

# Estimating marine resource use by the American crocodile *Crocodylus acutus* in southern Florida, USA

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**ABSTRACT:** Alligators and crocodiles differ in their physiological capacity to live in saline waters. Crocodiles can tolerate high-salinity water, at least for limited timeframes, whereas alligators and their close relatives cannot. Experiments have placed different crocodylians in various water salinities to document physiological responses, but no study has estimated the extent to which natural populations of crocodylids can live independent of fresh water. Here we estimated marine food and perhaps seawater contributions to a population of American crocodile *Crocodylus acutus* in southernmost Florida, USA. We evaluated the use of carbon, oxygen, and strontium isotopes as tracers of marine versus terrestrial sources. We compared *C. acutus* isotopic values to those of marine reptiles (marine iguanas and Pacific loggerhead turtles) and to American alligators, which require fresh water. We found that freshwater reptiles can be discriminated from those that drink seawater (or survive on metabolic and prey-included water in saline habitats) based on the magnitude of population-level oxygen isotope variation in bioapatite, whereas mean carbon isotope values discriminate between marine versus terrestrial food consumption. We used a 2 end-member (seawater and fresh water) mixing model to calculate percentage of marine resources used by *C. acutus*. Results indicate that adult *C. acutus* in southern Florida use marine food about 65% of the time and seawater or water gleaned from marine food about 80% of the time. This suggests that behavioral osmoregulatory techniques (i.e. seeking fresh water specifically for drinking, as suggested by other researchers) may not be necessary and that *C. acutus* is capable of being largely ecologically independent of fresh water.

**KEY WORDS:** Osmoregulation · Saltwater tolerance · Isotope · Reptile · Alligator · Marine iguana · Sea turtle

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## INTRODUCTION

Researchers have been intrigued by the ability of some crocodylians to spend extended periods of time in waters with salinities as high as seawater. Among the 2 most diverse families in the Crocodylia, the

Crocodylidae are better adapted to salty environments than Alligatoridae (reviewed by Taplin 1988, Mazzotti & Dunson 1989). This dichotomy has been demonstrated or inferred from differences in habitat use in the wild, from experiments, and based on their biogeography. With respect to habitat preferences,

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the crocodylids *Crocodylus acutus* and *C. porosus* are highly estuarine, and many other crocodylid species (e.g. *C. cataphractus*, *C. johnstoni*, *C. moreletti*, *C. niloticus*, and *C. palustris*), while typically associated with freshwater habitats, have estuarine populations (Taplin 1988, Mazzotti & Dunson 1989, Jackson et al. 1996). In contrast, no alligatorid species typically inhabit estuarine environments, and only *Alligator mississippiensis*, *Caiman crocodilus*, and *Caiman latirostris* have some populations that frequent brackish habitats (Jackson et al. 1996, Grigg et al. 1998, Elsey 2005).

With respect to biogeographic differences, crocodylids occupy every continent that offers hospitable temperatures, whereas modern alligatorids nearly all inhabit the Americas. The exception is the Chinese alligator *Alligator sinensis*, which presumably crossed from North America to Asia over the Bering land bridge during the Eocene (~56 to 34 Mya), when global temperatures were significantly higher than today (Brochu 2003). The limited biogeographic distribution of alligatorids applies to the fossil record as well (reviewed by Brochu 2000). This biogeography suggests that significant oceanic crossings are difficult or impossible for alligatorids, but unremarkable for crocodylids.

Physiological experiments have shown crocodylids to be especially suited for brackish and saline waters. Injection of a crocodylid with a methacholine chloride solution resulted in a hyperosmotic secretion via lingual salt glands (Taplin & Grigg 1981). Some estuarine crocodylids refuse to drink seawater when dehydrated (Mazzotti & Dunson 1984, Jackson et al. 1996); they only actively imbibe if water is less than 10 practical salinity units (psu). This often results in weight loss and/or dehydration for small fasted alligators kept in seawater (35 psu) under controlled settings (e.g. Dunson 1970, 1982, Evans & Ellis 1977). Larger crocodiles could remain healthy for months in waters of 26 psu and higher if fed (Dunson 1970, 1982). Other experiments demonstrate that *Crocodylus niloticus* can tolerate salinities as high as 35 psu if salinity is increased gradually and the animals have time to adapt (Leslie & Spotila 2000). Alligatorids have lingual glands that are stimulated by methacholine chloride injections, but their secretions are iso-osmotic with blood plasma and do not provide effective salt excretion (Taplin et al. 1985).

The adaptations of crocodylids to saline conditions (in particular the lingual salt glands) prompted Taplin et al. (1985) to suggest that crocodylids are derived from an ocean-going ancestor. Despite these adaptations, however, there is evidence that modern

crocodylids are very much tied to fresh water. There is no modern example of pelagic/marine crocodylians and even though *Crocodylus acutus* and *C. porosus* are primarily estuarine, the other crocodylids are most often found in fresh water. Given this apparent dependence on fresh water, it is difficult to imagine a recent pelagic ancestor to Crocodylidae. In fact, Mazzotti & Dunson (1989) argued that *Crocodylus* probably evolved saltwater adaptations the same way that turtles and snakes did, transitioning from fresh to estuarine, and finally seawaters. The evidence (physiology, ecology, biogeography) for adaptations to saltwater tolerance in *Crocodylus* is conspicuous, and the question of how tolerant natural populations are to saline waters remains open.

Results from several experiments imply that in natural conditions, adult crocodiles might be able to osmoregulate with very small amounts of low-salinity drinking water (Dunson 1970, 1982, Leslie & Spotila 2000), but we are aware of no studies that have attempted to quantify the salinity of the water ingested by crocodylids in the wild. Observations of aquatic animals (especially those that can submerge for long periods of time) are difficult, and most observations made at specific field sites did not attempt to follow individuals to estimate the amount of time they spent in brackish (salinities 0.5 to 30 psu) versus saline waters (salinities 30 to 50 psu, with seawater averaging 35 psu). Kushlan & Mazzotti (1989) compiled observational data for *Crocodylus acutus* in southern Florida (hereafter FL), USA, noting the number of sightings in waters of different salinity. Their data showed an overall preference for fresher water (average salinity = 14 psu), but substantial numbers of individuals were seen in higher-salinity water. The number of sightings in higher-salinity waters (average = 17.8 psu) increased during the nesting season (March to August), when individuals were more commonly observed on the shoreline along Florida Bay, where nesting sites are common. That study estimated time spent in different salinity waters but did not attempt to estimate ingestion of marine resources (food and water).

Radio- or satellite-tracking is increasingly being used to study animal habitat preferences and migration. However, studies of taxa with estuarine adaptations have largely been limited to freshwater populations of those species (e.g. Rodda 1984, Brien et al. 2008) with some exceptions. Read et al. (2007) focused on the homing abilities of translocated individuals of *Crocodylus porosus*, but their study is not applicable to discerning the proportion of time spent in fresh versus saline water. Kay (2004) studied 16

*C. porosus* to discern preferred habitats and home ranges. The relative proportions of time spent in each salinity zone were not explicitly discussed, but the data suggest that the time spent in each zone is highly variable from individual to individual. Kushlan & Mazzotti (1989) focused on *C. acutus* in southern FL, using observational data (see above) and telemetry data from 10 individuals to better understand nesting ecology, but they did not tie their data to salinity gradients.

Determining the habitats in which crocodylians are feeding or drinking observationally is even more difficult. Although there may be cues associated with some feeding behaviors, it is challenging to determine by observation whether an aquatic animal is drinking the water in which it is immersed. Such problems have been addressed using tracers (natural or artificial) to track the source of an animal's food and water inputs. Here, we used stable isotope ratios of carbon, oxygen, and strontium (Sr) in biological apatite, the mineral in bones and teeth, as natural tracers to estimate the amount of marine resources used by the crocodylid *Crocodylus acutus* in southern FL. We compared the isotope ratios of *C. acutus* to those of 2 fully marine reptiles (marine iguanas *Amblyrhynchus cristatus* and Pacific loggerhead turtles *Caretta caretta*). We further compared *C. acutus* to the alligatorid *Alligator mississippiensis*, an obligate freshwater drinker (Laurén 1985), with similar opportunistic generalist feeding ecology (McNease & Joanen 1977, Thorbjarnarson 1988a) and similar home range requirements (Joanen & McNease 1989, Kushlan & Mazzotti 1989, Cherkiss et al. 2011), in both inland/freshwater and coastal ecosystems. Before presenting our analysis, we offer a brief overview of the isotopic systems we used and what aspects of animal ecology and physiology they record.

### Carbon isotope ratios in bioapatite

Carbon in biological hydroxyapatite (or bioapatite),  $\text{Ca}_5(\text{PO}_4)_3(\text{OH}, \text{CO}_3)$  is derived from blood bicarbonate, which in air-breathing terrestrial animals is supplied by the oxidation of food (i.e. respiration). In mammals, the carbon isotope ( $\delta^{13}\text{C}$ ) value of bioapatite is tightly correlated with that of bulk diet, offset by a trophic discrimination ( $\sim 9\%$  for carnivores,  $\sim 14\%$  for herbivores; reviewed by Koch 2007). Differences in the  $\delta^{13}\text{C}$  value of primary producers cascade up food webs with small  $^{13}\text{C}$  enrichments ( $\sim 1\%$  per trophic step) to label large carnivores

such as crocodylians. Clementz & Koch (2001) showed that bioapatite  $\delta^{13}\text{C}$  values from mammals are useful in identifying the different marine, estuarine, and terrestrial ecosystems in which animals forage.

Carbon fixation by primary producers sorts carbon isotopes in different but predictable ways. Plants using the  $\text{C}_3$  photosynthetic pathway in terrestrial and freshwater ecosystems (trees, shrubs, cool growing season grasses) average  $\sim -27\%$ .  $\text{C}_4$  plants (warm growing season grasses) average  $\sim -12\%$ , and CAM plants (some succulents) have variable values between  $-27$  and  $-11\%$  (reviewed by Farquhar et al. 1989). Marine  $\delta^{13}\text{C}$  values vary with the source of carbon, photosynthetic processes, and other factors (reviewed by Newsome et al. 2010). Macroscopic marine plants have higher  $\delta^{13}\text{C}$  values (i.e. kelp averages  $\sim -17\%$ , seagrass averages  $\sim -10\%$ ), whereas marine phytoplankton has lower values of  $-20$  to  $-26\%$ , depending on oceanographic setting and plankton type (Clementz & Koch 2001, Page et al. 2008). River ecosystems contain carbon both from autochthonous aquatic ( $\text{C}_3$ ) sources ( $\sim -27\%$ ) and allochthonous terrestrial sources ( $\text{C}_3$  or  $\text{C}_4$  vegetation) from riverbanks ( $\sim -27$  to  $\sim -13\%$ ; reviewed by Fry & Sherr 1984, Doi 2009). Estuaries mix river and marine waters, and the sources of carbon can be allochthonous or autochthonous, but autochthonous  $\delta^{13}\text{C}$  values in estuarine habitats are higher than in freshwater rivers due to the marine influence (e.g. Silva et al. 2011).

We know of only 2 papers that have analyzed bioapatite  $\delta^{13}\text{C}$  values to study modern reptiles. Smith et al. (2002) presented a straightforward comparative study of the diets of terrestrial mammals, birds, and lizards in the Chihuahuan Desert. The situation is more complex for ectotherms living in water, which is relatively rich in dissolved inorganic carbon (DIC), a potential alternate source of carbon to blood bicarbonate. Biasatti (2004) studied bone bioapatite  $\delta^{13}\text{C}$  values in sea turtles. She found large differences in diet-to-apatite fractionation among taxa and across space within taxa, which she attributed to differences in the labeling of bone carbonate by respired carbon versus seawater DIC. She did not exclude an alternate hypothesis, that differences in digestive physiology between herbivorous and carnivorous turtles could explain the observed pattern. However, in a study of entirely carnivorous (though not air-breathing) sharks, Vennemann et al. (2001) also detected large differences in bioapatite  $\delta^{13}\text{C}$  values that they attributed to differences in labeling by respired carbon versus seawater DIC.

### Oxygen isotope ratios in bioapatite

The oxygen isotope ( $\delta^{18}\text{O}$ ) value of bioapatite depends on the temperature at which the mineral forms and the  $\delta^{18}\text{O}$  value of the body fluid from which it precipitates (reviewed by Kohn & Cerling 2002, Koch 2007). The  $\delta^{18}\text{O}$  value of body water is influenced by ingested water (which differs among potential sources, such as food versus drinking water) and the input of food oxygen and inspired  $\text{O}_2$  via respiration, and by the physiology of water turnover and economy (Kohn 1996). Aquatic ectotherms, such as crocodylians, form bioapatite from body water that is close in  $\delta^{18}\text{O}$  value to environmental water at a temperature close to ambient. Wild alligator bone phosphate  $\delta^{18}\text{O}$  values are consistent with those expected given environmental temperatures (Stoskopf et al. 2001). In wild and captive crocodylians, Amiot et al. (2007) correlated phosphate  $\delta^{18}\text{O}$  values to those of ambient water ( $R^2 = 0.84$ ), and attributed residual variation to differences in temperature, diet, and/or physiology. Overall, we expect the  $\delta^{18}\text{O}$  value of an aquatic reptile to reflect the  $\delta^{18}\text{O}$  value of the water it ingests, either by drinking or in the fluids or metabolic water from diet items.

Coastal ocean water in tropical and subtropical regions has a fairly constant  $\delta^{18}\text{O}$  value close to 0‰ (LeGrande & Schmidt 2006). Meteoric water  $\delta^{18}\text{O}$  values, in contrast, vary with latitude, altitude, and distance from the evaporative source. Precipitation and surface water in the tropics is typically  $^{18}\text{O}$ -depleted relative to ocean water by up to 5‰, although values greater than 0‰ can occur in tropical and subtropical surface waters that experience strong evaporative water loss (reviewed by Gat 1996). These isotopic differences between fresh water and seawater, and the fact that they are recorded in bioapatite, have been used to study habitat use and drinking water sources in aquatic and semi-aquatic fossil vertebrates (Thewissen et al. 1996, 2007, Roe et al. 1998, Clementz et al. 2003, 2006, Billon-Bruyat et al. 2005, Amiot et al. 2010).

### Sr isotope ratios in bioapatite

Sr in bioapatite occurs as a substitution for calcium. Assuming biological fractionation of Sr is negligible (Capo et al. 1998), Sr incorporated into bioapatite will reflect the bioavailable Sr derived from the soil (or

water) of an animal's environment (reviewed by Bentley 2006, Koch 2007). For animals such as crocodiles, which ingest and dissolve calcium-rich bone, the dominant source of Sr will be diet and not ingested water.

Similar to carbon and oxygen, variations in the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of animals are controlled by the mixing of isotopically distinct sources. Environmental  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios can differ geographically due to regional differences in geologic substrate, whereas seawater has a relatively uniform  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio ( $\sim 0.7092$ ), although this marine value varies on a geological timescale (Burke et al. 1982). Animals feeding exclusively in a marine ecosystem should have  $^{87}\text{Sr}/^{86}\text{Sr}$  values similar to that of seawater. Therefore, these taxa can be distinguished from those feeding in (or inhabiting) terrestrial or estuarine ecosystems (e.g. Koch et al. 1992, Schmitz et al. 1997, Poyato-Ariza et al. 1998, Kennedy et al. 2002, Clementz et al. 2003, Price & Gestsdóttir 2006, Kocsis et al. 2007).

## MATERIALS AND METHODS

### Samples

We sampled 4 species of reptile: the crocodylians *Alligator mississippiensis* and *Crocodylus acutus*, and the marine reptiles *Amblyrhynchus cristatus* and *Caretta caretta*. *C. acutus* specimens were collected in southern FL (Fig. 1) and curated by the Florida Museum of Natural History at the University of

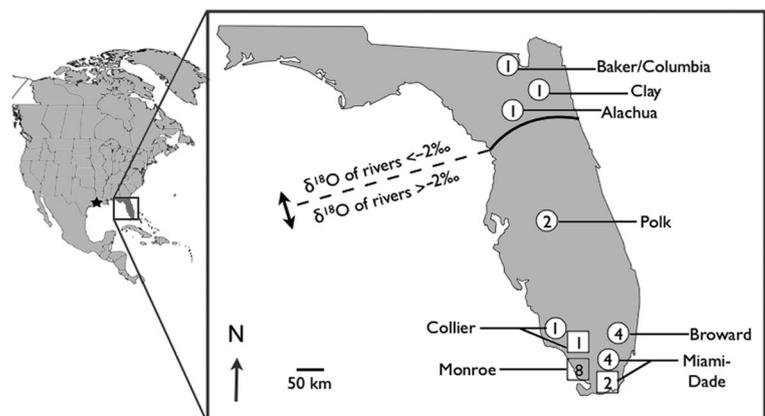


Fig. 1. Localities where crocodylians were sampled from Florida and Louisiana, USA. Contour line from Kendall & Coplen (2001) that separates river waters with  $\delta^{18}\text{O}$  values  $> -2\text{‰}$  from those  $< -2\text{‰}$ . Numerals in circles represent the number of alligators sampled from that county; numerals in squares represent the number of crocodiles sampled in that county. ★: location of Rockefeller Wildlife Refuge (RWR)

Florida (UF). Care was taken not to select samples of *C. acutus* from the Turkey Point nuclear power station canal. The effluent water from the power plant is artificially warm, and we were concerned about the effects on the isotopic composition of bioapatite carbonate from these individuals. *A. mississippiensis* specimens were collected in FL (Fig. 1) and at the Rockefeller Wildlife Refuge (RWR) in Louisiana (hereafter LA). These specimens were divided into those that were collected in coastal habitats and those that were collected at inland sites (>16 km from the coast, i.e. farther than the typical home range of *A. mississippiensis*; Joanen & McNease 1989).

*Crocodylus acutus* and UF-curated *Alligator mississippiensis* are identified by UF museum numbers, and some additional information on these specimens may be available through the Florida Museum of Natural History. These animals are all adults, although sex for most individuals is unknown. Most of the UF crocodylians were collected in the 1980s. Less information is available for the RWR animals. Most of the RWR *A. mississippiensis* specimens are represented only by teeth, and in most cases the sex of the animal and accurate size information are not available. Most animals were estimated to be adults in a field setting by RWR staff. Body length measurements where available (4 non-adults and several adults) are reported in Table A1 in Appendix 1. The RWR crocodylians were collected between 2005 and 2009. 'UW' numbers are uncataloged specimens at the University of Wyoming Geological Museum. These specimens are teeth only, and no size data exist, but tooth sizes are consistent with sub-adults or small adults from RWR.

Marine iguana *Amblyrhynchus cristatus* specimens were collected from the Galapagos Islands and curated by the California Academy of Sciences (CAS). Loggerhead turtles *Caretta caretta* were col-

lected in Baja California Sur, Mexico, and are curated by the Long Marine Laboratory (LML) at the University of California, Santa Cruz (UCSC). Locality information and number of individuals analyzed are reported in Table 1. We analyzed teeth or bone samples from 5 individuals as a minimum population size to determine mean isotopic values and standard deviations that accurately reflect the variability in a natural population (Clementz & Koch 2001).

For the 2 crocodylians and the marine iguana, tooth enamel and dentine were combined for isotopic analysis of the carbonate fraction of tooth mineral bioapatite. We expect similar mean values and variability in tooth enamel and dentine because they contain the same mineral phase and form in the same narrow temporal window (~10 mo for tooth formation; Edmund 1962). We studied teeth because ultimately we will use our results to inform studies of fossil material, and tooth enamel is much more resistant to post-mortem alteration than other biogenic tissues (Wang & Cerling 1994, Koch et al. 1997). Although we used both enamel and dentine for this study, fossil workers should take care to use only enamel because dentine is highly subject to diagenetic alteration. Because sea turtles are edentulous, we used bone from the humerus. Mean values for bone apatite carbonate should be directly comparable to those for tooth enamel and dentine. Bone tissue is deposited in concentric layers in turtle bones and turns over slowly (De Ricqlés 1976, Butcher et al. 2008). If only one growth increment is subsampled and analyzed, then variability between bone and teeth might be roughly equivalent. However, if bone is homogenized and analyzed, then bone samples represent a much longer time interval than does a single reptile tooth. Our turtle bone samples were homogenized and thus they likely have lower intrinsic variability than reptile tooth samples.

Table 1. Stable isotope means (‰ relative to V-PDB) and standard deviations of carbonate in bioapatite in teeth (bone in the case of loggerhead turtles *Caretta caretta*) of the reptile specimens used in this study. CAS: California Academy of Sciences; UCSC: University of California, Santa Cruz; UF: University of Florida; RWR: Rockefeller Wildlife Refuge (Louisiana); na: not available

Species	n	General locality	Institution	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$
<i>Amblyrhynchus cristatus</i>	5	Galapagos Islands	CAS	$-4.2 \pm 2.2$	$-0.8 \pm 0.6$
<i>Caretta caretta</i>	31	Baja California	Long Marine Lab (UCSC)	$-9.0 \pm 0.6$	$1.3 \pm 0.6$
<i>Dermodochelys coriacea</i> <sup>a</sup>	5	Guyana and Florida	na	$-9.6 \pm 2.2$	na
<i>Crocodylus acutus</i>	9	Southern Florida	UF	$-9.6 \pm 3.9$	$0.5 \pm 0.8$
<i>Alligator mississippiensis</i> (coastal)	16	Florida and Louisiana	RWR & UF	$-10.3 \pm 2.6$	$-0.9 \pm 1.7$
<i>Alligator mississippiensis</i> (inland)	12	Florida	UF	$-15.7 \pm 1.8$	$-0.2 \pm 2.2$

<sup>a</sup>Leatherback turtle data from Biasatti (2004)

### Isotopic analysis

Approximately 10 mg of powder were drilled from each alligator tooth. For the marine iguanas, a single whole tooth was used, and ~20 mg of bone were drilled from the loggerhead turtle humeri. The powdered samples were soaked in 30% H<sub>2</sub>O<sub>2</sub> for 48 h to oxidize organic matter. They were then rinsed 5 times with distilled water and soaked for 24 h in 1 M calcium acetate/acetic acid buffer solution (pH 5) to remove carbonate contaminants. Finally, the powdered samples were rinsed 5 times in distilled water and freeze dried. Marine iguana teeth were soaked for 48 h in 30% H<sub>2</sub>O<sub>2</sub> then freeze dried. Whole tooth samples were not soaked in acetic acid due to fears of sample loss. The whole tooth samples were taken from wild-caught marine iguanas, and 30% H<sub>2</sub>O<sub>2</sub> has a pH of ~5, so carbonate contamination is unlikely.

For each analysis, ~1.5 mg of pre-treated powder, or a whole individual tooth, were used. Analyses were conducted on an ISOCARB carbonate preparation system linked to either a Micromass Optima or Prism gas source isotope ratio mass spectrometer in the Stable Isotope Laboratory at UCSC. During analysis, samples were dissolved in a continuously-stirred, common acid bath containing 100% phosphoric acid at 90°C. Reaction time was 800 s, and to reduce sample-to-sample memory, blanks were run between samples. The resulting CO<sub>2</sub> was cryogenically purified and then admitted to the mass spectrometer for analysis. The inter-lab isotope standard was NBS-19, our internal standard was Carrera Marble, and values are reported relative to Vienna PeeDee Belemnite (V-PDB). We also used a bioapatite internal standard of elephant dentine (n = 9, for both δ<sup>13</sup>C and δ<sup>18</sup>O; for the bioapatite standard, 1 SD is ±0.3‰).

For Sr isotope analysis, ~1 mg of drilled dental powder was dissolved in ~2.5 N HCl and concentrated HNO<sub>3</sub>, then evaporated to dryness on a hot plate. The samples were redissolved in 2.5 M HCl, and Sr was isolated using Teflon shrink tubing cation exchange columns packed with Eichrom Sr Spec resin. The elutant was collected and dried down on a hot plate. Samples were redissolved in 2% HNO<sub>3</sub>, and Sr concentration was adjusted to ~100 ppm. Sr isotope composition was measured at the Keck Isotope Laboratory at UCSC on a Finnigan Neptune multicollector inductively coupled plasma mass spectrometer (ICP-MS) at low resolution. Corrections were made for interfering Kr masses. Results were normalized to the international standard, NIST 987, and precision was calculated to be better than 0.00005 (1 SD).

Carbon isotope values were calculated using the following formula:

$$\delta^{13}\text{C} = \left[ \left( \frac{^{13}\text{C}/^{12}\text{C}_{\text{sample}}}{^{13}\text{C}/^{12}\text{C}_{\text{standard}}} - 1 \right) \times 1000 \right] \quad (1)$$

where the standard is V-PDB. δ<sup>18</sup>O values were calculated using the same convention, but the isotopic ratio is <sup>18</sup>O/<sup>16</sup>O. Units are reported in parts per thousand (‰). In the case of Sr, the isotope ratio (<sup>87</sup>Sr/<sup>86</sup>Sr) is reported rather than normalizing to a standard.

Because crocodylians replace their teeth continuously, it is possible that analysis of multiple teeth from 1 individual could supply as much information about a population as analysis of a single tooth from multiple individuals, assuming that the individual behaved in a manner that was representative of the population as a whole. We tested this possibility by analyzing multiple teeth from 1 *Alligator mississippiensis* and comparing the variance in both δ<sup>13</sup>C and δ<sup>18</sup>O values to the variance of 14 individuals from the same population. Multiple teeth from the same individual had significantly less variability than the population as a whole for both δ<sup>13</sup>C and δ<sup>18</sup>O values, leading us to conclude that for isotopic analyses, it is not reasonable to bolster sample size by treating multiple teeth from a single animal with the same weight as a tooth from a different individual (see Appendix 2).

## RESULTS AND DISCUSSION

Mean carbon and oxygen isotope values and associated standard deviations for all reptile groups are reported in Table 1 and Fig. 2.

### Marine reptiles

Pacific loggerhead turtles forage in the eastern Pacific but nest on Japanese or Australian beaches (although the vast majority of the loggerheads off Baja California, where our study samples were collected, nest in Japan; Bowen et al. 1995). Loggerheads are generally carnivorous and may feed at high trophic levels compared to other genera of sea turtles (Godley et al. 1998, Frick et al. 2009).

Pacific loggerhead turtle bone carbonates have a mean δ<sup>18</sup>O value near 0‰ and low standard deviation (1.3 ± 0.6‰), which is expected for an animal that gets water and food entirely from the sea (see discussion about the significance of population δ<sup>18</sup>O variance below). Pacific loggerhead δ<sup>13</sup>C values (mean = -9.0 ± 0.6‰) are similar to values for bone

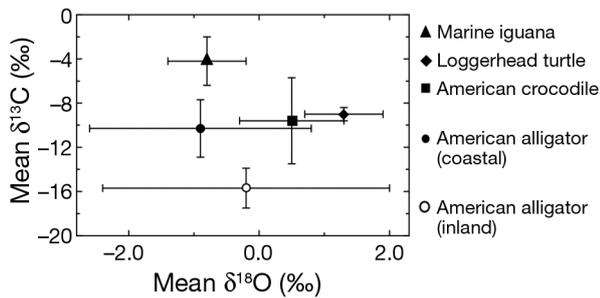


Fig. 2.  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values (mean  $\pm$  SD) for the reptiles sampled in this study (marine iguana *Amblyrhynchus cristatus*, loggerhead turtle *Caretta caretta*, American crocodile *Crocodylus acutus*, and American alligator *Alligator mississippiensis*). Note relatively high  $\delta^{13}\text{C}$  values of species with access to marine food webs

carbonate from marine carnivorous leatherback turtles, which forage in the Atlantic Ocean and the Gulf of Mexico, from a previous study (Biasatti 2004).

Marine iguanas live exclusively in the Galapagos Islands and have an amphibious lifestyle, basking on land but drinking and foraging in seawater (Carpenter 1966, Shoemaker & Nagy 1984). Similar to the Pacific loggerhead samples, marine iguanas have relatively invariant  $\delta^{18}\text{O}$  values near 0‰ ( $-0.8 \pm 0.6\text{‰}$ ), consistent with seawater ingestion and marine foraging. Marine iguanas are herbivorous, feeding almost exclusively on marine brown and red algae (Wikelski et al. 1993). The  $\delta^{13}\text{C}$  values of marine algae can vary widely. Kelps can have very high  $\delta^{13}\text{C}$  values ( $\sim -12\text{‰}$ ), whereas intertidal red algae can have extremely low values ( $\sim -30\text{‰}$ ; Raven et al. 2002). Marine iguana tooth bioapatite carbonate ranged from  $-9.6$  to  $-0.3\text{‰}$  (mean =  $-4.2 \pm 2.2\text{‰}$ ). Values near 0‰ likely indicate high reliance on kelp, whereas lower values could be a mix of kelp and red algae. In herbivorous mammals,  $^{13}\text{C}$ -enrichment in bone and tooth carbonate relative to diet can be higher than in carnivores, due to loss of  $^{13}\text{C}$ -depleted methane during gut fermentation of plant material (Hedges 2003, Clementz et al. 2009). Although this phenomenon has not been documented in reptiles, it may be a factor in explaining the high  $\delta^{13}\text{C}$  values in marine iguanas.

### Spatial gradients in crocodylians

We compared isotopic values for saltwater tolerant *Crocodylus acutus* to obligate freshwater drinking *Alligator mississippiensis* in similar environments. When comparing  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of *C. acutus* to all *A. mississippiensis*, it is appropriate to use a mul-

tivariate analysis of variance (MANOVA) because  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values generally covary. When analyzed for differences by taxon, significant differences were found between *C. acutus* and all *A. mississippiensis* ( $F_{2,34} = 4.8$ ,  $p = 0.0148$ ). Results for *C. acutus* versus the alligators split into different ecological zones (inland and coastal) were also calculated (MANOVA, Pillai's Trace Test; approximate  $F_{4,68} = 7.6$ ,  $p < 0.0001$ ). Post hoc pairwise comparisons found that the multivariate mean for saltwater-tolerant (marine/estuarine) *C. acutus* is significantly higher than for the inland ( $F$ -test;  $F_{2,33} = 13.9$ ,  $p < 0.0001$ ) but not the coastal *A. mississippiensis* populations ( $F$ -test;  $F_{2,33} = 2.3$ ,  $p = 0.1172$ ). Multivariate means for *A. mississippiensis* from coastal settings are significantly higher than those from inland settings (MANOVA  $F_{2,33} = 13.0$ ,  $p < 0.0001$ ).

When *Alligator mississippiensis* is split into inland and coastal populations, the covariance is removed. Based on mean  $\delta^{13}\text{C}$  values, coastal *A. mississippiensis* and *Crocodylus acutus* are not significantly different (Table 1), indicating that  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  do not covary in this smaller subset of the data, so MANOVA is no longer required. A simple  $t$ -test between the mean  $\delta^{18}\text{O}$  values of the 2 groups indicates that coastal *A. mississippiensis* have a significantly lower mean value than *C. acutus* ( $t_{22,8} = -2.9$ ,  $p = 0.009$ ). The similarity in  $\delta^{13}\text{C}$  values between coastal alligators and crocodiles indicates that they have comparable amounts of marine carbon in their diets. The difference in  $\delta^{18}\text{O}$  values among crocodylian groups is more difficult to interpret, with latitude, precipitation, and evaporation all potentially playing a role.

We expected that seawater ingestion (either directly or via water in food and metabolic water) would yield higher  $\delta^{18}\text{O}$  values than freshwater consumption. However, the inland population of *Alligator mississippiensis* has a mean  $\delta^{18}\text{O}$  value that is not significantly different than the mean for saltwater-drinking marine iguanas ( $p = 0.61$ ). A possible explanation is that some inland alligators may ingest water that is  $^{18}\text{O}$ -enriched relative to precipitation by evaporation. Inland water sources in central and southern FL may have values of up to  $+4\text{‰}$ , whereas the amount-weighted mean annual  $\delta^{18}\text{O}$  of precipitation is  $-2.7$  to  $-2.5\text{‰}$  (Meyers et al. 1993, Gremillion & Wanielist 2000).

Mean  $\delta^{18}\text{O}$  values from coastal and inland populations of *Alligator mississippiensis* are not significantly different ( $p = 0.35$ ), which might be expected for freshwater consumers, but a latitudinal difference in sampling sites complicates this interpretation.

With 1 exception, our coastal alligators are from LA, whereas the majority of our inland alligators are from southern FL. The meridional change in precipitation  $\delta^{18}\text{O}$  values across the region results in river waters in northern FL and southern LA that are 2‰ lower than in southern FL (Kendall & Coplen 2001), and this difference may be transmitted to crocodylian bioapatite carbonate, assuming growth occurs at similar temperatures in both sites. If animals grow at lower temperatures at northern sites (LA sites average 5°C cooler than southern FL sites), the increase in water-to-apatite oxygen isotope fractionation with decreasing temperature, ~1‰ per 5°C (Friedman & O'Neil 1977), would partially offset this difference in environmental water.

To explore possible latitudinal effects, we divided inland FL alligators into northern and southern subsets based upon the Kendall & Coplen (2001) contour line separating river waters with  $\delta^{18}\text{O}$  values  $> -2\text{‰}$  from those  $< -2\text{‰}$  (Fig. 1). The southern FL inland alligators have mean  $\pm$  SD  $\delta^{18}\text{O}$  value of  $0.2 \pm 2.2\text{‰}$  ( $n = 9$ ), and northern FL inland alligators have a mean of  $-1.4 \pm 1.8\text{‰}$  ( $n = 3$ ). The means are not significantly different ( $t_{4,2} = -1.3$ ,  $p = 0.25$ ), but our northern FL sample is small, and the ~2‰ difference we observed is of the expected magnitude and sign given the difference between northern and southern FL river water  $\delta^{18}\text{O}$  values. Coastal alligators from RWR (which receives precipitation of roughly the same  $\delta^{18}\text{O}$  value as northern FL) average  $-0.9 \pm 1.6\text{‰}$ , not significantly different from northern FL inland alligators ( $t_{2,8} = 0.5$ ,  $p = 0.68$ ). The sole coastal alligator sample from southern FL had a  $\delta^{18}\text{O}$  value of  $-0.4$ , well within the range of values for coastal LA as well as southern FL inland alligators but about 1 SD lower than the *Crocodylus acutus* mean (Table 1).

Our results suggest that mean  $\delta^{18}\text{O}$  values are of limited use as an ecological tracer in our study area. In our data, a combination of latitudinal and evaporative differences in isotopic composition of precipitation and river water is likely driving the trends in  $\delta^{18}\text{O}$  values. Thus, we cannot separate organisms that obtain their water from the sea from those that drink fresh water in southern FL based on mean  $\delta^{18}\text{O}$  values. At higher latitudes (where the difference in the  $\delta^{18}\text{O}$  value between fresh water and seawater is larger), mean  $\delta^{18}\text{O}$  values may be a useful measure of the extent to which a vertebrate ingests seawater or obtains food and metabolic water from the sea.

While differences in mean  $\delta^{18}\text{O}$  values show limited utility in discriminating between alligators and crocodiles where they co-occur in southern FL, there are differences in standard deviations among taxa

(Table 1, Fig. 3). Animals that drink seawater or ingest seawater via prey body water (marine iguanas, sea turtles, and crocodiles) have low  $\delta^{18}\text{O}$  variability, whereas coastal and inland alligators show significantly higher variability (Table 1). This relationship is similar to that observed by Clementz & Koch (2001) for terrestrial versus aquatic mammals. Reptiles that drink seawater ingest a source that is isotopically homogeneous, providing an input to body water (and thus bioapatite) with a relatively constant  $\delta^{18}\text{O}$  value. Even if food-included water and metabolic water from marine food items contribute to body water, these sources will have  $\delta^{18}\text{O}$  values that will be more homogeneous than those of terrestrial or freshwater prey. Note that the marine iguana tooth  $\delta^{18}\text{O}$  SD is as small as the SD for loggerhead turtle bone (Table 1, Figs. 2 & 3), which is integrated over a longer time period than teeth (and thus averages out short-term changes in temperature or water source). In contrast, many terrestrial water sources vary latitudinally and seasonally, or based on differences in source (recent precipitation versus groundwater).

Coastal alligators from RWR, inland alligators from northern FL, and inland alligators from southern FL all exhibit similar variance (Levene test for unequal variance  $F_{2,24} = 0.43$ ,  $p = 0.65$ ). We conclude that use of seawater (directly or via diet) by *Crocodylus acutus* has significantly reduced its  $\delta^{18}\text{O}$  variation in comparison to the freshwater-drinking alligators ( $F_{26,8} = 0.17$ ,  $p = 0.014$ ).

We expected that  $\delta^{13}\text{C}$  values would vary inversely with distance to the coast, such that high carbon isotope values were expected when marine resources were consumed and low values when terrestrial/freshwater resources were consumed. Even if DIC plays a significant role in labeling crocodylian bioap-

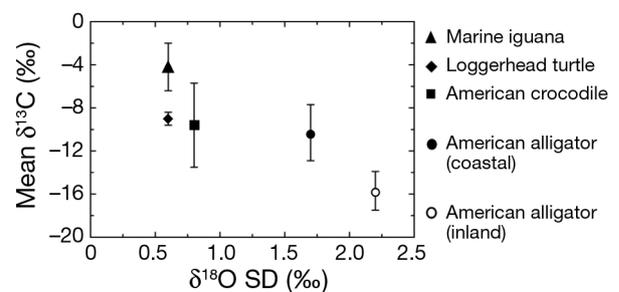


Fig. 3. Mean  $\delta^{13}\text{C}$  value versus the standard deviation of  $\delta^{18}\text{O}$  values. Note that American crocodile *Crocodylus acutus* plots closer to the marine reptiles (marine iguana *Amblyrhynchus cristatus* and loggerhead turtle *Caretta caretta*) than either coastal or inland alligators *Alligator mississippiensis*

atite, the expectation is the same. Estuaries in both western LA and FL have very similar DIC  $\delta^{13}\text{C}$  values, increasing as salinity increases (Sackett et al. 1997, Kaldy et al. 2005). The expected trend is observed and significant ( $t_{32.8} = -7.3$ ,  $p < 0.0001$ ), clearly separating the inland populations from the coastal and marine crocodylian populations and species (Fig. 2). The high  $\delta^{13}\text{C}$  values observed in coastal alligators and crocodiles likely reflect consumption of marine resources. An alternative explanation, however, is that  $\text{C}_4$  vegetation could be influencing the aquatic food webs in which the alligators and/or crocodiles are feeding.  $\text{C}_4$  grasses do occur in FL, but in coastal swamps and marshes, freshwater  $\text{C}_3$  macrophytes and algae form the base of the food web (Williams & Trexler 2006, Troxler & Richards 2009). We are aware of no evidence suggesting that terrestrial  $\text{C}_4$  plants increase in riparian and swamp communities near the ocean. Thus, we contend that the conspicuously higher  $\delta^{13}\text{C}$  values of crocodylians near the coast are due to marine carbon influence in brackish water and estuaries.

Sr isotope ratios provide 1 test of the hypothesis that marine resources, rather than  $\text{C}_4$  resources, are the source of high  $\delta^{13}\text{C}$  values in coastal crocodylians. Crocodylians ingest bone, which is much more concentrated in Sr than water (FL river freshwater  $\approx 0.5$  ppm Sr, seawater  $\approx 7$  ppm Sr, Bryant & Mueller 1995; bone  $\approx 200$  ppm Sr, Blincoe et al. 1973). Therefore, food will control the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of bones and teeth, as well as their  $\delta^{13}\text{C}$  values. If high  $\delta^{13}\text{C}$  values reflect use of marine resources, we would expect  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios to approach the value of modern seawater (0.7092) as  $\delta^{13}\text{C}$  values rise. Although in restricted basins the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio can be altered by local river influence, the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of water in

Biscayne Bay (a popular nesting site for *Crocodylus acutus*) is indistinguishable from seawater (Stalker et al. 2009). Although we are aware of no coastline measurements of seawater from RWR, measurements near the Mississippi Delta in eastern LA (where river influence should be much higher than at RWR) indicate that by the time water reaches 35 psu, the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio is the same as seawater (Xu & Marcantonio 2007).

$^{87}\text{Sr}/^{86}\text{Sr}$  ratios of FL crocodylians (Table 2) are consistent with those of terrestrial mammals collected across FL by Hoppe et al. (1999). Among all coastal crocodylians (filled symbols in Fig. 4A), there is a trend towards higher  $\delta^{13}\text{C}$  values as  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios approach 0.7092 ( $R^2 = 0.6$ ). We contend that this correlation is evidence that high  $\delta^{13}\text{C}$  values for coastal crocodylians (both alligators and crocodiles) are due to the influence of marine carbon. The inland alligators, which have no access to marine resources unless they are migratory (open symbols in Fig. 4A), do not show this pattern. The inland alligators from southern FL have  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in the same range as coastal crocodylians. The bedrock in this region is Late Quaternary limestone, with  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios essentially identical to modern seawater (Weedman et al. 1999). Thus, alligators with entirely terrestrial/freshwater diets that are supplied with Sr only from bedrock soils (e.g. sink hole or standing water wetland versus a river draining a large area with disparate  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios) could have ratios similar to modern seawater (0.7092). We observed  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios higher than modern seawater in 2 southern FL alligators (open circle and filled triangle, Fig. 4A) as well as the 3 crocodiles from southern FL (filled diamonds, Fig. 4A). The implication is that soil is being labeled with a Sr source other than seawater or

Table 2. *Alligator mississippiensis* and *Crocodylus acutus*. Sr, C, and O isotope data from tooth bioapatite and locality information for individual alligators and crocodiles. FL: Florida; LA: Louisiana; RWR: Rockefeller Wildlife Refuge

Species	ID no.	Ecology	$^{87}\text{Sr}/^{86}\text{Sr}$	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	Location
<i>A. mississippiensis</i>	UF10941	Coastal	0.7093	-13.6	-0.4	FL, Collier Co.
<i>A. mississippiensis</i>	RWR10-O	Coastal	0.7091	-9.9	1.0	LA, RWR
<i>A. mississippiensis</i>	RWR 30	Coastal	0.7095	-12.9	-1.0	LA, RWR
<i>A. mississippiensis</i>	RWR 17	Coastal	0.7092	-9.9	1.0	LA, RWR
<i>C. acutus</i>	UF56671-D	Coastal	0.7094	-12.9	-1.1	FL, Collier Co.
<i>C. acutus</i>	UF54201-A	Coastal	0.7092	-8.0	0.4	FL, Monroe Co.
<i>C. acutus</i>	UF56580-A	Coastal	0.7095	-14.5	-0.4	FL, Monroe Co.
<i>A. mississippiensis</i>	UF87886-B	Inland	0.7093	-17.0	1.2	FL, Broward Co.
<i>A. mississippiensis</i>	UF42548-B	Inland	0.7085	-13.0	-3.5	FL, Clay Co.
<i>A. mississippiensis</i>	UW G3	Inland	0.7091	-15.4	2.4	FL, Everglades
<i>A. mississippiensis</i>	UW G2	Inland	0.7091	-16.3	2.5	FL, Everglades
<i>A. mississippiensis</i>	UF42872-A	Inland	0.7101	-11.6	-4.5	FL, Polk Co.

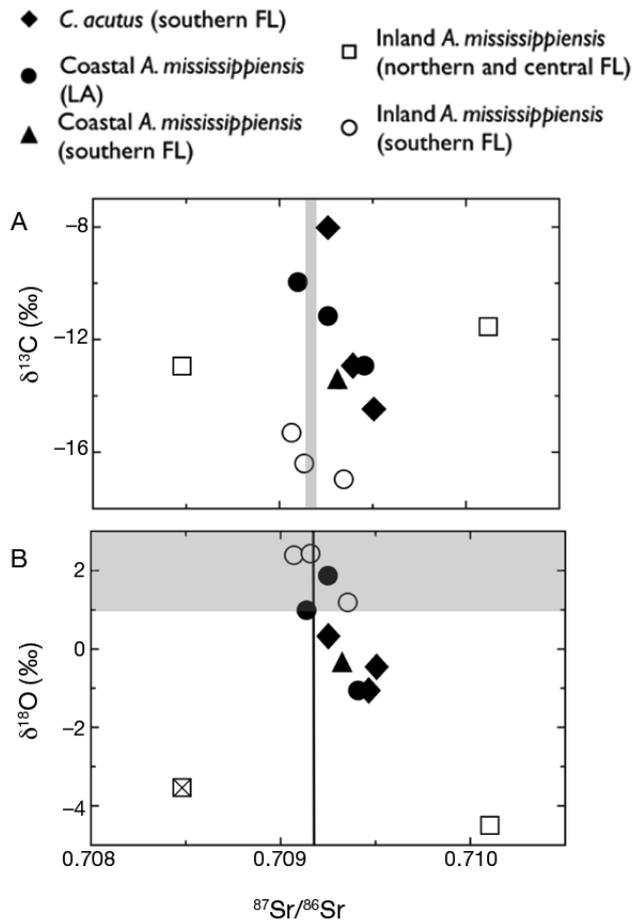


Fig. 4. *Alligator mississippiensis* and *Crocodylus acutus*.  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios versus (A)  $\delta^{13}\text{C}$  and (B)  $\delta^{18}\text{O}$  values for several crocodylians. Filled symbols are coastal individuals and open symbols are inland individuals. Vertical line at  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of 0.7092 is ratio of modern seawater. Horizontal shading in (B) indicates  $\delta^{18}\text{O}$  values likely affected by evaporation. In (A), for all coastal crocodylians there is a trend towards higher  $\delta^{13}\text{C}$  values as  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios approach 0.7092 ( $R^2 = 0.6$ ). In (B), all crocodylians (with the exception of 1 outlier ☒ from northern Florida) show a trend towards higher  $\delta^{18}\text{O}$  as  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios approach 0.7092 ( $R^2 = 0.87$ )

bedrock. African dust does reach southern FL in quantities that could label soil with  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios that average around 0.7179, substantially higher than seawater (Muhs et al. 2007, Pett-Ridge et al. 2009). This provides a plausible mechanism for having high  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in southern FL soils and therefore southern FL alligators and crocodiles with  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios higher than seawater.

Because it is likely that crocodylian Sr is largely supplied by food, not water, we were surprised by the tight correlation between  $\delta^{18}\text{O}$  values (a proxy for drinking water) and  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios ( $R^2 = 0.87$ ) when eliminating the 1 outlier from northern FL. While

there are relationships between salinity and both  $\delta^{18}\text{O}$  values and  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios, the tight linear correlation in Fig. 4B is puzzling. The relationship between salinity and  $\delta^{18}\text{O}$  value in a 2 end-member mixing scenario is a straight line, because the concentration of oxygen does not change between seawater and fresh water. The relationship between  $^{87}\text{Sr}/^{86}\text{Sr}$  and salinity, on the other hand, is typically exponential or logarithmic because Sr is an order of magnitude more concentrated in seawater than in fresh water (Bryant & Mueller 1995). Therefore, if the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of crocodylian teeth is being primarily controlled by drinking water, we would expect to see an exponential relationship between  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$ , but instead we see a linear trend. Because Sr concentration in bone does not change appreciably between marine and terrestrial environments (Burton & Price 1999), the observed linear trend supports our inference that the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio is controlled by food rather than water Sr.

We offer 2 scenarios to explain the linear correlation of  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$ . First, for coastal crocodylians (filled symbols in Fig. 4B) the influences of marine food and marine oxygen—either from food and water in the case of *Crocodylus acutus* or from food in the case of *Alligator mississippiensis*—are providing information about the same sources (marine versus terrestrial/ freshwater environment) and some correlation among all 3 tracers ( $\delta^{18}\text{O}$ ,  $\delta^{13}\text{C}$ , and  $^{87}\text{Sr}/^{86}\text{Sr}$ ) is to be expected. Second, for the inland alligators (open symbols in Fig. 4B), excepting the outlier from northern FL, the linear trend is probably a combination of latitudinal variation in  $\delta^{18}\text{O}$  value of surface waters and southern FL geology. A transect of bedrock from central to southern FL moves from Paleogene sedimentary rocks to Pleistocene limestone, and the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio shifts from  $\sim 0.7095$  to  $\sim 0.7092$  (Scott 1992, Hoppe et al. 1999). Along the same transect,  $\delta^{18}\text{O}$  values of surface waters increase because of a change in the  $\delta^{18}\text{O}$  value of precipitation and increased evaporation from north to south (Meyers et al. 1993, Gat 1996, Kendall & Coplen 2001). Note that for the  $\delta^{18}\text{O}$  values higher than  $\sim 1\text{‰}$  (grey area in Fig. 4B), it is unlikely that the source of water is marine, but more likely that evaporation has increased the freshwater  $\delta^{18}\text{O}$  value (Meyers et al. 1993).

Results from the Sr isotope analyses generally corroborate the idea that animals with higher  $\delta^{13}\text{C}$  are influenced by marine carbon. However, because southern FL bedrock and ocean water have essentially the same  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio and because our *Crocodylus acutus* data come from southern FL, we chose

to omit the Sr isotope system when estimating the contribution of marine resources to *C. acutus* (Table 3).

### Comparing different species and locations

To estimate the amount of marine resources used in *Crocodylus acutus* (see 'Marine resource use in crocodylians' below), it is necessary to compare different species from different locations. In estimating the source of the water ingested by *C. acutus*, we will compare the variability in  $\delta^{18}\text{O}$  values from the *C. acutus* population in southern FL (SD = 0.8) to the coastal population of obligate freshwater-drinking *Alligator mississippiensis*—primarily from RWR in LA (SD = 1.6)—and to the population of marine iguanas (SD = 0.6). Loggerhead turtles were excluded as a marine end-member for  $\delta^{18}\text{O}$  because the calculation involves variability, and we were concerned about biases due to differences in time averaging for teeth (short) in crocodylians and iguanas versus bone (long) in loggerhead turtles. However, addition of the loggerhead turtle data does not change the result of the analysis, as the loggerhead population has the same SD as the marine iguana population (see 'Materials and methods'). We chose to compare *C. acutus* to a population of coastal alligators (for which we have no population-level data in FL). Alternatively, *C. acutus* could be compared to an obligate freshwater-drinking reptile from nearby. If one chooses to compare *C. acutus* to southern FL inland alligators (which are geographically closer, but in our opinion, less ecologically similar), the result would shift towards *C. acutus* ingesting more marine water due to the larger SD of the southern FL alligators (SD = 2.2).

When choosing our terrestrial end-member for the food ( $\delta^{13}\text{C}$ ) mixing analysis, we cannot assume that coastal alligators are feeding exclusively in a terrestrial food web. Alligators have been sighted in the ocean, on beaches, and frequently in brackish water (Elsey 2005). Because they are not capable of drinking saline water, it is likely they are foraging on marine food in these areas. Therefore, we used the inland *Alligator mississippiensis* as our terrestrial carbon end-member ( $\delta^{13}\text{C} = -15.8\text{‰}$ ).

Choosing the marine end-member for the food mixing analysis is more nuanced. We are reluctant to compare carnivorous crocodylians to herbivorous marine iguanas because there is potential for gut fermentation of plant matter by the iguanas to raise the  $\delta^{13}\text{C}$  value of bioapatite (see 'Marine reptiles'). Therefore, we chose to compare the crocodylians to carnivorous marine turtles. This comparison is further complicated because baseline  $\delta^{13}\text{C}$  values can vary by location for marine food webs. To estimate baseline  $\delta^{13}\text{C}$  values across locations, we used sediment organic matter from the areas of interest as a proxy for the changes in relative baseline  $\delta^{13}\text{C}$  values of those food webs.

Organic sediment from the coast of southern FL has an average  $\delta^{13}\text{C}$  value of  $-18.5\text{‰}$  (Macko et al. 1984). Organic sediment off the Pacific coast of Baja has an average  $\delta^{13}\text{C}$  value of  $-20.0\text{‰}$  (Voss et al. 2001). Therefore we adjusted the Pacific loggerhead turtles by  $+2.5\text{‰}$  when comparing them to *Crocodylus acutus* from southern FL. Our sample of loggerhead turtles is broad ( $n = 31$ ), but relatively distant from southern FL, and problems can arise when comparing even the same species from different ocean basins (Wallace & Saba 2009). To mediate the effects of 2 ocean basins and distance in general, we

Table 3. *Crocodylus acutus*. Values and results for the mixing equation  $C = M(X) + T(1-X)$  (Eq. 2, see 'Results and Discussion') to estimate the amount of marine resources consumed by *C. acutus* in southern Florida (FL). Note that for oxygen, the mixing equation uses variability (1 SD) for each population's  $\delta^{18}\text{O}$  values, and for carbon, the equation uses mean  $\delta^{13}\text{C}$  values (‰ relative to V-PDB). Error (1 SD) on  $\delta^{13}\text{C}$  marine contribution estimate derived from IsoError (Phillips & Gregg 2001) using inputs from Table 1. LA: Louisiana

	Marine end-member	Marine isotope value ( $M$ )	Terrestrial end-member	Terrestrial isotope value ( $T$ )	<i>C. acutus</i> isotope value	% Marine contribution ( $X$ )
$\delta^{18}\text{O}$ ‰ SD (water)	Marine iguanas	0.6	Coastal LA and FL alligators	1.6	0.8	80
$\delta^{13}\text{C}$ ‰ mean (food)	Adjusted average of sea turtles from this study and Biasatti (2004). See 'Results and discussion'	-6.6	Inland alligators	-15.8	-9.6	65 ± 9

averaged our data set with  $\delta^{13}\text{C}$  carbonate values from leatherback turtles *Dermochelys coriacea* primarily collected in Guyana from Biasatti (2004) ( $\delta^{13}\text{C} = -9.6 \pm 2.2$ ,  $n = 5$ ). Organic sediments off the coast of Guyana average  $-21.0\text{‰}$  (Medina et al. 2005), and we adjusted the leatherback  $\delta^{13}\text{C}$  values accordingly ( $+3.5\text{‰}$ ) for comparison with *C. acutus* from southern FL.

### Marine resource use in crocodylians

We used simple 2 end-member linear mixing equations to estimate the percent of marine resource use by *Crocodylus acutus*. We assumed that the animals have only 2 inputs (marine and terrestrial) and then calculated the proportional contribution of the marine end-member ( $X$ ) to *C. acutus* isotope values. The equation takes the following form:

$$C = M(X) + T(1-X) \quad (2)$$

where  $C$  is the isotopic value for *C. acutus*,  $M$  is the isotopic value for the marine end-member, and  $T$  is the isotopic value for the terrestrial end-member. Table 3 contains the end-members we selected as well as their isotopic values and the calculated percent of marine contribution to *C. acutus*.

Our approach to carbon isotope mixing is straightforward, and follows many prior studies (e.g. Phillips & Koch 2002). Our approach to oxygen isotope mixing, in contrast, is not standard and merits further comment. We did not use mean  $\delta^{18}\text{O}$  values in our mixing model because we suspected that inland alligators have variably  $^{18}\text{O}$ -enriched values due to latitudinal and evaporative effects. Instead we used the  $\delta^{18}\text{O}$  SD as a proxy for ingestion of marine versus fresh water. While not an 'input' to an individual animal with a defined isotopic value, such as food carbon or water oxygen, variance at the population level is a characteristic that shows a clear and predictable difference between marine and terrestrial populations.

We estimated that the average *Crocodylus acutus* ingests 80% seawater (either via drinking or from food or metabolic water), based on the  $\delta^{18}\text{O}$  SD mixing model. Given that we calculated this number based upon the variance that the population exhibits in  $\delta^{18}\text{O}$  value, there is currently no way to estimate the error envelope surrounding our calculated value of seawater consumed. The estimate of 80% seawater consumption could manifest itself in a multitude of ways. The 2 extreme cases are (1) that the average *C. acutus* ingests seawater 80% of the time and

freshwater 20% of the time, or (2) that *C. acutus* ingests water that is an 80/20 mix of seawater/freshwater 100% of the time. Obviously, a scenario between those 2 possibilities is most likely. If our results indicate direct ingestion of seawater, the value is high relative to published estimates for Crocodylidae. Though no prior study has attempted to measure the actual intake of salt water by *C. acutus* in the field, most researchers argue that while *C. acutus* are often present where salinities are high, they probably do not consume this water, but instead imbibe when they return to low-salinity water (Dunson 1982, Mazzotti & Dunson 1984, 1989, Kushlan & Mazzotti 1989, Jackson et al. 1996). However, most assumptions about low saltwater tolerance in Crocodylidae are derived from laboratory studies on small individuals or hatchlings (e.g. Dunson 1970, Evans & Ellis 1977). In contrast, Dunson (1982) found that adult *C. acutus* could remain healthy for extended periods of time in water salinities as high as 26 psu (74% seawater), perhaps indicating a greater tolerance for ingestion of saltwater.

Alternatively, the bulk of the  $\delta^{18}\text{O}$  signal in *Crocodylus acutus* could be from water included within or derived from food. If the prey is marine, then ultimately that water is sourced from the ocean and will have a  $\delta^{18}\text{O}$  value close to 0‰. However, this water is potentially less salty than seawater. Many estuarine and marine crustaceans maintain high hemolymph concentrations of salinity ranging from 70% that of seawater to iso-osmotic with seawater (Mantel & Farmer 1983). Marine 'fish' maintain blood salt concentrations from about 15 to 50% that of ocean water (Ditrich 2007). Although crocodylians may ultimately be finding some sources of water that are less than the 35 psu of seawater in the ocean (potentially through prey body-water), this study is primarily concerned with the ability of crocodylians to be ecologically divorced from freshwater ecosystems. Thus, our result of 80% marine water consumption should not necessarily be viewed as commentary on the salt-excreting physiological abilities of *C. acutus*, but rather as a percentage of time that *C. acutus* could be acting as a functional marine reptile living independently of a freshwater ecosystem.

Our estimate for the percentage of marine carbon derived through diet for the average *Crocodylus acutus* is  $65 \pm 9\%$  (error on the mixing model estimate was calculated using IsoError; Phillips & Gregg 2001). Comparisons of this value with more traditional studies of *C. acutus* diet (e.g. gut content analysis) are problematic. Many gut content studies report data as a percentage of the number of preda-

tor stomachs in which a particular prey item was found. Unfortunately, this is the case for both stomach content studies done for *C. acutus* at coastal sites (Seijas 1988, Thorbjarnarson 1988b). This data set does not control for the fact that large prey items, even if only found in a small percentage of stomachs, could be more important dietary items than small ubiquitous prey items like insects. Nonetheless, our estimate of 65% marine diet is plausible in the context of these studies. Thorbjarnarson (1988b) reported 71% of crocodile stomachs containing crustaceans, which were predominately the marine crab *Uca bergersii*, whereas the vast majority of other prey comprised smaller insects. Seijas's (1988) data are more difficult to compare. In that study, *C. acutus* was shown to be highly reliant on crabs and fish, especially in larger animals, but no information was given regarding the freshwater or marine status of the prey for the coastal sites analyzed.

The estimate of a 65% marine diet based on carbon isotope mixing is lower than our estimate for marine influence on ingested water (80%). The apparent implication is that *Crocodylus acutus* drinks in saltier water than it feeds, which would be curious behavior. But the reality is that terrigenous plumes can extend far from the mouths of rivers in FL (e.g. Jolliff et al. 2003) carrying terrestrial carbon with them, and in Florida Bay (a nesting area for the *C. acutus* population), sediments can contain as much as 60% terrestrial (rather than marine) organic matter (Xu et al. 2006), which can provide an allochthonous source of carbon to the marine food web. Therefore, *C. acutus* could easily receive both its food and water from 1 geographic area without receiving equal marine inputs from each. Note that this dietary paradox of a high percentage of terrestrial dietary items is seemingly supported by the crocodile Sr isotope results, which are higher than marine values (Table 2, Fig. 4). This is most likely due to our small sample size coupled with individual variation. The 3 crocodiles sampled for Sr analysis had the lowest  $\delta^{18}\text{O}$  values of the crocodiles analyzed in this study, and 2 of the 3 (UF 56580 and UF 56671D) had lower than average  $\delta^{13}\text{C}$  values (Table A1 in Appendix 1) and are therefore probably habitually more terrestrial than average.

Our results indicate that *Crocodylus acutus* and coastal *Alligator mississippiensis* are eating similar amounts of marine food,  $65 \pm 9\%$  and  $77 \pm 20\%$ , respectively. When calculating the *A. mississippiensis* mixture, the sea turtles are adjusted to the marine sediment in the northwest Gulf of Mexico:  $\delta^{13}\text{C} = -20.6$  (Macko et al. 1984; see 'Comparing different species and locations'). This adjustment results in a

higher estimate of marine food than that of *C. acutus* (though within error) despite a lower mean  $\delta^{13}\text{C}$  value. Although several gut content studies have been done on coastal LA alligators (e.g. McNease & Joanen 1977, Wolfe et al. 1987, Elsey et al. 1992), comparisons of our isotopic data to the gut content analyses are not straightforward due to gut contents typically being binned by large phylogenetic rather than ecological groups (e.g. 'fish' may include freshwater and marine species). Gut content studies are wide ranging with respect to preferred prey items in their reported results. Elsey et al. (1992) and some surveys of McNease & Joanen (1977) would fall within our estimate's error envelope of percent reliance on marine prey (~65–70% and ~60%, respectively); other surveys from McNease & Joanen (1977) and Wolfe et al. (1987) would indicate much heavier reliance on aquatic mammals (specifically nutria) than marine fish or crustaceans.

In contrast to the hypothesis of Dunson & Mazzotti (1989), our data suggest that levels of incidental ingestion of seawater during feeding must be quite low, otherwise we would expect a comparable degree of variance in  $\delta^{18}\text{O}$  values between crocodiles and alligators from the same region. Likewise, despite the fact that these animals ingest prey body-water that must have a marine  $\delta^{18}\text{O}$  value (which presumably makes the mean tooth carbonate  $\delta^{18}\text{O}$  values more similar between the 2 populations), the difference in variance between coastal alligators and crocodiles suggests that this prey body-water makes only a minor contribution to the oxygen and water mass balance of these animals. Our data cannot exclude the possibility that in an estuarine environment, marine organisms could make their way into waters of varying salinity, resulting in *Alligator mississippiensis* consuming foods similar in  $\delta^{13}\text{C}$  value to *Crocodylus acutus* but in lower salinity waters. In any case, alligator populations in coastal settings are using fresh water to an extent that coastal populations of *C. acutus* are not—even when the amounts of marine food being consumed are comparable.

The similar amounts of marine food in the diets of the 2 species imply that where their ranges overlap, there could be significant competition for resources and perhaps space. This possibility has largely been unexplored, probably because the area where these 2 animals' ranges overlap is small compared to the full range of each species. However, the range of *Crocodylus acutus* in FL appears to be thermally constrained (Kushlan & Mazzotti 1989). Yet if rapid climate change in the future results in warmer winters, *C. acutus* population ranges in FL could expand. If

this happens, we have little knowledge of how *C. acutus* and *Alligator mississippiensis* would interact competitively.

## CONCLUSIONS

Our results support the conclusion of the study of Clementz & Koch (2001) on mammals, which suggested that mean  $\delta^{18}\text{O}$  values offer a weak monitor of marine versus terrestrial water consumption. However, as in Clementz & Koch (2001), the standard deviations around the mean  $\delta^{18}\text{O}$  values are informative. In reptiles, populations of animals consuming seawater (directly or via food) have lower variance than animals that consume fresh water.

We estimated that 80% of the water ingested by the average *Crocodylus acutus* in southern FL is marine water, although this estimate may include water derived from marine prey, which can be of lower salinity than seawater. The average southern FL *C. acutus* contains  $65 \pm 9\%$  prey items originating in the marine environment. Estimates of marine food use by *C. acutus* and *Alligator mississippiensis* are comparable. We hypothesize that significant competition for resources may occur in areas where these species overlap. Although that area is currently small, climate change could expand the range of *C. acutus* in FL, and further study of competitive interactions between the 2 American crocodylians is warranted. Our estimates of marine resource use by *C. acutus* in southern FL is high compared to prior hypotheses, but *C. acutus* is observed in waters that are 28 psu or higher, and laboratory data confirm that adult *C. acutus* can remain healthy in waters of approximately this salinity. According to our data, it appears that extensive behavioral osmoregulation by frequently returning to fresh water to drink or avoiding drinking water containing more than 10 psu is not required of *C. acutus*. For much of the time, *C. acutus* may be capable of being ecologically isolated from freshwater ecosystems.

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## Appendix 1

Table A1. *Alligator mississippiensis* and *Crocodylus acutus*. Summary information for the crocodylians analyzed in this study. ID number followed by 'avg' indicates that multiple teeth were analyzed and averaged to achieve the reported  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values. TL: total length; n: number of teeth analyzed per individual; na: not available; LA: Louisiana; N. (S.) FL: northern (southern) Florida. Some UF specimens have size or weight data associated with their entries in the Florida Museum of Natural History catalog

ID	Size class	TL (m)	$\delta^{13}\text{C} \pm \text{SD}$	$\delta^{18}\text{O} \pm \text{SD}$	$^{87}\text{Sr}/^{86}\text{Sr}$	Habitat	Location	n
<b><i>A. mississippiensis</i></b>								
RWR2	Adult <sup>a</sup>	na	-8.2 ± 0	-0.9 ± 0	-	Coastal	LA	1
RWR3	Adult <sup>a</sup>	na	-7.6 ± 0	-1.8 ± 0	-	Coastal	LA	1
RWR4	Adult <sup>a</sup>	na	-7 ± 0	-2.3 ± 0	-	Coastal	LA	1
RWR5	Adult <sup>a</sup>	na	-12 ± 0	-3.1 ± 0	-	Coastal	LA	1
RWR6	Adult <sup>a</sup>	na	-7.7 ± 0	-1.7 ± 0	-	Coastal	LA	1
RWR7	Adult <sup>a</sup>	na	-12.2 ± 0	-3.8 ± 0	-	Coastal	LA	1
RWR8	Adult <sup>a</sup>	na	-7.3 ± 0	-1.9 ± 0	-	Coastal	LA	1
RWR9	Adult <sup>a</sup>	na	-7.6 ± 0	0.3 ± 0	-	Coastal	LA	1
RWR 10 avg	Adult	2.0	-10.1 ± 0.8	-0.3 ± 0.6	0.7091	Coastal	LA	19
RWR 13 avg	Juvenile	1.1	-10.7 ± 0.6	2 ± 0.7	-	Coastal	LA	2
RWR 17 avg	Subadult	1.3	-12.6 ± 1.1	1.4 ± 1.2	0.7092	Coastal	LA	3
RWR 21 avg	Juvenile	0.9	-15.1 ± 0.8	-0.8 ± 0.0	-	Coastal	LA	2
RWR 28 avg	Subadult	1.3	-11.1 ± 1.6	1.9 ± 0.2	-	Coastal	LA	2
RWR 30 avg	Adult	2.4	-12.9 ± 0.1	-1 ± 0.8	0.7095	Coastal	LA	2
UF 35153 avg	Adult	na	-9.3 ± 0.3	-1.4 ± 0.4	-	Coastal	LA	3
UF 10941 avg	Adult	na	-13.6 ± 4.8	-0.4 ± 0.1	0.7093	Coastal	S. FL	2
UF 9225	Adult	na	-15.5 ± 0	-0.3 ± 0	-	Inland	N. FL	1
UF 34886	Adult	na	-17.4 ± 0	-0.5 ± 0	-	Inland	N. FL	1
UF 42548B	Adult	na	-13 ± 0	-3.5 ± 0	0.7085	Inland	N. FL	1
UF 42872B	Adult	na	-11.6 ± 0	-4.5 ± 0	0.7101	Inland	S. FL	1
UF 105541 avg	Adult	na	-17.1 ± 0.4	0.4 ± 0.7	-	Inland	S. FL	3
UF 84197 avg	Adult	na	-16.7 ± 1.0	-1.2 ± 1.7	-	Inland	S. FL	3
UF 87886 avg	Adult	na	-17 ± 1.0	1.2 ± 1.2	0.7093	Inland	S. FL	3
UF 98341	Adult	na	-17.6 ± 1.0	-1 ± 0.4	-	Inland	S. FL	3
UW G1	Subadult <sup>b</sup>	na	-15.6 ± 0	0.7 ± 0	-	Inland	S. FL	1
UW G2	Subadult <sup>b</sup>	na	-16.3 ± 0	2.5 ± 0	0.7091	Inland	S. FL	1
UW G3	Subadult <sup>b</sup>	na	-15.4 ± 0	2.4 ± 0	0.7091	Inland	S. FL	1
UW G4	Subadult <sup>b</sup>	na	-15.7 ± 0	1.6 ± 0	-	Inland	S. FL	1
<b><i>C. acutus</i></b>								
UF 33655 avg	Adult	na	-10.9 ± 0.1	1.1 ± 0.5	-	Coastal	S. FL	2
UF 54201 avg	Adult	na	-8 ± 0.3	0.3 ± 0.6	0.7092	Coastal	S. FL	4
UF 56428 avg	Adult	na	-6.8 ± 0.4	0.6 ± 0.4	-	Coastal	S. FL	2
UF 56429 avg	Adult	na	-2.5 ± 1.6	1.4 ± 0.2	-	Coastal	S. FL	4
UF 56580 avg	Adult	na	-14.5 ± 0.9	-0.4 ± 0.3	0.7095	Coastal	S. FL	3
UF 63929 avg	Adult	na	-14.3 ± 1.0	1.12 ± 0.8	-	Coastal	S. FL	3
UF 66034 avg	Adult	na	-8.3 ± 0.0	0.4 ± 0.3	-	Coastal	S. FL	2
UF 98068 avg	Adult	na	-8.3 ± 1.4	1.2 ± 1.2	-	Coastal	S. FL	4
UF 56671D	Adult	na	-12.8 ± 0	-1 ± 0	0.7094	Coastal	S. FL	1
<sup>a</sup> Field estimate								
<sup>b</sup> Tooth size comparison estimate								

## Appendix 2. Information from an individual versus the population

Because crocodylians replace their teeth continuously, it is possible that analysis of multiple teeth from 1 individual could supply as much information about a population as analysis of a single tooth from multiple individuals, assuming that the individual behaved in a manner that was representative of the population as a whole. There is growing recognition of the importance of individual specialization, however, in shaping the ecology of a population and the dynamics of food webs (Bolnick et al. 2003). Furthermore, crocodylians can travel large distances (Rodda 1984, Kushlan & Mazzotti 1989, Kay 2004, Brien et al. 2008), and mere proximity does not ensure similarity in diet, migratory habits, or other aspects of ecology. To assess the possibility of using multiple teeth from single individuals to bolster sample size, we analyzed the  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of teeth from 14 alligators from Rockefeller Wildlife Refuge (RWR) in coastal Louisiana (Table A2). For 1 alligator (RWR 10), we analyzed 19 teeth (Table A3), and then compared within-individual to within-population variance. RWR 10 was included in the population statistics, but the values from the 19 teeth were averaged to give a single value for that individual. Two-sample *F*-tests for variance were performed for  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values. In both statistical analyses,  $p < 0.01$ , indicating that the level of variance present in the 2 populations is different. Isotopic values from multiple samples from 1 individual were significantly less variable than values for single teeth from different individuals. Therefore, it is not reasonable to bolster sample size by incorporating multiple teeth from the same individual.

Table A2. *Alligator mississippiensis*. Isotope data from 14 individual alligators from Rockefeller Wildlife Refuge (RWR), Louisiana (note UF specimen was collected at RWR but is curated at the University of Florida)

Individual	$\delta^{13}\text{C}$ (‰)	$\delta^{18}\text{O}$ (‰)
RWR 2	-8.2	-0.9
RWR 3	-7.6	-1.8
RWR 4	-7.0	-2.3
RWR 5	-12.1	-3.1
RWR 6	-7.7	-1.7
RWR 7	-12.2	-3.8
RWR 8	-7.3	-1.9
RWR 9	-7.6	0.3
RWR 13	-10.7	2.0
RWR 21	-15.1	-0.8
RWR 28	-11.1	1.9
RWR 30	-12.9	-1.0
UF 35153	-9.3	-1.4
RWR 10 (mean)	-10.1	-0.3
Mean	-9.9	-1.1
SD	2.5	1.7

Table A3. *Alligator mississippiensis*. Isotope data from 19 teeth from 1 individual alligator from Rockefeller Wildlife Refuge (RWR)

Tooth	$\delta^{13}\text{C}$ (‰)	$\delta^{18}\text{O}$ (‰)
RWR 10A	-8.9	-0.9
RWR 10B	-10.1	-0.8
RWR 10C	-11.1	1.7
RWR 10D	-9.4	0.3
RWR 10E	-9.6	-0.3
RWR 10F	-9.8	-0.6
RWR 10G	-9.4	-0.8
RWR 10H	-9.5	-0.5
RWR 10I	-9.5	-0.6
RWR 10J	-10.2	-0.3
RWR 10K	-11.1	-0.4
RWR 10L	-11.1	-0.6
RWR 10M	-10.0	-0.1
RWR 10N	-9.2	-0.5
RWR 10O	-9.9	1.0
RWR 10P	-11.5	-0.5
RWR 10Q	-10.4	-1.3
RWR 10R	-10.3	-0.5
RWR 10S	-11.4	-0.2
Mean	-10.1	-0.3
SD	0.8	0.6

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