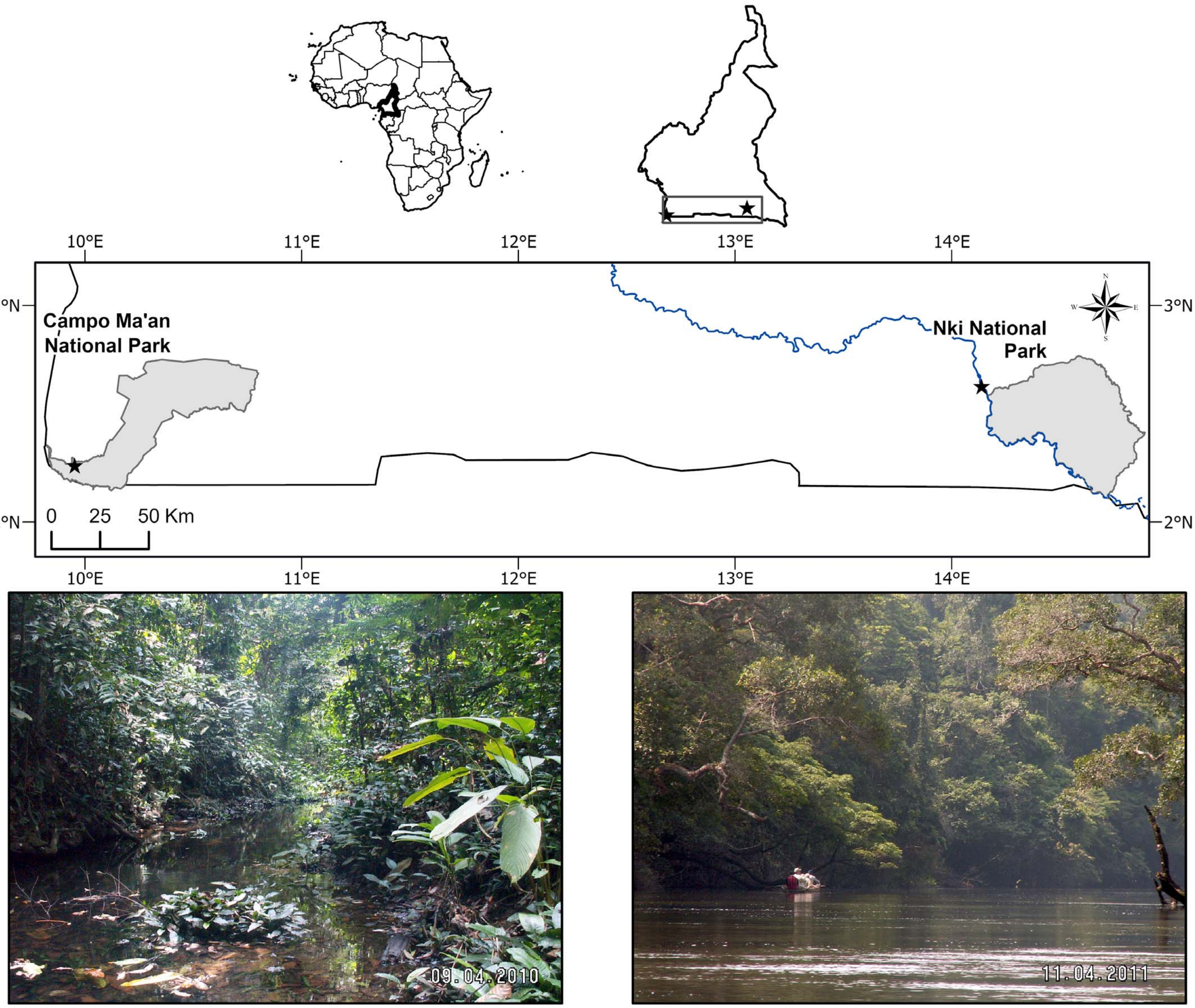

FIG. 1. Map showing locations and photos of the aquatic habitats of the Campo Ma’an and Dja River sites in Cameroon.

logic regime is seasonal and bimodal, with peak discharges in October and July (451–487 m^3/sec) (Seyler et al., 1993). The river is slightly acidic (6.39–6.89 pH), with low conductivity (48 μS/cm) and low suspended sediment levels (26.0 mg/L), but high particulate (2.3 mg/L) and dissolved (6.0 mg/L) organic carbon levels.

The second site was a first-order stream within the Ntem River basin inside the Campo Ma’an National Park between latitudes 2°10′–2°52′N and longitudes 9°50′–10°54′E. Compared with the Dja River, the Ntem has lower discharge with less seasonal variation (290 m^3/sec) and lower acidity (5–6 pH) and conductivity (20–30 μS/cm). Streams of the Ntem Basin are clear, with low dissolved nutrient concentrations, dense canopy cover, and large inputs of allochthonous organic material (Toham and Teugels, 1997, 1998; Brummett and Teugels, 2004). The substrate consists of leaf litter, woody debris, and fine organic particulate matter over sand and gravel.

Both study sites are at low elevations with landscapes dominated by humid evergreen forest. The climate is similar at the two sites; mean annual precipitation is 1,700 mm/yr and mean annual temperature is 25°C. In both regions, there are two dry seasons from late November to late February and from late June to early August. We obtained samples during the dry season of 2011–2012. We sampled at the Dja River site during November and December 2011, and at the Campo Ma’an site during February 2012.

Sample Collection.—At each location we obtained two to five replicate samples of basal production sources, including grasses, leaves, mosses, aquatic macrophytes, course particulate organic matter, detritus, and tissues from dwarf crocodiles and their potential prey—macroinvertebrates, amphibians, and fishes (Schmidt, 1923; Riley and Huchzermeyer, 2000; Pauwels et al., 2007; Eaton, 2010; Table 1). A minimum of two samples was obtained for items that were difficult to find at the sites. We captured potential prey using a seine, dip nets, or by hand, and captured dwarf crocodiles by lassoing (Vliet, 2007; Fitzgerald, 2012). We took samples of muscle tissue from the dorsolateral region from some of the fishes that were sampled at our study sites for a separate ichthyological survey of the Dja River and surrounding streams. Fishes were euthanized in MS-222. Macroinvertebrates and fish tissues were preserved whole in salt; specimens were crushed within plastic bags containing salt

TABLE 1. Descriptions of samples collected for isotope analyses from two study sites in Cameroon. Hemipterans, two frog species, and six species of fishes plus the shrimp had indistinguishable isotopic ratios and were labeled as aqI for aquatic invertebrates, Am for amphibians, and F for fishes, respectively, for plotting the values in figures.

Site	Taxon or sample name	Abbreviation	*N*
Dja River	Detritus	Dtrt	2
Dja River	Broadleaf tree sp. A	BrdA	3
Dja River	Broadleaf tree sp. B	BrdB	3
Dja River	Broadleaf tree sp. C	BrdC	3
Dja River/Campo Ma’an	Moss	Mss	3
Dja River	Grass	Grss	1
Dja River	Lepidoptera (hairy caterpillar)	Lpd	1
Dja River	Orthoptera (Gryllidae cricket)	Ort	1
Dja River	Hemiptera (Naucoridae water bug)	Ncd (AqI)	5
Dja River	Hemiptera (Nepidae water scorpion)	Npd (AqI)	4
Dja River	Odonata (dragonfly larvae)	Odt	1
Dja River	Hymenoptera (Formicidae ant)	Frm	3
Dja River	*O. osborni*_keratin	OstK	3
Dja River	*O. osborni*_collagen	OstC	3
Dja River/Campo Ma’an	Gastropoda (aquatic snail)	AqGstp	5/1
Dja River	*Hylarana* sp. (frog)	Hlr (Am)	1
Dja River	*Conraura crassipes* (frog)	Cnr (Am)	1
Dja River	Atyidae (shrimp)	Aty (F)	2
Dja River	*Hemichromis elongates* (fish)	Hm (F)	4
Dja River/Campo Ma’an	*Hepsetus odoe* (fish)	Hod (F)	1/1
Dja River	*Tilapia* sp. (fish)	Tlp (F)	3
Dja River/Campo Ma’an	*Clarias* sp. (fish)	Clr (F)	3/1
Dja River	*Ctenopoma* sp. (fish)	Ctn (F)	2
Dja River	*Micralestes* sp. (fish)	Mcrl (F)	7
Campo Ma’an	Emergent macrophyte	Fcwt	1
Campo Ma’an	Course particulate organic matter	CPOM	1
Campo Ma’an	*Achatina* sp. (terrestrial snail)	Ach	1
Campo Ma’an	*Macrobrachium vollenhovenii* (shrimp)	Mvol	2
Campo Ma’an	*Potamonemus* sp. (crab)	Pot	1
Campo Ma’an	*Grayia ornate* (snake)	Gry	1
Campo Ma’an	*Scotobleps gabonicus* (frog)	Sct	1
Campo Ma’an	*Labeo* sp. (fish)	Lab	2
Campo Ma’an	*Epiplatys sexfasciatus* (fish)	Esp	1
Campo Ma’an	*Brycinus* sp. (fish)	Bry	2
Campo Ma’an	*O. tetraspis*_keratin	OstK	27
Campo Ma’an	*O. tetraspis*_collagen	OstC	29

to allow penetration of dissolved ions. Caudal scutes were removed from live *Osteolaemus* species, and morphometric measurements were taken in the field before release of animals. We removed scutes with a scalpel or surgical scissors and stored the tissue in small vials containing ethylenediaminetetraacetic acid/dimethyl sulfoxide solution. The scalpel or scissors was sanitized before and after each procedure. Wounds were cleaned with ethanol, and a topical antibiotic was applied. For each captured crocodile we recorded head and body measurements. Head measurements included snout length and width, cranial platform width, and distance between the eyes. Body measurements included snout–vent length (SVL), total length, and weight. No crocodiles were euthanized during this study. All tissue samples except for those from *Osteolaemus* species were preserved in salt and stored in plastic bags for subsequent processing. *Osteolaemus* tissue samples were preserved in 95% ethanol for use in a concurrent genetic study (Smolensky et al., 2015). These preservation methods have been shown to have negligible effect on δ^{13}C and δ^{15}N isotope ratios of fish and bird muscle tissue for trophic studies (Hobson et al., 1997; Arrington and Winemiller, 2002; Edwards et al., 2002).

Samples were rinsed and soaked with deionized water for 24 h to remove salt and then dried at 60°C for 48 h. Caudal scutes of *Osteolaemus* species have an outer keratinous epidermis and a dermal collagen core that may yield isotope signatures different from muscle tissue (Hobson and Clark, 1992a,b; Tieszen et al., 1983). Scute samples were first soaked in a 0.1 M NaOH solution for 12 h, rinsed with deionized water, and then manually separated into keratin and collagen before drying (Radloff et al., 2012). Collagen samples were placed in glass vials, and keratin samples and any remaining material were ground to a homogenous fine powder using a ball-mill grinder. Subsamples were placed in Ultra-Pure tin capsules (Costech Analytical Technologies). Samples were sent to the Analytical Chemistry Laboratory, University of Georgia for mass spectrometry analysis of stable isotope ratios (δ^{13}C and δ^{15}N).

Statistical Analyses.—We obtained dual isotope (δ^{13}C, δ^{15}N) mixing models of prey sources using the program IsoSource, which calculates proportional contributions of sources to the sample mixtures (Phillips and Gregg, 2003). We ran separate mixing model analyses for each site. Biplots of δ^{13}C and δ^{15}N indicated that certain taxa (e.g., fish, amphibians) had similar isotopic ratios (Fig. 2) and subsequently, we averaged these to reduce the number of alternative sources used in the analyses. Eight and seven potential sources were included for the Dja River and Campo Ma’an sites, respectively. We corrected isotope values for trophic fraction using values $\Delta\delta C = 0.61$‰ and $\Delta\delta N = 1.22$‰, on the basis of an experimental study of isotopic discrimination and tissue turnover in American Alligators (*Alligator mississippiensis*; Rosenblatt and Heithaus, 2013). We set mass balance tolerance levels to ± 0.1‰ and ± 0.4‰ for the Dja River and Campo Ma’an sites, respectively. We allowed larger tolerances for

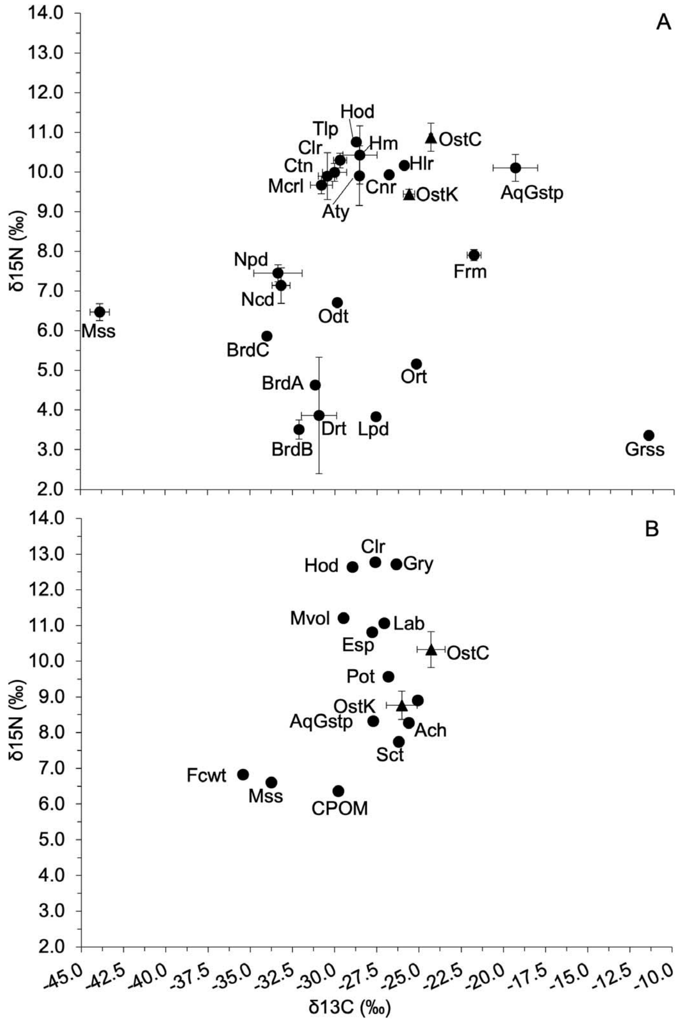

FIG. 2. Biplot of stable isotope ratios of basal production sources, dwarf crocodiles (*Osteolaemus osborni*, *O. tetraspis*) (triangles), and their potential prey (circles) from the Dja River (A) and creek at Campo Ma'an (B). Abbreviations of samples are listed in Table 1. Values were not adjusted for trophic fractionation. Points are means; error bars are standard deviations.

Campo Ma'an samples to minimize exclusion of potential sources and to accommodate the greater source variability at this site (Fig. 2B) (Phillips and Gregg, 2003). We iteratively adjusted the source proportions by 1% increments to generate IsoSource model outputs representing feasible solutions (Phillips and Gregg, 2003). We used these solutions to create histograms and determine minimum and maximum percentile contributions of each potential prey source (Figs. 3–4). Visualizations were created in Microsoft Excel and Inkscape.

We examined our data for ontogenetic isotopic shifts for *O. tetraspis* at the Campo Ma'an site, as we considered the sample of 24 individuals large enough to reveal ontogenetic shifts. We used Kruskal-Wallis tests to determine if there were differences in isotope signatures among males, females, and juveniles for both tissue types. We used multivariate adaptive regression analyses (MARS; Friedman, 1991) to investigate ontogenetic diet shifts on the basis of isotope signatures (δ^{13}C and δ^{15}N) and SVL for each tissue type. The MARS analysis was conducted in Program R (R Development Core Team, 2011). One outlier for the nitrogen isotopic ratio of collagen clustered with keratin samples, and therefore was excluded from this analysis. We estimated crocodile TPs using the following equation (Post, 2002):

$$\mathrm{TP} = \lambda + (\delta^{15}\mathrm{N}_{\mathrm{secondary\ consumer}} - \delta^{15}\mathrm{N}_{\mathrm{base}})/\mathrm{TEF},$$

where TP is trophic position, λ and $\delta^{15}\mathrm{N}_{\mathrm{base}}$ are the TP and δ^{15}N the isotope signature of the organism used as the baseline. We used Orthoptera (cricket, Gryllidae) and larval *Scotobleps gabonicus* (Gaboon Forest Frog tadpoles) from the Campo Ma'an site as the baseline organisms for trophic level 2 (primary consumer) because the crickets are primarily herbivorous and the tadpoles are herbivorous (Fig. 2). The $\delta^{15}\mathrm{N}_{\mathrm{secondary\ consumer}}$ term is the isotope signature of the dwarf crocodile specimen's tissue, and TEF is the trophic enrichment factor (1.22) for crocodilians (Rosenblatt and Heithaus, 2013). We tested for a statistically distinguishable difference in TPs between juveniles and adults at Campo Ma'an with a Mann-Whitney *U*-test. Statistical analyses were conducted in Program R (R Development Core Team, 2011).

RESULTS

Food Web Structure.—We sampled *O. tetraspis* at Campo Ma'an stream (12 males, 7 females, and 3 juveniles, 2 of which were of unknown sex) and *O. osborni* at the Dja River (2 adult females and a juvenile male). *Osteolaemus osborni* abundance was low at our site on the Dja River and they were difficult to catch. Both species had carbon isotope signatures indicating assimilation of material derived from terrestrial as well as aquatic sources (Fig. 2). However, the proportional contributions of aquatic and terrestrial sources differed between the sites. At the Dja site, two trophic pathways likely supported biomass of *O. osborni*—an aquatic pathway containing fishes supported by food chains originating from broadleaf trees and detritus from the riparian zone and a terrestrial pathway involving terrestrial insects supported by basal sources intermediate between detritus and C4 grasses in the riparian zone (Fig. 2A). At Campo Ma'an, *O. tetraspis* biomass appeared to be supported by an aquatic pathway involving fishes supported by detritus from basal sources in the riparian zone (Fig. 2B). Consumer taxa from the ephemeral stream at Campo Ma'an had heavier nitrogen isotopic ratios compared with consumers from the Dja River. For example, the shrimp *Macrobrachium vollenhovenii* and fish *Labeo batesii* from the stream at Campo Ma'an are both omnivores that consume algae (Toham and Teugels, 1998; Jimoh et al., 2011), and their δ^{15}N values were higher than those of predatory naucorid and nepid hemipteran insects from the Dja River (Fig. 2). *Hepsetus odoe*, a predatory fish, occurred at both sites where it had similar δ^{13}C values, but δ^{15}N values were 1.89‰ higher for specimens from Campo Ma'an.

Osteolaemus species from the two sites had similar isotopic values for both tissue types. The *Osteolaemus* species do not appear to be apex predators, because their δ^{15}N values were significantly lower than those of predatory fishes and snakes (Fig. 2). Mean TP was higher for crocodiles from the Dja River (keratin = 5.51, SD = 0.09; collagen = 6.73, SD = 0.28, n = 3) compared with those from Campo Ma'an (keratin = 3.86, SD = 0.34; collagen = 5.11, SD = 0.47, n = 24). Samples from both sites contained adults and juveniles.

Proportional Contributions of Alternative Prey to Crocodile Biomass.—At both sites the two tissue types resulted in different estimates for relative contributions of alternative sources to crocodile biomass (Table 2). Model solutions predicted that the

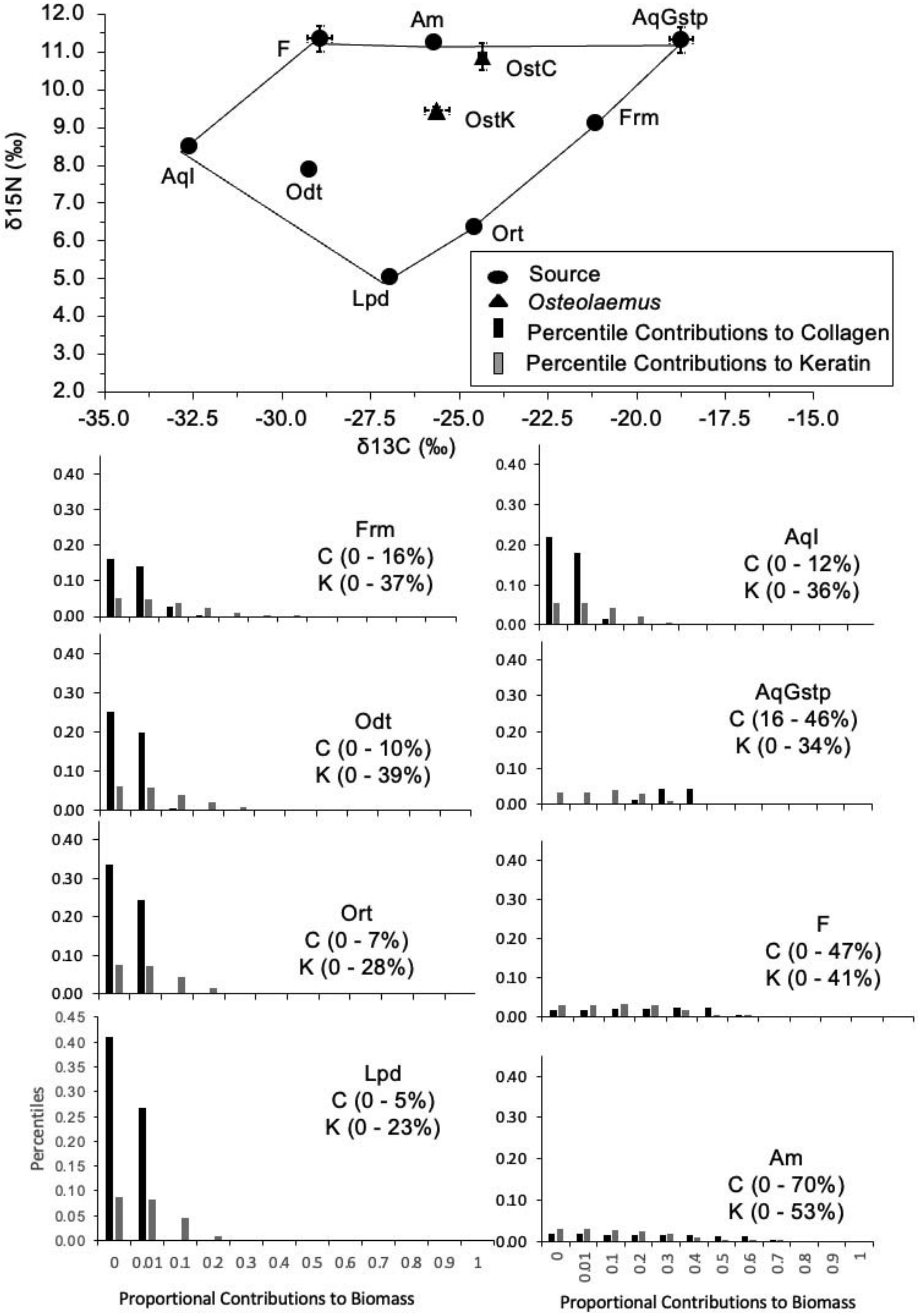

FIG. 3. Polygons and histograms of proportional contributions of sources to *Osteolaemus osborni* ($n = 3$) isotope signatures of collagen (OstC) and keratin (OstK) at the Dja River site. The convex hull area connects sources delineating the mixing space for dwarf crocodile diet. Values of potential prey sources were adjusted for trophic fractionation. Histograms show the mean percentile of feasible proportional contributions of sources, with ranges of values in parentheses. Abbreviations for sources are listed in Table 1.

potential contribution to Dja River *O. osborni* biomass was less than 10% for the potential prey categories that were surveyed and included in the analysis (Figs. 3–4). Aquatic gastropods, fishes, and amphibians had higher maximum feasible contributions to biomass of *O. osborni* than terrestrial invertebrates, particularly when estimates were based on collagen tissue. However, the wide distribution of estimated proportional contributions (e.g., range for fish = 0–47%; Table 2) reduced resolution for estimates of prey contributions to *O. osborni* biomass. At Campo Ma'an, *O. tetraspis* isotopic ratios plotted outside the mixing model polygon, indicating consumption of one or more important sources that were not collected from the study site (Fig. 4). Even considering the broad confidence intervals for model estimates, the amphibian (Sct = 67–94%) and fish (Bry = 71–95%) sources are inferred to be the major contributors to *O. tetraspis* biomass at Campo Ma'an.

Ontogenetic Niche Shifts.—Relationships between *O. tetraspis* SVL and isotopic ratios for both elements and tissue types were

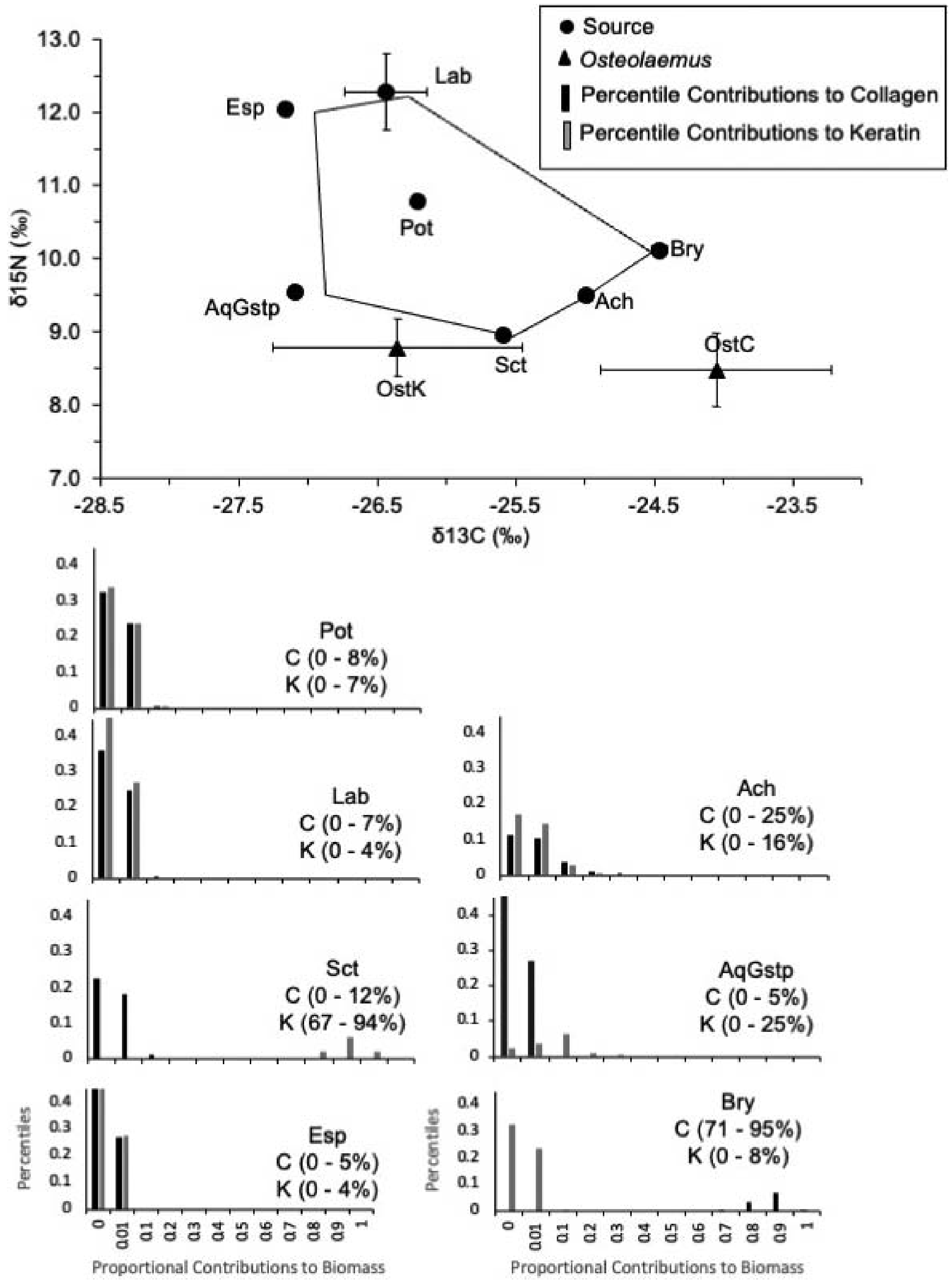

FIG. 4. Polygons and histograms of proportional contributions of sources to *Osteolaemus tetraspis* ($n = 24$) isotope signatures of collagen (OstC) and keratin (OstK) at Campo Ma'an. The convex hull area connects sources delineating the mixing space for dwarf crocodile diet. Values of potential prey sources were adjusted for trophic fractionation. Histograms show the mean percentile of feasible proportional contributions of sources, with ranges of values in parentheses. Abbreviations for sources are listed in Table 1.

statistically significant. Collagen was enriched in ^{15}N and ^{13}C relative to keratin, and values for both tissue types had narrow ranges across size classes (^{15}N enrichment = 1.33‰, keratin minimum = 8.81, maximum = 9.51; ^{13}C enrichment = 2.23‰, keratin minimum = −27.23, maximum = −25.00; Fig. 5). Nitrogen and carbon isotope signatures initially declined with body size across small juvenile size classes and subsequently increased in larger juveniles and adults (Fig. 5). This isotopic shift occurred between 46.5 and 51 cm SVL, near the transition between juvenile and adult stages (Table 2). Less notable shifts occurred at 38 cm and 58.5 cm SVL for certain elements and tissue types (Table 2). There were no differences in carbon and nitrogen isotope signatures for keratin and collagen between sexes ($\delta^{13}C_{\mathrm{keratin}}$: $H = 4.96$, $P = 0.19$; $\delta^{13}C_{\mathrm{collagen}}$ $H = 6.50$, $P = 0.10$; $\delta^{15}N_{\mathrm{keratin}}$ $H = 5.54$, $P = 0.15$; $\delta^{15}N_{\mathrm{collagen}}$ $H = 0.79$, $P = 0.86$). Estimated TPs of juveniles and adults did not differ significantly ($U_{\mathrm{keratin}} = 84.5$, $P = 0.22$; $U_{\mathrm{collagen}} = 33.5$, $P = 0.27$).

DISCUSSION

In the Dja River, dwarf crocodiles apparently consumed prey associated with aquatic food chains (e.g., aquatic gastropods) as well as prey associated with terrestrial (e.g., insects, frogs) food chains, with both kinds of pathways originating with organic matter largely derived from riparian vegetation. For example, aquatic gastropods had relatively high values for both δ^{13}C and

TABLE 2. Results from multivariate adaptive regression splines of the relationship between snout–vent length (SVL, cm) and isotopic ratios of caudal scutes from 24 *Osteolaemus tetraspis*. Analyses were done separately for keratin and collagen tissues.

Isotope and tissue type	Model	Basis function (BF)	Knot	R^2	F	P
δ^{15}N Keratin	$Y = 8.74 + 0.04 \times \text{BF2} - 0.35 \times \text{BF3} + 0.38 \times \text{BF5}$	BF2 = max(0, 51.00 − SVL)	51.00	0.44	3.72	0.04
		BF3 = max(0, SVL − 46.50)	46.50			
		BF5 = max(0, SVL − 47.62)	47.62			
δ^{15}N Collagen	$Y = 8.66 + 0.06 \times \text{BF1} + 0.01 \times \text{BF2} - 0.06 \times \text{BF3} + 0.01 \times \text{BF5}$	BF1 = max(0, SVL − 50.50)	50.50	0.48	3.34	0.06
		BF2 = max(0, 50.50 − SVL)	50.50			
		BF3 = max(0, SVL − 46.55)	46.55			
δ^{13}C Keratin	$Y = -25.86 - 0.03 \times \text{BF1} + 0.09 \times \text{BF2} - 0.08 \times \text{BF4}$	BF1 = max(0, SVL − 21.50	21.50	0.48	5.31	0.01
		BF2 = max(0, SVL − 38.00)	38.00			
		BF4 = max(0, SVL − 58.50)	58.50			
δ^{13}C Collagen	$Y = -24.31 + 0.01 \times \text{BF2} - 0.03 \times \text{BF3} + 0.06 \times \text{BF5}$	BF2 = max(0, 46.05 − SVL)	46.50	0.44	3.52	0.05
		BF3 = max(0, SVL − 51.00)	51.00			
		BF5 = max(0, SVL − 47.45)	47.45			

δ^{15}N, indicating likely assimilation of material originating from terrestrial plants. Less apparent were the production sources contributing to biomass of *O. tetraspis* inhabiting the ephemeral stream at Campo Ma'an. *Osteolaemus tetraspis* samples fell outside the polygon defined by potential sources, which implies assimilation of material from one or more sources that were missing from our data set (Phillips and Gregg, 2003).

The wide ranges of feasible contributions of potential prey produced by IsoSource models suggest three potential explanations. Dwarf crocodiles probably have broad diets, which would be consistent with observations of opportunistic foraging by crocodilians globally. Alternatively, our analysis might have excluded one or more important food resources. Finally, the lack of isotopic differences among sources limited the mixing model's ability to discriminate among sources to estimate their proportional contributions to consumer biomass. All these possibilities are plausible and not mutually exclusive. Sampling dwarf crocodiles and their prey at more sites could help disentangle the influence of opportunistic foraging habits of *Osteolaemus* and the contributions of potential prey sources. *Osteolaemus osborni* from Gabon and Democratic Republic of Congo were reported to consume millipedes and insects in swamp habitats, and to consume crabs and gastropods in creeks (Luiselli et al., 1999; Riley and Huchzermeyer, 2000; Pauwels et al., 2007). *Osteolaemus* species at both of our study sites probably have broad diets that include prey biomass derived from aquatic and terrestrial primary production, with *O. osborni* from the Dja River more strongly linked to the aquatic food web. In both study systems, arthropods appear to be important in the diet of *Osteolaemus* adults as well as juveniles. *Osteolaemus* were feeding across a range of trophic levels, which indicates the larger body sizes of these crocodiles continued to consume arthropod prey even as they incorporated vertebrate prey. A similar pattern was documented in a study of diet and ontogenetic niche shifts in *Caiman crocodilus* (Fitzgerald, 1988).

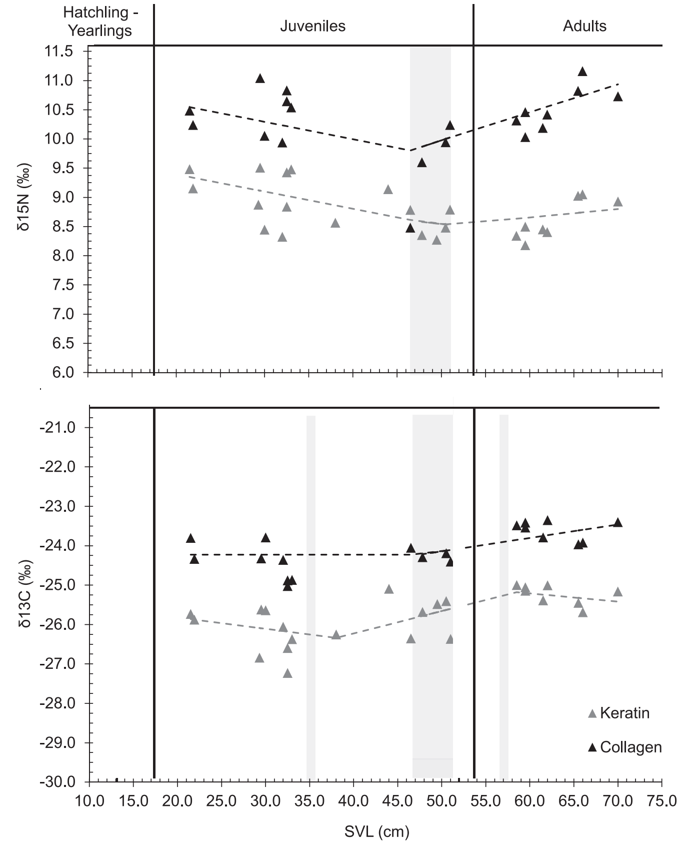

FIG. 5. Relationship between body size and δ^{15}N and δ^{13}C from keratin and collagen tissues of *Osteolaemus tetraspis* at Campo Ma'an National Park. Broken lines are multivariate adaptive regression splines (MARS). Gray bars indicate break points in the data from MARS on the basis of both tissue types.

Hydrology, sediment loads, canopy cover, and other environmental factors influence proportional contributions of autochthonous and allochthonous sources of primary production to aquatic food webs (Jepsen and Winemiller, 2007; Lau et al., 2009; Roach and Winemiller, 2015). The Dja River and streams of Campo Ma'an are characterized by low nutrient concentrations, low suspended sediment loads, and dense forest canopy cover (Seyler et al., 1993; Toham and Teugels, 1998; Brummett and Teugels, 2004; Coynel et al., 2005). The small stream at Campo Ma'an also has ephemeral flows with much lower discharge than the Dja River. Allochthonous production sources (riparian trees) likely supported a significant fraction of the biomass of aquatic macroinvertebrates, fish, amphibians, and *O. tetraspis*. We were unable to obtain a sufficient quantity of periphyton to include in our analyses to estimate contribution of autochthonous production to food webs. The lack of visible periphyton on substrates in these dimly lit streams, together with previously cited literature, suggest that aquatic primary production was extremely low in both locations.

We identified an ontogenetic niche shift in one *Osteolaemus* species (*O. tetraspis*; we had insufficient sampling of *O. osborni*) consistent with those reported for other crocodilians (Cott, 1961;

Fitzgerald, 1988; Platt et al., 2006; Subalusky et al., 2009). Isotope signatures of small juveniles were lower than those of larger juvenile and adult *O. tetraspis*. Nonetheless, overlap of isotopic signatures of juveniles and adults suggests broad dietary overlap between life stages, a pattern observed in other crocodilian species (Cott, 1961; Radloff et al., 2012; Webb et al., 1991). Although crocodilians consume progressively larger prey as they grow, most continue to include invertebrates and small prey in their diets throughout life (Fitzgerald, 1988). Additionally, invertebrates and other small prey often are present within the alimentary canal of fish consumed by crocodilians. The initial low values and subsequent increase in isotopic signatures with body size that we observed could be associated with dietary differences between size classes, or physiological changes associated with growth that affect isotopic incorporation and routing (Reich et al., 2008; Rio et al., 2009; Rio and Carleton, 2012).

Stable isotope analysis is useful for estimating temporal variation of assimilation of foods, but resolution depends on isotopic differences among potential sources, rates of tissue turnover, trophic fractionation, and other factors (DeNiro and Epstein, 1978, 1981; Tieszen et al., 1983; Hobson and Clark, 1992a). A challenge for estimating relative contributions of food types in a generalist predator is to collect representative samples from the full spectrum of available prey categories. Our study was limited by time constraints and funding for sampling at many sites. We compared a sample of each species in different ecological settings, relatively small streams in the Ntem River basin and the relatively large Dja River. We could not control for differences between the two sites in terms of prey types and prey availability between sites. Further research with replicated samples in multiple ecological settings would help refine our understanding of similarities and differences in trophic ecology among populations of *Osteolaemus* species. More extensive data sets that include additional alternative prey categories from both aquatic and terrestrial environments at multiple sites could improve IsoSource estimates if there is sufficient isotopic discrimination among sources.

Given the typically broad diets of crocodilians, resolution probably will remain low for estimates of prey assimilated by various size classes of dwarf crocodiles. Analysis of gut contents of dwarf crocodiles would provide much greater resolution of diet composition and inform collection of prey for future isotopic studies. However, gut contents analysis also has limitations, including differential digestion efficiency for prey types that can bias estimates of dietary importance, and the fact that gut contents only reveal a brief snapshot of the organism's feeding history (Garnett, 1985; Jackson et al., 1974). Often, many stomachs are empty, so that large sample sizes are needed to reveal dietary variation (Fitzgerald, 1989; Wallace and Leslie, 2008). Stable isotope analysis is useful for estimating temporal variation of assimilation of foods, but resolution depends on the isotopic differences of food types and among potential sources, rates of tissue turnover, trophic fractionation, and other factors (DeNiro and Epstein, 1978, 1981; Tieszen et al., 1983; Hobson and Clark, 1992a). Pairing of analyses of gut contents and isotopes would provide greater insights into the trophic ecology of crocodilians.

Ours is one of a few studies of isotopes from crocodilians to report isotope signatures from both collagen and keratin (Adame et al., 2018; Woodborne et al., 2021). Although caudal scutes are commonly used for isotopic analyses of crocodilians, scutes have rarely been separated into keratin and collagen fractions (Wheatley et al., 2011; Radloff et al., 2012; Caut, 2013; Smolensky et al., 2015). In contrast to Adame et al. (2018), who found no difference, we found that collagen was more ^{15}N and ^{13}C enriched than keratin in the ontogenetic series of *O. tetraspis*. Variation in tissue turnover rate (half-life) and trophic isotopic fractionation influence patterns of isotopic variation and inferences about trophic ecology (Tieszen et al., 1983; Kelly, 2000; Dalerum and Angerbjörn, 2005). Previous studies indicated that keratin has a faster turnover rate than collagen and therefore should reflect material assimilated over the previous few months, whereas collagen could reflect the diet over as much as a year (Spearman, 1966; Radloff et al., 2012; Rosenblatt and Heithaus, 2013). Indeed, Woodborne et al. (2021) analyzed collagen and keratin in detail in *Crocodilus niloticus* and concluded that diet-to-tissue determinations were dependent on the type of tissue analyzed.

In conclusion, our isotopic analysis of *Osteolaemus* tissues indicated relatively small trophic niche differences among size classes within populations and between species inhabiting different environmental conditions. For crocodilians and other animals with broad diets, inclusion of additional elements, such as hydrogen or sulfur, could further improve discrimination of food resources and assimilation estimates. An important question for future research is the influence of hydrologic regime on contributions of terrestrial and aquatic production sources and alternative prey to biomass of African dwarf crocodiles.

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Literature Cited

Adame, M. F., T. D. Jardine, B. Fry, D. Valdez, G. Lindner, J. Nadji, and S. E. Bunn. 2018. Estuarine crocodiles in a tropical coastal floodplain obtain nutrition from terrestrial prey. PLoS One 13:e0197159.

Arrington, D. A., and K. O. Winemiller. 2002. Preservation effects on stable isotope analysis of fish muscle. Transactions of the American Fisheries Society 131:337–342.

Baxter, C. V., K. D. Fausch, and W. C. Saunders. 2005. Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. Freshwater Biology 50:201–220.

Brummett, R., and G. Teugels. 2004. Rivers of the Lower Guinean rainforest: biogeography and sustainable exploitation. Proceedings of the Second International Symposium on the Management of Large Rivers for Fisheries, pp. 149–172.

Caut, S. 2013. Isotope incorporation in broad-snouted caimans (crocodilians). Biology Open 2:629–634.

Cott, B. H. 1961. Scientific results of an inquiry into the ecology and economic status of the Nile crocodile (*Crocodilus niloticus*) in Uganda and Northern Rhodesia. Transactions of the Zoological Society of London 29:211–356.

Coynel, A., P. Seyler, H. Etcheber, M. Meybeck, and D. Orange. 2005. Spatial and seasonal dynamics of total suspended sediment and organic carbon species in the Congo River. Global Biogeochemical Cycles 19:1–17.

Dalerum, F., and A. Angerbjörn. 2005. Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia 144:647–658.

DeNiro, M. J., and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42: 495–506.

DeNiro, M. J., and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta 45:341–351.

Eaton, M. J. 2010. Dwarf Crocodile *Osteoalemus tetraspis*. Pp. 127–132 in S. C. Manolis and C. Stevenson (eds.), Crocodiles. Status Survey and Conservation Action Plan. Crocodile Specialist Group, Australia.

Edwards, M. S., T. F. Turner, and Z. D. Sharp. 2002. Short- and long-term effects of fixation and preservation on stable isotope values of fluid-preserved museum specimens. Copeia 2002:1106–1112.

Fittkau, E. J. 1973. Crocodiles and the nutrient metabolism of Amazonian waters. Amazoniana 4:103–133.

Fitzgerald, L. A. 1988. Dietary patterns of *Caiman crocodilus* in the Venezuelan llanos. Ph.D. diss., University of New Mexico, USA.

Fitzgerald, L. A. 1989. An evaluation of stomach flushing techniques for crocodilians. Journal of Herpetology 23:170–172.

Fitzgerald, L. A. 2012. Finding and capturing Reptiles. Pp. 77–88 in R. W. McDiarmid, M. S. Foster, C. Guyer, J. W. Gibbons, and N. Chernoff (eds.), Measuring and Monitoring Biological Diversity: Standard Methods for Reptiles. University of California Press, USA.

Friedman, J. H. 1991. Multivariate adaptive regression splines. Annals of Statistics 19:1–67.

Garnett, S. 1985. The consequences of slow chitin digestion on crocodilian diet analyses. Journal of Herpetology 19:303–304.

Hobson, K. A., and R. G. Clark. 1992a. Assessing avian diets using stable isotopes I: turnover of ^{13}C in tissues. Condor 94:181–188.

Hobson, K. A., and R. G. Clark. 1992b. Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. Condor 94:189–197.

Hobson, K. A., M. L. Gloutney, and H. L. Gibbs. 1997. Preservation of blood and tissue samples for stable-carbon and stable-nitrogen isotope analysis. Canadian Journal of Zoology 75:1720–1723.

Jackson, J. F., H. W. Campbell, and K. E. Campbell. 1974. The feeding habits of crocodilians: validity of the evidence from stomach contents. Journal of Herpetology 8:378–381.

Jepsen, D. B., and K. O. Winemiller. 2007. Basin geochemistry and isotopic ratios of fishes and basal production sources in four neotropical rivers. Ecology of Freshwater Fish 16:267–281.

Jimoh, A. A., E. O. Clarke, O. O. Whenu, and H. B. Adeoye. 2011. Food and feeding habits of the African river prawn (*Macrobrachium vollenhovenii*, Herklots, 1857) in Epe Lagoon, southwest Nigeria. International Journal of Fisheries and Aquaculture 3:10–15.

Kelly, J. F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology 78:1–27.

Kofron, C. P. 1992. Status and habitats of the three African crocodiles in Liberia. Journal of Tropical Ecology 8:265–273.

Lau, D. C., K. M. Leung, And D. Dudgeon. 2009. What does stable isotope analysis reveal about trophic relationships and the relative importance of allochthonous and autochthonous resources in tropical streams? A synthetic study from Hong Kong. Freshwater Biology 54:127–141.

Luiselli, L., G. C. Akani, And D. Capizzi. 1999. Is there any interspecific competition between dwarf crocodiles (*Osteolaemus tetraspis*) and Nile monitors (*Varanus niloticus ornatus*) in the swamps of central Africa? A study from southeastern Nigeria. Journal of Zoology 247: 127–131.

Magnusson, W. E., E. V. Da Silva, and A. P. Lima. 1987. Diets of Amazonian crocodilians. Journal of Herpetology 21:85–95.

Magnusson, W. E., and A. P. Lima. 1991. The ecology of a cryptic predator, *Paleosuchus trigonatus*, in a tropical rainforest. Journal of Herpetology 25:41–48.

Matich, P., M. R. Heithaus, and C. A. Layman. 2011. Contrasting patterns of individual specialization and trophic coupling in two marine apex predators. Journal of Animal Ecology 80:294–305.

Pauwels, O. S., B. Barr, M. L. Sanchez, and M. Burger. 2007. Diet records for the dwarf crocodile (*Osteolaemus tetraspis tetraspis*) in Rabi Oil Fields and Loango National Park, Southwestern Gabon. Hamadryad 31:258–264.

Phillips, D. L., and J. W. Gregg. 2003. Source partitioning using stable isotopes: coping with too many sources. Oecologia 136:261–269.

Platt, S. G., T. R. Rainwater, A. G. Finger, J. B. Thorbjarnarson, T. A. Anderson, and S. T. McMurry. 2006. Food habits, ontogenetic dietary partitioning and observations of foraging behaviour of Morelet's crocodile (*Crocodylus moreletii*) in northern Belize. Herpetological Journal 16:281–290.

Polis, G. A. 1984. Age structure component of niche width and intraspecific resource partitioning: can age groups function as ecological species? American Naturalist 123:541–564.

Polis, G. A., C. A. Myers, and R. D. Holt. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. Annual Review of Ecology and Systematics 20:297–330.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703–718.

Post, D. M. 2003. Individual variation in the timing of ontogenetic niche shifts in largemouth bass. Ecology 84:1298–1310.

Quevedo, M., R. Svanbäck, and P. Eklöv. 2009. Intrapopulation niche partitioning in a generalist predator limits food web connectivity. Ecology 90:2263–2274.

R Development Core Team. 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org.

Radloff, F. G., K. A. Hobson, and A. J. Leslie. 2012. Characterising ontogenetic niche shifts in Nile crocodile using stable isotope (δ^{13}C, δ^{15}N) analyses of scute keratin. Isotopes in Environmental and Health Studies 48:439–456.

Reich, K. J., K. A. Bjorndal, and C. M. Del Rio. 2008. Effects of growth and tissue type on the kinetics of ^{13}C and ^{15}N incorporation in a rapidly growing ectotherm. Oecologia 155:651–663.

Riley, J., and F. W. Huchzermeyer. 2000. Diet and lung parasites of Swamp forest dwarf crocodiles (*Osteolaemus tetraspis osborni*) in the Northern Congo Republic. Copeia 2000:582–586.

Rio, C. M. D., and S. A. Carleton. 2012. How fast and how faithful: the dynamics of isotopic incorporation into animal tissues. Journal of Mammalogy 93:353–359.

Rio, C. M. D., N. Wolf, S. A. Carleton, and L. Z. Gannes. 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biological Reviews 84:91–111.

Roach, K. A., and K. O. Winemiller. 2015. Hydrologic regime and turbidity influence entrance of terrestrial material into river food webs. Canadian Journal of Fisheries and Aquatic Sciences 72:1099–1112.

Rosenblatt, A. E., and M. R. Heithaus. 2011. Does variation in movement tactics and trophic interactions among American alligators create habitat linkages? Journal of Animal Ecology 80:786–798.

Rosenblatt, A. E., and M. R. Heithaus. 2013. Slow isotope turnover rates and low discrimination values in the American alligator: implications for interpretation of ectotherm stable isotope data. Physiological and Biochemical Zoology 86:137–148.

Schmidt, K. P. 1923. Contributions to the herpetology of the Belgian Congo based on the collection of the American Museum Congo Expedition, 1909–1915 Part II—Snakes. Bulletin of the American Museum of Natural History 49:1–146.

Schriever, T. A., and D. D. Williams. 2013. Ontogenetic and individual diet variation in amphibian larvae across an environmental gradient. Freshwater Biology 58:223–236.

Seyler, P., J. Olivry, and L. Nkamdjou. 1993. Hydrogeochemistry of the Ngoko River, Cameroon: chemical balances in a rainforest equatorial basin. Hydrology of Warm Humid Regions, pp. 87–105. IAHS publication, Proceedings of the Yokohama Symposium.

Shirley, M. H., W. Oduro, and H. Y. Beibro. 2009. Conservation status of crocodiles in Ghana and Cote-d'Ivoire, West Africa. Oryx 43:136–145.

Smolensky, N. 2014. Co-occurring cryptic species pose challenges for conservation: a case study of the African dwarf crocodile (*Osteolaemus* spp.) populations in Cameroon. Oryx 49:584–590.

Smolensky, N. L., L. A. Hurtado, and L. A. Fitzgerald. 2015. DNA barcoding of Cameroon samples enhances our knowledge on the distributional limits of putative species of *Osteolaemus* (African dwarf crocodiles). Conservation Genetics 16:235–240.

Spearman, R. 1966. The keratinization of epidermal scales, feathers and hairs. Biological Reviews 41:59–95.

Subalusky, A. L., L. A. Fitzgerald, and L. L. Smith. 2009. Ontogenetic niche shifts in the American Alligator establish functional connectivity between aquatic systems. Biological Conservation 142:1507–1514.

Thorbjarnarson, J., and M. J. Eaton. 2004. Preliminary examination of crocodile bushmeat issues in the Republic of Congo and Gabon. Proceedings of the 17th Working Meeting of the Crocodile Specialist Group., pp. 237–247. IUCN, Australia.

Tieszen, L. L., T. W. Boutton, K. Tesdahl, and N. A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. Oecologia 57:32–37.

Toham, A. K., and G. G. Teugels. 1997. Patterns of microhabitat use among fourteen abundant fishes of the lower Ntem River Basin (Cameroon). Aquatic Living Resources 10:289–298.

Toham, A., and G. Teugels. 1998. Diversity patterns of fish assemblages in the Lower Ntem River Basin (Cameroon), with notes on potential effects of deforestation. Archiv für Hydrobiologie 141:421–446.

Tucker, A. D., C. J. Limpus, H. I. McCallum, and K. R. McDonald. 1996. Ontogenetic dietary partitioning by *Crocodylus johnstoni* during the dry season. Copeia 1996:978–988.

Vliet, K. A. 2007. Crocodilian capture and restraint. Pp. 313–323 in G. West, D. Heard, and N. Caulkett (eds.), Zoo Animal and Wildlife Immobilization and Anesthesia. Wiley, USA.

Wallace, K. M., and A. J. Leslie. 2008. Diet of the Nile Crocodile (*Crocodylus niloticus*) in the Okavango Delta, Botswana. Journal of Herpetology 42:361–368.

Webb, G. J. W., G. J. Hollis, and S. C. Manolis. 1991. Feeding, growth and food conversion rates of wild juvenile saltwater crocodiles (*Crocodylus porosus*). Journal of Herpetology 25:462–473.

Wheatley, P. V., H. Peckham, S. D. Newsome, and P. L. Koch. 2011. Estimating marine resource use by the American crocodile *Crocodylus acutus* in southern Florida, USA. Marine Ecology Progress Series 447:211–229.

Woodborne, S., H. Botha, D. Huchzermeyer, J. Myburgh, G. Hall, and A. Myburgh. 2021. Ontogenetic dependence of nile crocodile (*Crocodylus niloticus*) isotope diet-to-tissue discrimination factors. Rapid Communications in Mass Spectrometry 35:e9159.

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