LIVING ON THE EDGE: TROPHIC ECOLOGY OF *ALLIGATOR MISSISSIPPIENSIS* (AMERICAN ALLIGATOR) WITH ACCESS TO A SHALLOW ESTUARINE IMPOUNDMENT

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ABSTRACT

I used a combination of stomach content and stable isotope analyses to examine intrapopulation and temporal variation in use of estuarine prey resources by *Alligator mississippiensis* (American Alligator) with access to a shallow estuarine impoundment located on the northeast Atlantic coast of Florida. I used a multi-tissue stable isotope approach to examine temporal trends in trophic interactions. This study took place within the Guana River Wildlife Management Area located in Ponte Vedra, Florida. From 2010 to 2012, I collected stomach contents from 44 *A. mississippiensis* and stable isotope samples from a total of 127 individuals. Stomach contents indicated the principal prey taxa consumed on a short-term basis were invertebrates (i.e., insects and crustaceans) and small baitfish. Individuals of all sizes used estuarine, as well as freshwater prey resources; however, the importance of estuarine prey to the diet increased through ontogeny. Juvenile and sub-adult stomach contents predominantly contained freshwater insects, while adult diets mainly contained estuarine baitfish and crustaceans. I used stable carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) measured in three tissues (blood plasma, red blood cells, scute keratin) differing in turnover rates to assess overlap and temporal variation in the isotopic niche and inferred trophic interactions of *A. mississippiensis* sub-populations. Isotopic niche and inferred trophic interactions of *A. mississippiensis* varied among size classes, sexes, years, and capture habitats. Overlap in isotopic niches of sub-populations was highest between sexes of similar body size and for individuals captured in similar habitats within the same years. Temporal shifts in trophic interactions were most prevalent for juveniles and sub-adults, while adults demonstrated a higher degree of temporal stability in trophic interactions and niche specialization. These findings represent one of the few studies to examine intrapopulation variation in use of estuarine prey resources by *A. mississippiensis*. Results of this study should be useful to habitat managers designing and implementing conservation programs for coastal ecosystems in the southeastern United States, especially in light of the expected alterations in coastal habitat structure due to sea level rise and global climate change.

Key words: *Alligator mississippiensis*, crocodilian, Florida, habitat connectivity, niche specialization, salt marsh, stable isotope analysis, trophic interactions.

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INTRODUCTION

Highly mobile top predators, particularly those that can attain high population densities, can have strong effects on ecosystem structure and function through their interactions with prey, translocation of acquired nutrients, and by connecting disparate habitats (Terborgh and Estes, 2010; Schmitz et al., 2010; Rosenblatt et al., 2013a). The American Alligator (*Alligator mississippiensis*) is an abundant large-bodied, top predator inhabiting the Atlantic Coastal Plain of the southeastern United States, ranging from the Rio Grande River basin in Texas to the Albermarle Sound in North Carolina (Ross and Ernst, 1994; Powell et al., 2016). *Alligator mississippiensis* uses a wide variety of freshwater habitats including lakes, ponds, rivers, wetlands, swamps, and marshes (Ross and Ernst, 1994). In addition, although lacking the lingual salt-secreting glands in species of true crocodiles (e.g., *Crocodylus porosus*-Taplin et al., 1981; *C. acutus*-Taplin et al., 1982), *A. mississippiensis* frequent brackish (salinity 5–30 ppt) to marine (salinity >30 ppt) habitats. They include tidal rivers and creeks, salt marshes, mangroves, estuaries, and near-shore ocean waters (hereafter, estuarine habitats) throughout their native range (Chabreck, 1971; Birkhead and Bennett, 1981; Jacobson, 1983; Tamarack, 1989; Elsey, 2005; Rosenblatt and Heithaus, 2011; Rosenblatt et al., 2013b; Fujisaki et al., 2014, 2016).

Alligator mississippiensis occurs along nearly 1,500 km of the southeastern United States coastline where they have the potential to forage from freshwater into estuarine habitats. This behavior has historically received little attention because *A. mississippiensis* lacks specialized means for mitigating detrimental physiological effects of exposure to high salinities (Taplin et al., 1982; Laurén, 1985; Mazzotti and Dunson, 1989). Recently, however, multiple researchers have demonstrated that *A. mississippiensis* have the potential to provide several important ecologic functions as top predators in coastal ecosystems, chiefly by providing functional linkages between disparate ecosystems and exerting top-down pressures on important salt marsh mesopredators (Rosenblatt and Heithaus, 2011; Nifong and Silliman, 2013; Rosenblatt et al., 2013a; Fujisaki et al., 2016). However, strength and relative importance of these functions for coastal ecosystems and their inhabitants is contingent on prevalence of and variation in cross-ecosystem estuarine foraging behaviors by coastal *A. mississippiensis* populations (Nifong et al., 2015). Therefore, understanding intrapopulation and temporal variation in use of estuarine prey by *A. mississippiensis* will assist in designing comprehensive and effective conservation strategies for coastal ecosystems in the southeastern United States.

Stable isotope analysis (SIA), most often of the elements carbon (C) and nitrogen (N), is increasingly being used by ecologists to examine trophic interactions of consumers and assess food web structure (Layman et al., 2012). Isotopic composition of a consumer's tissues closely reflects the isotopic composition of their diet over the time period of tissue generation (Peterson and Fry, 1987). The ratio of ^{15}N to ^{14}N (expressed relative to a standard, $\delta^{15}N$) is often used to infer trophic position due to the preferential excretion of lighter ^{14}N during protein synthesis and resulting step-wise enrichment of ^{15}N in consumer tissues up food chains ($\Delta^{15}N_{tissue\text{-}diet} \approx 3.4 \pm 1‰$, Post, 2002). In addition to informing trophic position, $\delta^{15}N$ can also be used to indicate habitat use since primary producer $\delta^{15}N$ values (i.e., baseline for calculating trophic position) are dependent on $\delta^{15}N$ values of nitrogen sources, nitrogen concentrations in the environment, and relative rates of denitrification/nitrification within the nitrogen pool, all of which can vary depending on habitat type and location (Fry, 2006). The ratio of ^{13}C to ^{12}C (expressed relative to a standard, $\delta^{13}C$) is often used to indicate use of particular carbon pools or habitats, since $\delta^{13}C$ varies among primary producers with different photosynthetic pathways (i.e., C3, C4, CAM), as well as habitat type, and changes relatively little during incorporation into consumer tissues ($\Delta^{13}C_{tissue\text{-}diet} \approx 0 \pm 1‰$, Deniro and Epstein, 1978; O'Leary, 1981).

Together, $\delta^{13}C$ and $\delta^{15}N$ values are used by ecologists to define an organism's or a population's

isotopic niche (δ-space, bivariate space created by plotting an organisms isotope values) which has been proposed as an extension of the n-dimensional hypervolume describing an organism's realized ecological niche (Hutchinson, 1957; Newsome et al., 2007). This extension is possible through transformation of δ-values (raw isotope data) using isotopic mixing models into estimates of dietary proportions (p), essentially redefining δ-space to represent differences in resource use (p-space, Newsome et al., 2007). Transformation from δ- to p-space allows researchers to infer similarities and differences in use of resources based solely on size and location of the isotopic niche in δ-space. Changes or differences in position and size of a consumer's isotopic niche within δ-space serves as a proxy for changes or differences in a consumer's dietary and ecological niches (Jackson et al., 2011). Decreases in isotopic niche size can be interpreted as increased specialization on or loss of particular resources, while increases in the size of the isotopic niche represents the broadening of a consumer's dietary or ecological niche (Layman et al., 2012). In addition, overlap in the isotopic niche among species, populations, or sub-populations represents similarity in trophic interactions and ecological niches of the groups being compared. By measuring stable isotope values of various tissues from the same individual that differ in turnover rate (i.e., time it takes for complete breakdown and replacement of molecules within a tissue), it is possible to compare a consumer's trophic interactions over different time scales (Tieszen et al., 1983; Dalerum and Angerbjörn, 2005).

Isotopic turnover rates of *A. mississippiensis* tissues are relatively slow when compared to other organisms (Rosenblatt and Heithaus, 2013). Of the tissues studied thus far, blood plasma has the fastest complete turnover, roughly 250 days for both stable carbon and nitrogen isotopes. Complete turnover of red blood cells are 2–4 times longer than blood plasma (566 days for $\delta^{13}C$ and 1,109 for $\delta^{15}N$), and turnover of scute tissue is the longest for $\delta^{13}C$ (590 days) and intermediate for $\delta^{15}N$ (414 days). When compared with 'snap-shot' data such as stomach contents that integrate dietary interactions over the previous two weeks to one month, the temporal dynamics of *A. mississippiensis* trophic interactions can be evaluated and characterized.

Here, I employ a multidimensional approach to examine intrapopulation and temporal variation in use of estuarine prey by *A.* mississippiensis in freshwater habitats with access to an estuarine impoundment on the northeast Atlantic coast of Florida. Specifically, I use a combination of stomach content analysis (SCA) and multi-tissue SIA to address the following questions on use of estuarine prey and aspects of *A. mississippiensis* trophic ecology: 1) To what extent does use of estuarine prey vary by size class, sex, and capture habitat? 2) Is *A. mississippiensis* a dietary specialist or generalist at the sub-population level? 3) What is the amount of overlap in the isotopic niches of *A. mississippiensis* sub-populations? 4) To what extent does use of estuarine prey and the isotopic niche of *A. mississippiensis* sub-populations vary temporally? I compare my results to recent findings from other coastal *A. mississippiensis* populations and discuss the potential mechanisms driving the observed patterns and their ecological implications.

MATERIAL AND METHODS

Site description

Guana Lake (30.086603° N, 81.3434877° W), a 930 ha estuarine impoundment located within the 4,900 ha Guana River Wildlife Management Area (GRWMA) in Ponte Vedra, Florida, was created in 1957 by damming the upper reaches of the tidal Guana River (Fig. 1A, 1B). The shallow (mean water depth ~2 m) estuarine impoundment supports dense mats of submerged aquatic vegetation, chiefly Widgeon grass (*Ruppia maritima*) and various macroalgae. It is bordered by a variety of freshwater and brackish adapted macrophytes in the northern reaches and salt marsh plants such as smooth cordgrass (*Spartina alterniflora*) and black needle rush (*Juncus roemerianus*) in the southern reaches. Guana Lake and the freshwater wetlands located within the surrounding forest matrix support robust populations of small baitfish (e.g., mullet, mosquitofish, top-minnows), game fish, wading birds, migratory waterfowl, small

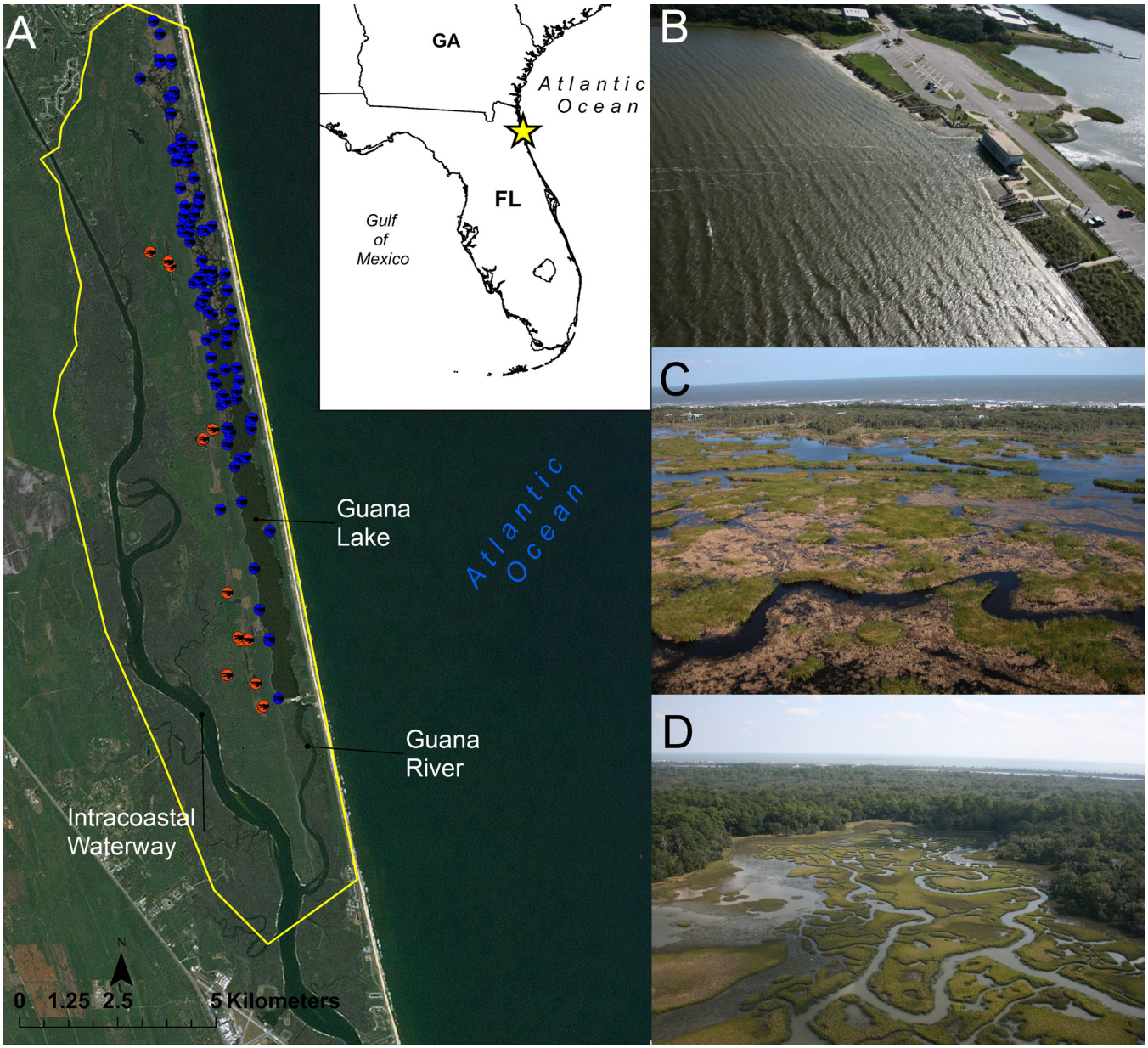

Figure 1. A. Map of study area. Blue symbols are estuarine and orange symbols are freshwater capture locations for individual *A. mississippiensis* within GRWMA (many capture locations overlap). The yellow polygon outlines the boundary of the northern component of the Guana Tolomato Matanzas National Estuarine Research Reserve which includes GRWMA. Inset map shows the general location of study area along the Florida coastline, denoted by the yellow star. B. Aerial photo of the water control structure separating Guana River and Guana Lake. C. Aerial photo of Guana Lake (foreground) and Atlantic Ocean (background). D. Aerial photo of Guana Lake (foreground) and adjacent forested peninsula (background). Photographs courtesy of Justin Ellenberger.

mammals, reptiles, amphibians, and a diverse assemblage of invertebrate taxa (e.g., decopod crustaceans, gastropods, bivalves, Frazel, 2009). Within Guana Lake, a north-south salinity gradient is maintained by freshwater inputs (~0 ppt salinity) from rainwater runoff to the north and saline water (15–34 ppt) inputs from the tidal Guana River to the south. The magnitude and extent of the salinity gradient is heavily influenced by the amount of precipitation, temperature, and evaporation. In drought conditions, water salinity can reach as high as 54 ppt and average ~40 ppt throughout the impoundment (JCN, pers. obs.). Guana Lake is bordered by the Atlantic Ocean to the east; a forested peninsula separates the impoundment from the Intracoastal Waterway to the west (Figs. 1C, 1D). Throughout the forested habitats on the adjacent peninsula there are numerous natural and man-made freshwater wetlands, marshes, and ponds in addition to an extensive network of ditches constructed for water drainage and mosquito control (Frazel, 2009; Means et al., 2009). In total, the peninsula supports 134 ephemeral freshwater wetlands ranging in hydroperiod from 50 to 200 days (Means et al., 2009). Other than a small number of drainage ditches servicing semi-permanent ponds and wetlands, all freshwater habitats are essentially isolated from the surrounding estuarine habitats, including the Guana Lake impoundment. For purposes of this study, individuals captured anywhere within the Guana Lake impoundment were considered estuarine captures and individuals captured in transit between or within freshwater habitats were considered freshwater captures.

Animal capture, tissue and fluid collections

I captured individual *A. mississippiensis* opportunistically at night and during the day using standard collection methodologies, dependent on size and situation (e.g., hand capture, noose pole, snag hook). I collected blood samples (2–15 ml dependent on body size) from the internal jugular vein posterior to the terminus of the cranial table using a sterile syringe, transferred to Lithium Heparin coated vacutainers, and placed on ice until further processing. Following blood sampling, I took morphometric measurements (head length [HL] , head width [HW], snout-to-vent length [SVL], total length [TL], and tail girth [TG]) to the nearest 0.01 cm, determined sex by cloacal examination and/or palpation, and when possible measured body mass to nearest 0.01 kg using a spring scale (Pesola® AG, Schindellegi, Switzerland). I marked individuals and collected keratinized scute tissue for SIA by removing three caudal scutes, one from each of the three caudal tail whirls, with a sterile scalpel. The particular cutting pattern followed a numerical sequence to provide a three-digit identification number for each individual captured. I placed removed scutes immediately on ice then transferred them to -10 °C freezer until further processing for SIA.

Stomach content analysis (SCA)

I collected stomach contents from captured individuals using the hose-Heimlich technique (for a full description see Nifong et al., 2012). The hose-Heimlich technique is successful at removing 91% to 100% of prey remains and a reliable method for characterization of short-term food habits over the past two weeks to one month (Rice, 2004; Nifong et al., 2012). Stomach contents were preserved in 70% ethanol until processing. Numerical counts for prey items were assigned based on enumeration of either whole prey items or specific body portions unique to one individual for a particular prey taxon (i.e., presence of atlas bone of vertebrates or paired eye stalks of crustaceans). Gravimetric abundance measures (i.e., wet mass) closely approximates volumetric measurements and is a preferred method for many invertebrate taxon with chitinous exoskeleton such as insects (Garnett, 1985). After blotting contents on paper towels to remove excess water, I measured wet mass of prey items to the 0.01 g on a digital balance (PGL, 2002, Adam Equipment Inc., Oxford, Connecticut, USA). For purposes of this study, prey were first identified to the lowest taxonomic subdivision possible (genus or species in most cases) and then categorized as either estuarine or freshwater prey according to the species' known habitat use patterns, presence in specific habitats during isotope sampling efforts, and personal observations. I quantified diets for alligator sub-populations (i.e., size classes, capture

habitat, year) by calculating %N, %W, %FO, and percent Index of Relative Importance (%IRI) for each prey category as follows (Cortés, 1997; Liao et al., 2001):

$$\%N = \frac{100N_i}{\sum_{i=1}^{n} Ni}, \quad (1)$$

$$\%W = \frac{100W_i}{\sum_{i=1}^{n} Wi}, \quad (2)$$

$$\%FO = \frac{100FO_i}{\sum_{i=1}^{n} FOi}, \quad (3)$$

$$\%IRI = 100 \times IRI_i \sum_{i=1}^{n} IRI_i, \quad (4)$$

where n is the number of prey categories identified for a sub-population, W_i and N_i are the total wet mass and number of individuals of prey i in a sub-population, respectively, FO_i is the number of stomach contents containing prey i in a sub-population divided by the total number of individuals sampled in a sub-population, and $IRI_i = \%FO_i(\%W_i + \%N_i)$.

Stable isotope analysis (SIA)

I sampled representative primary producer and potential prey species opportunistically during 2010–2012 within GRWMA to determine habitat-specific isotope signatures and calculate end member (prey resource categories) parameter values (i.e., means and standard deviations) to be used in isotopic mixing model analyses. From freshwater wetlands I collected samples from the following primary producers: macroalgae, particulate organic matter (POM), *Quercus virginiana* (Live oak), and Cyperaceae (Sedge grasses). From estuarine habitats I sampled macroalgae, POM, *S. alterniflora*, and *R. maritima*. I collected live green tissue from 10–15 individual plants or clumps in the case of macroalgae using scissors or by hand and combined all material to form aggregate samples. I placed all samples on ice at time of collection and transferred to a -10° C freezer until further processing. Prior to SIA, I carefully washed all plant tissues with deionized water, gently rubbed samples free of any detritus, soil, or epiphytes, and dried samples at 60° C for 48 h. I ground and homogenized dried samples to fine powder using a Wiley Mill (Thomas Scientific, Swedesboro, New Jersey, USA).

I collected representative prey item samples using dip-nets, minnow traps, seine nets, single-line sampling, and obtained tissues from animals harvested by recreational hunters and fishermen. For small organisms such as grass shrimp and aquatic insects, aggregate samples consisted of 10–15 whole individuals. From larger animals such as fish, mammals, and large crustaceans, 1–3 g of bulk muscle tissue was taken from single individuals. I immediately placed all animal samples on ice and transferred to a -10° C freezer until further processing. Once thawed, I cleaned the samples with deionized water, removed indigestible portions (i.e., shell, scales, hair), and dried samples at 60° C for 48 h or until completely dehydrated. I homogenized samples by grinding to fine powder with mortar and pestle.

To examine temporal dynamics of *A. mississippiensis* trophic interactions, I measured and compared stable carbon and nitrogen isotope ratios in three body tissues (blood plasma-PL, red blood cells-RBC, keratinized epidermis of caudal scutes-SC) with different turnover rates (i.e., time it takes for complete breakdown and replacement of molecules within a tissue). I separated blood fractions (PL, RBC) by centrifuging whole blood samples for 5 min at 3,000 rpm within 24 h of collection and removing the lighter plasma layer using a sterile transfer pipet. I dried blood portions for 48 h at 60° C and homogenized to fine powder by crushing with a modified spatula as a pestle. Processing of keratinized scute samples for SIA followed methods described by Nifong et al. (2015).

I weighed and loaded approximately 500 to 800 µg of homogenized animal tissues or 1–3 mg of plant tissues into 9 × 5 mm tin capsules analysis at the University of Florida Geology Stable Isotope Laboratory, Gainesville, Florida. Analyses were performed using one of two systems either a Finnigan DeltaPlus XL isotope mass spectrometer with ConFlo III interface linked to a Costech ECS 4010 Elemental Combustion System (elemental analyzer) or Finnigan-MAT 252 isotope ratio mass

spectrometer coupled with a ConFlo II interface linked to a Carlo Erba NA 1500 CNS Elemental Analyzer. Stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) are expressed in standard per mil notation (‰):

$$\delta X(‰) = [\, R_{sample}/R_{standard} - 1 \,] \times 1000, \quad (5)$$

where X is the element of interest and R is the ratio of heavy to light isotopes ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) of the *sample* and *standard* (Vienna Pee Dee Belemnite used for $\delta^{13}C$ and Atmospheric Nitrogen-AIR for $\delta^{15}N$). Machine accuracy was measured and corrected for each 48-sample run using four measures of in-lab standard USGS-40 (l-glutamic acid) with $\delta^{13}C$ = -26.39 and $\delta^{15}N$ = -4.52. Analytical machine error for USGS-40 was 0.12±0.04 for $\delta^{15}N$ and 0.10±0.05 for $\delta^{13}C$ (mean±SD) across all runs.

Differences in concentration of lipids depleted in ^{13}C (lower $\delta^{13}C$ values) within organisms among tissues can confound the interpretation of $\delta^{13}C$ values used in trophic ecology studies (DeNiro and Epstein, 1977; Post et al., 2007). $\delta^{13}C$ of tissues rich in lipids (>5% lipid content) relative to proteins and carbohydrates are often skewed toward the more negative $\delta^{13}C$ values of lipids. Post et al. (2007) recommended lipids be extracted prior to analysis or their effect analytically corrected if the tissue's C:N is >3.5 (an indicator of tissues with more carbon-rich lipids). Mean±SD of C:N measured for *A. mississippiensis* PL, RBC, and SC samples were 3.75±0.14 (n = 47), 3.39±0.09 (n = 43), 3.22±0.11 SD (n = 34), respectively. Since the C:N values for *A. mississippiensis* tissues in this study were generally less than 3.5, I did not extract lipids or apply analytical corrections. Although mean C:N measured for PL samples was slightly greater than the recommended 3.5 threshold, I did not treat or correct PL samples for lipid content because Rosenblatt and Heithaus (2013) demonstrated lipid removal did not significantly affect $\delta^{13}C$ values of *A. mississippiensis* blood plasma.

Estimating proportional use of prey resources-Isotopic mixing model analysis

To estimate contributions of estuarine and freshwater prey to the diet of *A. mississippiensis* using isotopic data I used a Bayesian isotopic mixing model within the '*siar*' package (version 4.2) of R (hereafter, SIAR). I estimated the relative proportional contributions of prey resources to the diet of *A. mississippiensis* sub-populations and at different time-scales using cross-tissue comparisons (Parnell et al., 2010; Parnell and Jackson, 2013; Phillips et al., 2014). SIAR simulations were performed using non-adjusted isotope data and tissue-specific discrimination factors determined for *A. mississippiensis* (Rosenblatt and Heithaus, 2013). For mixing model end-members (i.e., discrete prey resource groups comprising the diet), I aggregated prey taxa into two categories (freshwater and estuarine), and calculated end-member means and standard deviations from representative prey sampled within each habitat type. Representative prey species were selected based on previous food habit studies of coastal *A. mississippiensis* populations, SCA data, and observations (Nifong et al., 2012, 2015; Rosenblatt et al., 2015). The posterior distributions produced by SIAR provided a range of plausible solutions for the proportional contribution of prey resource categories (end-members) to the diet of a consumer and incorporated variation in isotope values of both consumer and prey resources. It also incorporates variation in discrimination factors to provide robust estimates of resource-to-diet proportional contributions. I conducted SIAR model simulations separately for each tissue type and sub-population. Each simulation consisted of 500,000 iterations, a burn-in interval of 50,000, and thin-by interval of 15, resulting in a posterior distribution of 30,000 resource-to-diet estimates for each individual simulation.

Estimating niche specialization (E)

To assess whether *A. mississippiensis* are dietary generalists or specialists at the sub-population-level, I calculated the specialization index (ε) using posterior distributions from SIAR simulations (for details regarding formulation of these metrics see Newsome et al., 2012). The niche specialization index (ε) varies from 0 to 1 and measures the degree of dietary specialization at the population or group level. A value of 0 indicates consumers are complete generalists (i.e., feed on all

available prey resources in equal proportions) and a value of 1 indicates consumers are ultra-specialists (i.e., feed only on a narrow subset of available prey resources). However, due to how ε is calculated, this metric does not indicate on which prey resource individuals specialize, although this can be inferred from SIAR resource-to-diet contribution estimates. Variability in ε at the population or sub-population level can determine prevalence of individual-level specialization within the consumer group being compared (Newsome et al., 2012). I calculated ε for each sub-population and each tissue type. I compared specialization indices estimated for sub-populations to assess intra-population variation, and compared within sub-populations across tissue types to assess temporal variation in niche specialization.

Estimating the Isotopic niche and Niche overlap

To characterize the isotopic niche I estimated the standard ellipse area (SEAc) corrected for small sample sizes for each *A. mississippiensis* sub-population using the '*standard.ellipse'* function in the package '*siar*' of R (Jackson et al., 2011). Analogous to the standard deviation of univariate data, SEAc is calculated using the variance/covariance matrix of bivariate isotopic data and represents the core isotopic niche space (the space created by plotting bivariate $\delta^{13}C$ and $\delta^{15}N$ data) occupied by a consumer group or population (Jackson et al., 2011). Overlap in SEAc among consumer groups or populations in isotopic niche space is a measure of trophic similarity, with complete overlap representing extreme similarity and zero overlap representing extreme dissimilarity (Jackson et al., 2011). I calculated overlap of SEAc among sub-populations and within sub-populations among tissue types using the '*overlap*' function in '*siar*'.

Statistical methods

Prior to data analyses, I assigned isotopic data from captured individuals to size classes based on total length (TL) measurements as follows (Chabreck, 1966): juvenile (100 cm), sub-adult (100–183 cm), and adult (> 183 cm). To analyze isotopic data and establish *A. mississippiensis* sub-populations for comparisons, I first adjusted tissue isotope values for tissue-specific isotopic discrimination using published mean values (Rosenblatt and Heithaus, 2013). I then assessed effects of capture year, habitat type (estuarine or freshwater), size class, and sex using MANOVA tests on bivariate $\delta^{13}C$ and $\delta^{15}N$ data for each tissue separately. If significant effects were detected, I explored the effects of factors on univariate $\delta^{13}C$ and $\delta^{15}N$ values separately using ANOVA. Assumption of normality was checked using Shapiro-Wilk's test on residuals for each ANOVA model. Significant differences among levels of influential factors identified during ANOVA tests were evaluated post-hoc using Tukey's HSD test. Sub-populations were established based on significant differences among factor levels detected during post-hoc analyses. I examined the relationship between isotopic values ($\delta^{13}C$ and $\delta^{15}N$) and body size (total length [TL]) using Pearson's correlation test. I used non-parametric gamma correlation tests to assess the form of associations between sub-population's ε values (mean and standard deviation) and SEAc (Goodman and Kruskal, 1954). I further examined strength of ε–SEAc associations using linear regression. Significance of statistical test results were evaluated at $\alpha = 0.05$. Means are presented with ± one standard deviation.

RESULTS

Animal captures

From 26 May 2010 to 31 July 2012, I captured 127 *A. mississippiensis* within GRWMA (Fig. 1A). Individuals ranged in size (TL) from 31.7 cm to 331.0 cm, and captures were slightly male-biased (M:F = 1.2:1). Water salinity measured at capture locations within Guana Lake ranged from 0 to 54 ppt and were considerably elevated in 2010 and 2011 (mean = 34±14 ppt) compared to 2012 (1±4 ppt). Salinity consistently measured zero ppt at all freshwater capture locations. Due to logistical constraints, I collected stomach contents from 44 of the 127 individuals (35%) captured.

Stomach content analysis (SCA)

I collected stomach contents from 19 males and 25 females ranging in size (TL) from 72.0 cm to 270.0 cm; yielding samples from six juveniles,

11 sub-adults, and 27 adults. Stomach contents of one adult individual contained no prey items; all other contents contained remanence of prey. A total of 37 of the 44 stomach content samples were collected from individuals captured in estuarine habitats and seven from freshwater habitats. I collected no stomach contents in 2010, 12 contents in 2011, and 32 in 2012. Stomach contents of *A. mississippiensis* within GRWMA were chiefly comprised of invertebrates (i.e., crustaceans and insects) and baitfish (Table 1).

All size classes consumed estuarine prey to some degree, however, importance of estuarine prey to diet increased through ontogeny. Freshwater taxa were the most important prey in juvenile stomach contents with aquatic insects and hylid frogs contributing the most to %IRI of freshwater prey (Fig. 2A). Estuarine prey contributed little to the diet of juveniles while baitfish (*Menidia menidia* [Atlantic silverside]) contributed the most to the estuarine %IRI. Freshwater prey were important to sub-adults, and although aquatic insects contributed the most to %IRI of freshwater prey for this group, their stomach contents contained a greater variety of freshwater prey than juveniles (7 vs 4 prey taxa). Small baitfishes (i.e., poeciliids, atherinopsids, cyprinodontids) were the most important estuarine prey in sub-adult contents (combined estuarine baitfish %IRI ≈ 24%, accounting for 92% of the estuarine prey contribution to the %IRI). Estuarine prey taxa were the most important prey type to adult individuals, contributing 98% to the %IRI for this size class. Small baitfish accounted for the majority of the estuarine prey %IRI value of adults (combined baitfish %IRI ≈ 97%).

Sample sizes were too small to make meaningful within-size class comparisons among sexes, thus I combined data from all size classes to assess differences in %IRI of prey between males and females. Estuarine prey taxa were highly important for both sexes, however, females relied more heavily on estuarine prey than males (Fig. 2B). Similarly, I combined data by habitat type to compare food habits of all individuals captured in estuarine habitats to those captured in freshwater habitats. Capture habitat strongly affected %IRI values (Fig. 2C). Individuals captured in freshwater habits demonstrated stronger reliance on freshwater prey and individuals captured in estuarine habitats were more reliant on estuarine prey. Prey importance indices were nearly equal when calculated for individuals captured in different years (Fig. 2D). Individuals captured in 2011 appeared to maintain a more variable diet than those captured in 2012 (i.e., greater number of prey taxa in contents). In addition to prey items, a number of non-prey items were recovered from *A. mississippiensis* stomach contents including vegetative material (i.e., leaves, stems, and twigs), seeds, rocks, glass, plastic, fishing lures, shotgun shells, and gastric parasites.

Stable isotope analysis (SIA)

$\delta^{13}C$ values of primary production sources collected in estuarine habitats were more positive than values measured for freshwater primary producers (Table 2, Fig. 3). Similarly, mean $\delta^{15}N$ values were more positive in estuarine (+2.6±3.3‰) than in freshwater producers (+2.2±2.3‰). Isotope values of representative prey from each habitat type closely tracked values measured for dominant primary producers in each system (Table 3, Fig. 3). Mean estuarine prey ($\delta^{13}C$ = -17.9±2.5‰, $\delta^{15}N$ = +7.1±2.0‰) were enriched in both ^{13}C and ^{15}N (more positive δ-values) compared to mean freshwater prey ($\delta^{13}C$ = -27.6±1.9‰, $\delta^{15}N$ = +4.8±1.7‰).

Although 127 individual *A. mississippiensis* were captured during this study, due to logistical constraints I could only determine isotope values for all three tissue types (plasma-PL, red blood cells-RBC, and keratinized scute-SC) for 118 of them (Table 4, Fig. 3). This resulted in isotopic analyses being performed with data from 32 juvenile (8 female, 24 male), 28 sub-adult (10 female, 18 male), and 58 adult (35 female, 23 male) individuals.

Multivariate analyses (MANOVA) revealed significant effects of year, capture habitat type, sex, and size class on PL and SC isotope values for *A. mississippiensis* sub-populations (Table 5), whereas RBC isotope values were significantly influenced by capture year and size class. Univariate analyses (ANOVA) indicated capture

Table 1. Percent frequency of occurrence (%FO), percent numerical abundance (%N), percent gravimetric abundance (%W), and percent Index of Relative Importance (%IRI) calculated for prey taxa recovered from *A. mississippiensis* stomach contents collected from GRWMA. All values are percentages (0–100%). Bold values are diet metrics calculated for broad categories of estuarine and freshwater prey.

	Juvenile (n = 6)				Sub-adult (n = 11)				Adult (n = 26)			
Prey Item	**%FO**	**%N**	**%W**	**%IRI**	**%FO**	**%N**	**%W**	**%IRI**	**%FO**	**%N**	**%W**	**%IRI**
Estuarine Prey	**41.65**	**10.36**	**24.96**	**8.15**	**48.47**	**29.51**	**69.75**	**25.97**	**82.75**	**93.13**	**93.87**	**98.16**
Invertebrate												
Gastropoda	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.92	1.77	1.16	0.19
Nassariidae												
Arthopoda												
Ocypodidae	0.00	0.00	0.00	0.00	2.27	0.42	0.86	0.09	1.28	0.22	0.00	0.01
Uca sp.												
Palaemonidae	0.00	0.00	0.00	0.00	6.36	1.68	2.12	0.74	2.37	0.67	0.21	0.07
Palaemonetes sp.												
Panopidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.62	1.33	1.28	0.83
Panopious sp.												
Penaeidae	0.00	0.00	0.00	0.00	3.03	0.42	0.22	0.06	0.96	0.22	0.06	0.01
Penaeus sp.												
Portunidae	8.33	0.94	3.34	0.72	5.30	0.84	1.39	0.36	3.01	0.67	0.68	0.13
Callinectes sapidus												
Aquatic insects	16.66	0.94	1.53	0.83	0.00	0.00	0.00	0.00	5.45	0.89	0.42	0.24
Tabanidae (larvae)												
Vertebrate												
Actinopterygii												
Atherinopsidae	12.50	7.54	17.26	6.29	9.39	4.64	13.64	5.27	3.21	1.55	1.39	0.31
Menidia menidia												
Cyprinodontidae	0.00	0.00	0.00	0.00	8.64	4.64	15.08	5.23	8.97	49.45	53.27	30.59
Cyprinodon variegatus												
Fundulidae	0.00	0.00	0.00	0.00	2.27	0.42	4.39	0.34	0.64	0.22	1.21	0.03
Fundulus heteroclitus												
Mugilidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.28	0.22	10.04	0.44
Mugil cephalus												
Poeciliidae	4.16	0.94	2.83	0.31	9.39	16.03	32.03	13.86	34.74	34.81	22.18	65.70
Poecilia latipinna												
Mammalia												
Leporidae	0.00	0.00	0.00	0.00	1.82	0.42	0.02	0.02	1.28	0.22	0.00	0.01
Sylvilagus palustris												
Procyonidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.85	0.22	1.08	0.17
Procyon lotor												
Aves	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.17	0.67	0.89	0.22
Unidentified sp.												

Table 1. Continued.

Prey Item	Juvenile (n = 6)				Sub-adult (n = 11)				Adult (n = 26)			
	%FO	%N	%W	%IRI	%FO	%N	%W	%IRI	%FO	%N	%W	%IRI
Freshwater Prey	**58.32**	**89.61**	**75.00**	**91.81**	**51.51**	**70.46**	**30.26**	**74.01**	**17.24**	**6.87**	**6.12**	**1.83**
Invertebrate												
Hirudinea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.96	0.22	0.00	0.01
Arthropoda												
Cambaridae *Procambarus* sp.	5.55	0.94	0.03	0.11	0.00	0.00	0.00	0.00	1.92	0.22	1.37	0.10
Aquatic Insects	34.72	84.90	26.72	78.68	27.12	63.29	23.19	72.02	5.58	1.33	0.15	0.27
Dytiscidae												
Hydrophilidae												
Ostracoda	0.00	0.00	0.00	0.00	4.85	4.22	0.29	0.67	0.64	3.55	0.04	0.08
Vertebrate												
Actinopterygii												
Poeciliidae *Gambusia affinis*	5.55	0.94	0.30	0.14	9.39	1.27	1.23	0.72	0.00	0.00	0.00	0.00
Reptilia/Amphibia												
Hylidae *Hyla cineria*	12.5	2.83	47.95	12.88	1.82	0.42	0.53	0.05	1.28	0.44	0.26	0.03
Ranidae Unidentified sp.	0.00	0.00	0.00	0.00	3.03	0.42	3.24	0.34	0.00	0.00	0.00	0.00
Colubridae *Nerodia* sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.64	0.22	0.28	0.01
Kinosternidae *Kinosternon subrubrum*	0.00	0.00	0.00	0.00	2.27	0.42	1.10	0.11	5.58	0.67	2.13	0.52
Mammalia												
Muridae Unidentified sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.64	0.22	1.89	0.04
Aves Unidentified sp.	0.00	0.00	0.00	0.00	3.03	0.42	0.68	0.10	0.00	0.00	0.00	0.00

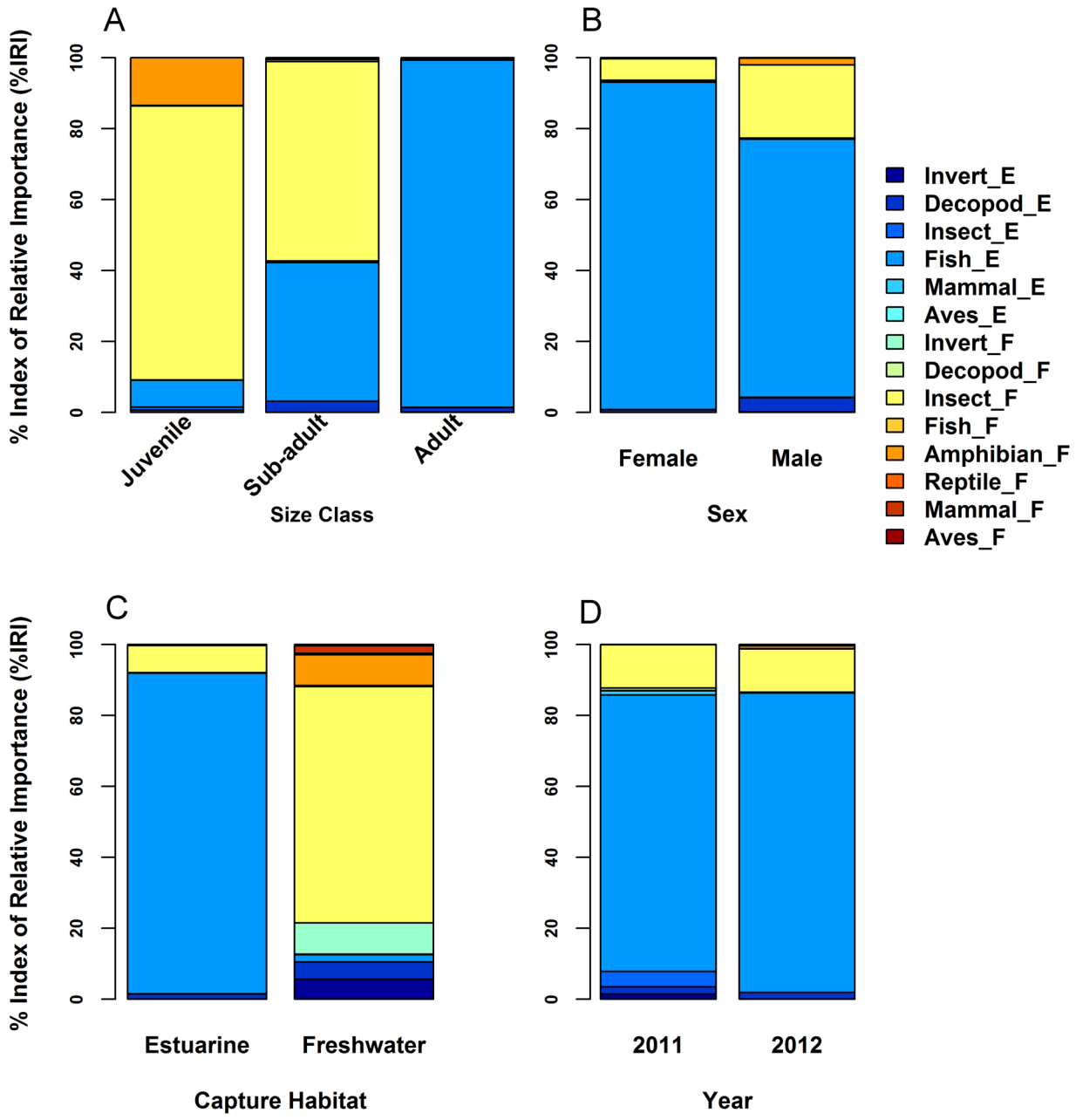

Figure 2. Bar-plots of percent Index of Relative Importance (%IRI) of prey categories determined for A) size classes, B) sexes, C) capture habitat, and D) capture years. Estuarine prey taxa, in cooler colors, are denoted by "_E"; freshwater prey taxa, in warmer colors, are denoted by "_F". For detailed information on prey taxa within the broader prey categories reported here see Table 1.

habitat type significantly affected carbon isotope values for all tissue types ($\delta^{13}C_{PL}$, $F_{1,111} = 13.79$, $P < 0.001$; $\delta^{13}C_{RBC}$, $F_{1,111} = 7.78$, $P = 0.049$; $\delta^{13}C_{SC}$, $F_{1,111} = 38.57$, $P < 0.001$). In addition to the effects of habitat type, $\delta^{13}C_{SC}$ values were also affected by capture year ($F_{2,111} = 17.18$, $P = 0.035$). Post-hoc analysis indicated $\delta^{13}C$ measured in all tissue types was significantly greater for individuals captured in estuarine habitats relative to freshwater habitats ($\delta^{13}C_{PL}$ and $\delta^{13}C_{SC}$, $P < 0.001$; $\delta^{13}C_{RBC}$, $P = 0.053$). Only a single pair-wise comparison among capture years was significant for $\delta^{13}C_{SC}$. Individuals captured in 2012 maintained more negative $\delta^{13}C_{SC}$ values than those captured in 2011 ($P = 0.035$).

Univariate tests indicated all factors significantly affected $\delta^{15}N_{PL}$ and $\delta^{15}N_{SC}$ values' whereas, $\delta^{15}N_{RBC}$ values significantly differed only as a function of capture year ($F_{2,111}$ = 17.24, P <0.001) and size class ($F_{2,111}$ = 25.48, P <0.001). Post-hoc analyses indicated capture year affected $\delta^{15}N$ values of all tissues similarly; $\delta^{15}N$ values of individuals captured in 2010 and 2012 were similar, and $\delta^{15}N$ measured in both these years were lower than values measured from individuals captured in 2011. Likewise, capture habitat had similar effects on both $\delta^{15}N_{PL}$ and $\delta^{15}N_{SC}$ values, individuals captured in estuarine habitats maintained higher $\delta^{15}N$ values than those captured in freshwater habitats. Sex significantly affected both $\delta^{15}N_{PL}$ ($F_{1,111}$ = 3.47, P = 0.041) and $\delta^{15}N_{SC}$ ($F_{1,111}$ = 2.54, P = 0.035) values; males maintained lower $\delta^{15}N$ values than females. Effect of size class on $\delta^{15}N$ values was similar across all three tissue types. $\delta^{15}N$ values of juvenile and sub-adult tissues were comparable, and $\delta^{15}N$ values of both these size classes were significantly lower than values measured in adult tissues. $\delta^{15}N$ values of all three tissue types were positively correlated to total length-TL (Pearson's: $\delta^{15}N_{SC}$, t_{116} = 9.01, r = 0.64, P <0.001; $\delta^{15}N_{PL}$, t_{116} = 6.26, r = 0.50, P <0.001; $\delta^{15}N_{RBC}$, t_{116} = 6.02, r = 0.49, P <0.001). I found no significant correlation between $\delta^{13}C$ values and TL for any tissue.

Using results of MANOVA and ANOVA, I created 16 sub-populations separating *A. mississippiensis* isotopic data based on capture habitat type (estuarine or freshwater), capture year (2012 and 2010 data), size class (juvenile and sub-adult data), and sex (male or female). A number of sub-populations, however, contained data from none or very few individuals. For example, no adult females were captured in freshwater habitats during 2011. If a group contained data from fewer than three individuals (minimum sample size recommended for SEAc calculations and SIAR, Parnell et al., 2009; Jackson et al., 2011), data from that group was combined with the closest related group (e.g., data combined for males and females within a size class captured in similar habitat in the same year). Data for adult males and females captured in freshwater habitats were combined for 2010 and 2012 captures, and data for juvenile and sub-adult sexes captured in freshwater habitats were combined for 2011. This resulted in a total of 13 sub-populations.

Estimating proportional use of Estuarine prey resources (SIAR): intrapopulation and Temporal variation

Median estimates of PL isotopic data for proportional contribution of estuarine prey to diet of each *A. mississippiensis* sub-population ranged from 0.49 to 0.75 (Table 6). Median estimates

Table 2. Stable isotope values measured in representative primary producers from estuarine and freshwater habitats within GRWMA. (-) denotes absence of data.

		$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)	
Primary producer taxa	**n**	**mean**	**SD**	**mean**	**SD**
Estuarine					
Macroalgae	5	+0.8	1.1	-16.2	2.3
POM	4	+2.0	0.3	-23.6	1.9
Spartina alterniflora (Smooth cord grass)	5	+7.4	1.6	-13.7	0.6
Ruppia maritima (Widgeon grass)	1	0.0	-	-18.4	-
Freshwater					
Carex sp. (Sedge grass)	3	+1.6	0.7	-28.4	1.6
Macroalgae	4	+4.4	4.1	-32.5	3.3
POM	1	+3.4	-	-28.9	-
Quercus virginiana (Live oak)	5	-0.8	1.4	-31.4	1.1

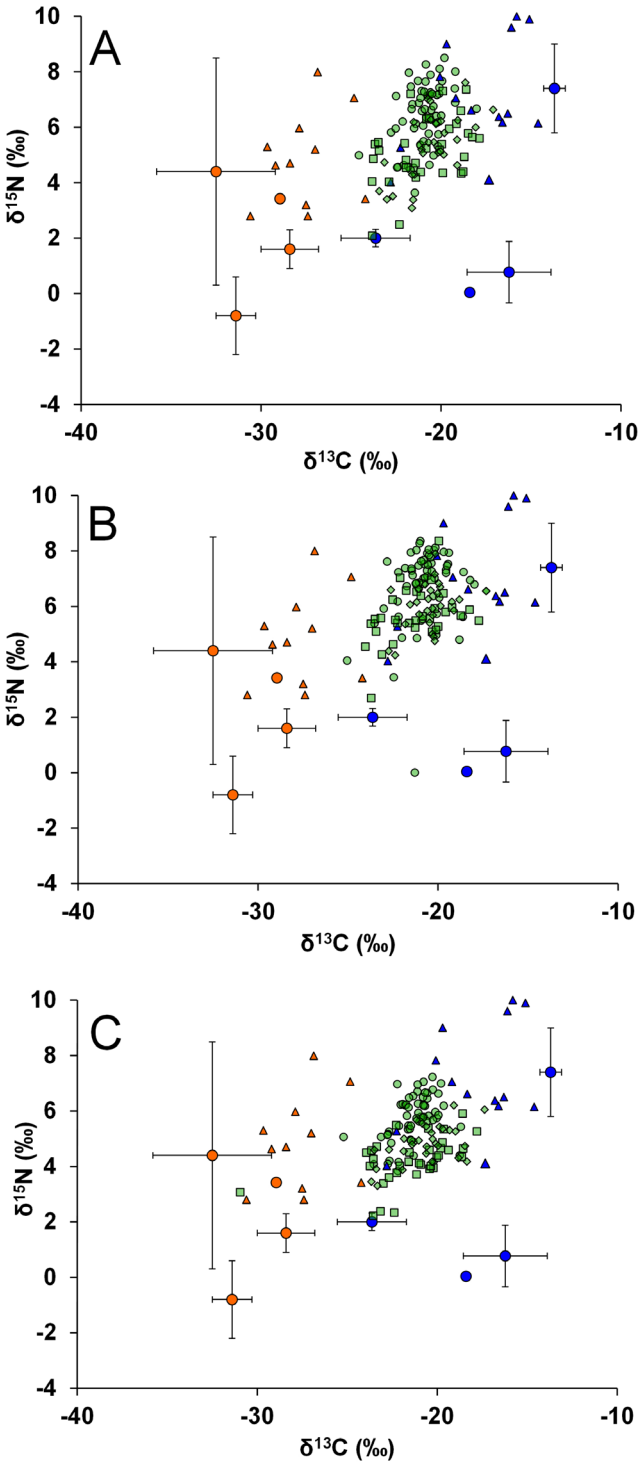

Figure 3. Stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) determined for primary producers (large circles: estuarine – blue and freshwater – orange), representative prey (triangles: estuarine – blue and freshwater – orange), and individual discrimination-adjusted *A. mississippiensis* (green symbols: square – juvenile, diamond – sub-adult, and small circle – adult): A) blood plasma-PL, B) red blood cell-RBC, and C) keratinized scute-SC isotope values. Excluding *A. mississippiensis* data, all points are mean values and error bars are ± 1 SD. *Alligator mississippiensis* data are individual values. Error bars are not included for representative prey taxa data for simplicity (for details on isotopic variation of prey see Table 3).

Table 3. Stable isotope values measured in representative prey taxa from estuarine and freshwater habitats within GRWMA. (-) denotes absence of data.

Taxa (common name)	n	δ^{15}N (‰) mean	SD	δ^{13}C (‰) mean	SD
Estuarine	110	+7.0	2.0	-17.9	2.6
Invertebrates					
Callinectes sapidus (Blue crab)	23	+10.0	0.8	-15.8	1.0
Cirripedia spp. (barnacles)	2	+4.0	0.9	-22.8	0.6
Litopenaeus setiferus (White shrimp)	1	+6.1	-	-14.6	-
Palaemonetes paludosus (Grass shrimp)	2	+6.4	0.2	-16.8	3.5
Panopeus herbstii (Atlantic mud crab)	8	+9.9	1.3	-15.1	1.1
Tabanidae (Horsefly larvae)	1	+5.3	-	-22.3	-
Uca pugnax (Fiddler crab)	17	+6.5	0.8	-16.3	1.2
Vertebrates					
Fundulus heteroclitus (Mummichug)	19	+9.6	0.8	-16.1	1.2
Lagodon rhomboides (Pinfish)	3	+6.6	1.0	-18.3	1.9
Menidia menidia (Atlantic silverside)	2	+7.1	0.3	-19.2	2.0
Micropogonias undulatus (Croaker)	8	+7.8	1.2	-20.1	0.9
Mugil cephalus (Striped mullet)	10	+4.1	0.4	-17.3	2.3
Procyon lotor (Raccoon)	12	+9.0	1.0	-19.7	1.3
Umbridae spp. (Mudminnow)	2	+6.2	0.1	-16.6	2.3
Freshwater	40	+4.8	1.7	-27.6	1.9
Invertebrates					
Aranae (spiders)	5	+5.2	1.7	-27.0	1.3
Coleoptera (Aquatic beetles)	4	+4.6	0.6	-29.2	0.8
Dytiscidae	6	+5.3	1.4	-29.7	1.3
Orthoptera (Grasshoppers)	3	+2.8	1.2	-27.4	1.1
Procambrus sp. (Crayfish)	9	+4.7	1.1	-28.4	1.5
Ranatra sp. (Water scorpion)	1	+6.0	-	-27.9	-
Vertebrates					
Gambusia affinis (Mosquito fish)	7	+8.0	2.0	-26.9	2.3
Kinosternon subrubrum (Mud turtle)	1	+3.2	-	-27.5	-
Lithobates sp. (true frog)	1	+2.8	-	-30.6	-
Nerodia fasciata (Banded water snake)	2	+7.1	0.6	-24.8	1.6
Pseudemys sp. (River cooter)	1	+3.4	-	-24.2	-

based on RBC isotope values ranged from 0.53 to 0.72. Estimates based on SC isotope values ranged from 0.34 to 0.69.

The greatest temporal shift in dietary preferences as inferred by differences in SIAR predictions based on isotope values of different tissue types was found for juvenile/sub-adult males captured in freshwater habitats during years 2010 and 2012. Estimated proportion of estuarine prey contributing to this sub-population's diet at short and intermediate time-scales (0.57 [0.45–0.72]) were nearly twice (1.6–1.7 times) the proportional contribution estimated for the longest time-scale (Table 6). Juvenile/sub-adult females captured in freshwater habitats during years 2010 and 2012 (sub-population 4) demonstrated a similar pattern, although not as pronounced as males. Short- and intermediate-term predictions for proportion of estuarine prey in sub-population 4's diet were only 1.2 to 1.4 times higher than long-term predictions

Table 4. Number of individuals (n) and stable isotope ($\delta^{13}C$ and $\delta^{15}N$) values of *A. mississippiensis* sub-populations for three tissue types (blood plasma-PL, red blood cells-RBC, and keratinized scutes-SC). All values are adjusted from tissue-specific discrimination using mean values determined by Rosenblatt and Heithaus (2013).

		$\delta^{15}N$ (‰)						$\delta^{13}C$ (‰)					
Sub-population	**n**	**PL_{mean}**	**PL_{SD}**	**RBC_{mean}**	**RBC_{SD}**	**SC_{mean}**	**SC_{SD}**	**PL_{mean}**	**PL_{SD}**	**RBC_{mean}**	**RBC_{SD}**	**SC_{mean}**	**SC_{SD}**
2010/2012	70	+5.3	1.1	+6.0	1.0	+4.8	1.1	-21.0	1.4	-21.0	1.5	-21.5	1.9
Juvenile/sub-adult	40	+5.0	1.2	+6.0	1.0	+4.4	0.9	-20.8	1.6	-20.9	1.5	-21.3	2.3
Estuarine	30	+5.3	1.0	+6.0	0.7	+4.8	0.7	-20.4	1.4	-20.7	1.2	-20.6	1.6
1-Male	20	+5.2	1.0	+6.0	0.8	+4.7	0.7	-20.5	1.6	-20.6	1.3	-20.7	1.8
2-Female	10	+5.5	0.9	+5.8	0.5	+5.0	0.6	-20.1	1.0	-20.8	1.1	-20.5	1.3
Freshwater	10	+4.1	1.3	+5.9	1.7	+3.3	0.8	-22.2	1.3	-21.4	2.1	-23.4	2.9
3-Male	7	+3.9	1.2	+6.0	1.9	+3.4	0.8	-22.0	1.3	-21.8	1.4	-23.4	3.5
4-Female	3	+4.6	1.5	+5.7	1.0	+3.0	0.7	-22.5	1.6	-20.5	3.4	-23.4	0.4
Adult	30	+5.6	0.9	+6.0	1.1	+5.4	0.9	-21.3	1.0	-21.1	1.6	-21.6	1.3
Estuarine	27	+5.6	1.0	+6.0	1.1	+5.4	1.0	-21.2	0.8	-21.0	1.4	-21.6	1.1
5-Male	9	+5.4	0.8	+5.9	0.9	+5.2	0.7	-21.3	0.7	-20.9	1.6	-21.8	0.9
6-Female	18	+5.7	1.0	+6.1	1.2	+5.5	1.1	-21.2	0.8	-21.1	1.3	-21.5	1.2
Freshwater													
7-Both sexes*	3	+5.6	0.7	+6.2	1.1	+5.5	0.9	-21.8	2.4	-21.6	3.0	-22.2	2.7
2011	48	+6.7	1.0	+6.7	1.1	+5.5	0.9	-20.6	1.4	-20.7	1.3	-20.9	1.3
Juvenile/sub-adult	20	+5.8	0.9	+5.6	0.6	+4.6	0.7	-20.5	1.9	-20.8	1.8	-20.8	1.8
Estuarine	13	+6.2	0.9	+5.7	0.7	+4.8	0.7	-19.7	1.2	-20.2	1.2	-20.1	1.2
8-Male	9	+6.4	0.8	+6.0	0.6	+4.8	0.6	-19.7	1.2	-20.1	1.1	-20.1	1.2
9-Female	4	+5.9	1.2	+5.0	0.6	+4.7	1.0	-19.6	1.2	-20.4	1.5	-20.1	1.3
Freshwater													
10-Both sexes‡	7	+5.2	0.5	+5.5	0.4	+4.3	0.4	-22.0	2.2	-21.9	2.2	-22.0	2.0
Adult	28	+7.3	0.6	+7.6	0.4	+6.1	0.5	-20.6	1.0	-20.6	0.9	-20.9	0.9
Estuarine	25	+7.3	0.6	+7.6	0.4	+6.1	0.5	-20.7	0.7	-20.7	0.6	-21.0	0.7
11-Male	10	+7.2	0.3	+7.7	0.5	+6.1	0.3	-20.9	0.9	-20.7	0.8	-21.1	0.5
12-Female	15	+7.4	0.7	+7.5	0.4	+6.2	0.6	-20.6	0.7	-20.6	0.6	-20.9	0.8
Freshwater													
13-Male†	3	+6.8	0.6	+7.2	0.4	+5.5	0.2	-19.8	2.1	-19.9	2.1	-20.5	2.1

*Data from two females and one male.
†Data from males only, no females.
‡Data from one female and six males.

Table 5. Results from MANOVA analyses on bivariate stable isotope values. Significant effects are in **bold**.

Variables	Blood Plasma-PL Wilk's λ	*F*	*df*	*df* error	*P-value*	Red Blood Cells-RBC Wilk's λ	*F*	*P-value*	Keratinized Scutes-SC Wilk's λ	*F*	*P-value*
Capture year	0.59	16.33	2	111	**<0.001**	0.85	4.70	**0.001**	0.82	5.70	**<0.001**
Habitat type	0.79	14.86	1	111	**<0.001**	0.96	2.40	0.096	0.70	23.09	**<0.001**
Sex	0.95	3.19	1	111	**0.045**	0.99	0.48	0.618	0.95	3.09	**0.049**
Size class	0.74	9.09	2	111	**<0.001**	0.75	8.34	**<0.001**	0.61	15.56	**<0.001**

(Table 6). Other sub-populations were relatively stable in their dietary preferences through time, however, overall proportional contribution of estuarine prey to any sub-population's diet based on SC isotope values were lower than predictions based on either PL or RBC isotope values.

Within size classes and habitat type for individuals captured during the same time period, estimated proportional contribution of estuarine prey to male and female diets were highly similar (Table 6). Median estimates for proportion of estuarine prey in diets of 2011 sub-populations were 9% greater than estimates based on 2010 and 2012 data for PL and SC isotope values, and 5% higher for those based on RBC isotope values. Individuals captured in freshwater habitats were predicted to use estuarine prey resources to a lesser extent than individuals captured in estuarine habitats. This pattern was relatively consistent across size-classes, sexes, and all time-scales, however, differences among juvenile/sub-adult sub-populations were greater than differences among adult sub-populations, and differences were more apparent at longer time-scales (estimates based on SC isotope values) (Table 6). The only sub-population for which this pattern did not hold was for adult males captured in freshwater habitats during 2011. Estimated proportion of estuarine prey in their diet was higher than males captured in estuarine habitats based on PL and RBC isotope values.

Examining dietary niche generalization and specialization: specialization Index (ε)

Niche specialization over the shortest time-scale (ε calculated using PL isotope values) sub-populations ranged from near complete generalists (mean ε_{PL} = 0.12±0.10) to moderate niche specialists (mean ε_{PL} = 0.50±0.13). Juvenile/sub-adults captured in freshwater habitats (all years) maintained the lowest ε_{PL} values indicating a more generalized diet comprised of near equal proportions of freshwater and estuarine prey (Table 7). Adults captured in estuarine habitats in years 2010 and 2012 also appeared to be more niche generalists. In general, shorter-term niche specialization (ε_{PL} approaching 1) was more prevalent for sub-populations captured in estuarine habitats. Niche specialization estimated at intermediate time-scales (ε_{RBC}) followed similar patterns to short-term estimates (ε_{PL}); however, a few sub-populations deviated from this pattern. In particular, juvenile and sub-adult females captured in estuarine habitats in all years were predicted to be stronger generalists over intermediate time-scales compared to shorter- and longer-term estimates (Table 7). Conversely, niche specialization over intermediate time-scales was higher than short- and long-term estimates for adults (both sexes) captured in estuarine habitats in all years, although differences were more pronounced for males compared to females (Table 7). In general, estimates of niche specialization over the long-term (ε_{SC}) were slightly lower than intermediate and short-term indices. Niche specialization over the longest time period (ε_{SC}) was estimated to be considerably higher than at intermediate and short time-scales for juvenile and sub-adult males captured in freshwater habitats during years 2010 and 2012. However, this was not the case for juveniles and sub-adults captured in similar habitats during 2011, wherein niche specialization was found to be relatively low across all three time scales (Table 7).

Table 6. SEAc estimates and SIAR model results for *A. mississippiensis* sub-populations inhabiting GRWMA.

	SEAc (‰²)			Estimated proportional contribution of estuarine/marine prey to diet: median (95% BCI)		
Sub-population Group	**PL**	**RBC**	**SC**	**PL**	**RBC**	**SC**
2010/2012						
Estuarine						
Juvenile/sub-adult						
1-Male	4.92	3.04	3.22	0.68 (0.59–0.76)	0.68 (0.60–0.75)	0.65 (0.55–0.74)
2-Female	3.16	1.68	1.79	0.70 (0.59–0.82)	0.64 (0.53–0.75)	0.66 (0.53–0.79)
Freshwater						
Juvenile/sub-adult						
3-Male	3.42	4.86	10.45	0.53 (0.40–0.68)	0.57 (0.45–0.72)	0.34 (0.04–0.61)
4-Female	7.01	2.74	1.47	0.49 (0.18–0.74)	0.57 (0.20–0.91)	0.42 (0.15–0.66)
Estuarine						
Adult						
5-Male	1.81	5.16	1.68	0.59 (0.48–0.70)	0.64 (0.51–0.77)	0.54 (0.44–0.66)
6-Female	2.71	4.46	3.71	0.61 (0.54–0.70)	0.63 (0.56–0.72)	0.59 (0.51–0.67)
Freshwater						
7-Adult*	5.12	18.72	11.95	0.53 (0.22–0.81)	0.56 (0.24–0.88)	0.51 (0.20–0.81)
2011						
Estuarine						
Juvenile/sub-adult						
8-Male	3.52	2.16	2.56	0.75 (0.63–0.88)	0.70 (0.58–0.83)	0.69 (0.54–0.83)
9-Female	4.81	0.54	4.11	0.72 (0.44–0.95)	0.63 (0.32–0.87)	0.66 (0.31–0.90)
Freshwater						
10-Juvenile/ sub-adult‡	4.06	2.78	3.07	0.52 (0.34–0.68)	0.53 (0.37–0.69)	0.51 (0.28–0.67)
Estuarine						
Adult						
11-Male	0.82	0.94	0.54	0.68 (0.58–0.80)	0.71 (0.60–0.83)	0.62 (0.52–0.73)
12-Female	1.43	0.72	1.63	0.71 (0.63–0.81)	0.71 (0.63–0.81)	0.64 (0.56–0.73)
Freshwater						
13-Adult†	6.45	0.16	2.70	0.70 (0.37–0.96)	0.72 (0.39–0.96)	0.61 (0.26–0.89)

*Data from two females and one male.
†Data from males only, no females.
‡Data from one female and six males

Isotopic niche, overlap, and temporal variation

To qualitatively assess the position of *A. mississippiensis* sub-populations in isotopic niche space (δ-space) relative to the locations of freshwater and estuarine resources, I plotted the SEAc calculated for each sub-population and each tissue type along with the mean isotope values of prey from each habitat (Fig. 4). The majority of *A. mississippiensis* sub-population's SEAc were positioned in-between freshwater and estuarine resources (slightly closer to the location of estuarine prey), indicating use of both resource pools by all sub-populations to some degree. The location of SEAc's calculated based on PL and SC isotope values were highly similar for 2010 and 2012 sub-populations (Figs. 4A, 4C); however, for 2011 sub-populations location of SEAc-$_{PL}$ was higher along the y-axis ($\delta^{15}N$) than SEAc-$_{SC}$. SEAc-$_{RBC}$ behaved somewhat differently than SEAc-$_{PL}$ and SEAc-$_{SC}$. For 2010 and 2012 sub-populations, SEAc-$_{RBC}$'s were higher along the y-axis than the positions of SEAc-$_{PL}$ and SEAc-$_{SC}$ (Figs. 4A-4C). SEAc-$_{RBC}$'s of 2011 sub-populations were similar in position to SEAc-$_{PL}$, which were both higher along the y-axis than SEAc-$_{SC}$ (Figs. 4D, 4F). Thus, individuals captured in 2011 demonstrated alternative foraging patterns over the short (SEAc-$_{PL}$) and intermediate (SEAc-$_{RBC}$) time scales compared to long-term patterns (SEAc-$_{SC}$).

SEAc size averaged 3.75±3.49‰² across all sub-populations and tissues (Table 6). Within a particular sub-population SEAc-$_{PL}$, which

Table 7. Niche specialization index (ε) calculations (mean ± SD) for *A. mississippiensis* sub-populations based on posterior distributions from SIAR model simulations for blood plasma-PL, red blood cell-RBC, and keratinized scute-SC isotope values. (-) denotes same classification as indicated above in each column.

Sub.pop #	Year	Size-class	Habitat	Sex	ε_{PL}	ε_{RBC}	ε_{SC}
1	2010/2012	Juv./sub.	E	♂	0.35 ± 0.09	0.35 ± 0.08	0.31 ± 0.10
2	-	-	-	♀	0.40 ± 0.12	0.29 ± 0.11	0.33 ± 0.12
3	-	-	F	♂	0.12 ± 0.10	0.18 ± 0.12	0.37 ± 0.26
4	-	-	-	♀	0.20 ± 0.18	0.31 ± 0.23	0.22 ± 0.18
5	-	Adult	E	♂	0.19 ± 0.11	0.29 ± 0.13	0.12 ± 0.09
6	-	-	-	♀	0.24 ± 0.08	0.28 ± 0.08	0.18 ± 0.08
7	-	-	F	♀+♂	0.22 ± 0.19	0.26 ± 0.21	0.22 ± 0.19
8	2011	Juv./sub.	E	♂	0.50 ± 0.13	0.41 ± 0.13	0.39 ± 0.14
9	-	-	-	♀	0.45 ± 0.22	0.30 ± 0.20	0.36 ± 0.21
10	-	-	F	♀+♂	0.13 ± 0.11	0.14 ± 0.11	0.15 ± 0.12
11	-	Adult	E	♂	0.36 ± 0.11	0.43 ± 0.12	0.24 ± 0.10
12	-	-	-	♀	0.43 ± 0.09	0.42 ± 0.09	0.29 ± 0.09
13	-	-	F	♀+♂	0.42 ± 0.25	0.45 ± 0.25	0.30 ± 0.22

represents integration of shorter-term trophic interactions (~250 days), was larger than both SEAc-$_{RBC}$ (8 of 13 sub-populations) and SEAc-$_{SC}$ (9 of 13 sub-populations), each of which represents integration of trophic interactions over successively longer time periods (~400-1,100 days depending on element and tissue). Conversely, SEAc-$_{PL}$ was smaller than SEAc-$_{RBC}$ for juvenile males captured in freshwater habitats during years 2010 and 2012, adults (both sexes) captured in either habitat during years 2010 and 2012, and adult males captured in estuarine habitats during 2011. SEAc-$_{PL}$ was smaller than SEAc-$_{SC}$ for juvenile males captured in freshwater habitats during years 2010 and 2012, adult females captured in estuarine habitats during 2010 and 2012, adults (both sexes) captured in freshwater habitats in years 2010 and 2012, and adult females captured in estuarine habitats in 2011 (Table 6). To a lesser extent SEAc-$_{RBC}$ was larger than SEAc-$_{SC}$ (5 out of 13 sub-populations). In particular, SEAc-$_{RBC}$ was larger than SEAc-$_{SC}$ for females captured in freshwater habitats during 2010 and 2012, adults (both sexes) captured in estuarine and freshwater habitats during 2010 and 2012, and adult males captured in estuarine habitats during 2011.

To examine similarity in isotopic niches occupied by different *A. mississippiensis* sub-populations, I estimated proportional overlap of SEAc for pairs of sub-populations for each tissue type (Table 8). Average proportional overlap in SEAc-$_{PL}$ with other sub-populations (mean of all pair-wise overlap calculations for each sub-population) ranged from 8% to 38% for adult females captured in estuarine habitats during 2011 and adult males captured in estuarine habitats during 2010 and 2012, respectively. Overlap in SEAc-$_{PL}$ of sub-populations was highest for different sexes within the same size-class captured in similar habitat types in the same years. For example, proportional overlap of SEAc-$_{PL}$ for juvenile, sub-adult males, and females captured in estuarine habitats during 2010 and 2012 (57% of sub-population 1's and 89% of sub-population 2's SEAc-$_{PL}$) was greater than overlap of sub-population 1 and juvenile and sub-adult males captured in estuarine habitats in 2011 (21% of sub-population 1's and 29% of sub-population 8's SEAc-$_{PL}$). Likewise, overlap in SEAc-$_{PL}$ was high among sub-populations (21–86%) within the same size class and of the same sex captured in different years (Table 8). The lowest proportional overlap in SEAc-$_{PL}$'s was among sub-populations of different size classes (regardless of sex), especially when making comparisons across years or capture habitat type.

Overlap between SEAc-$_{RBC}$ calculated for *A. mississippiensis* sub-populations (Figs. 4B, 4E)

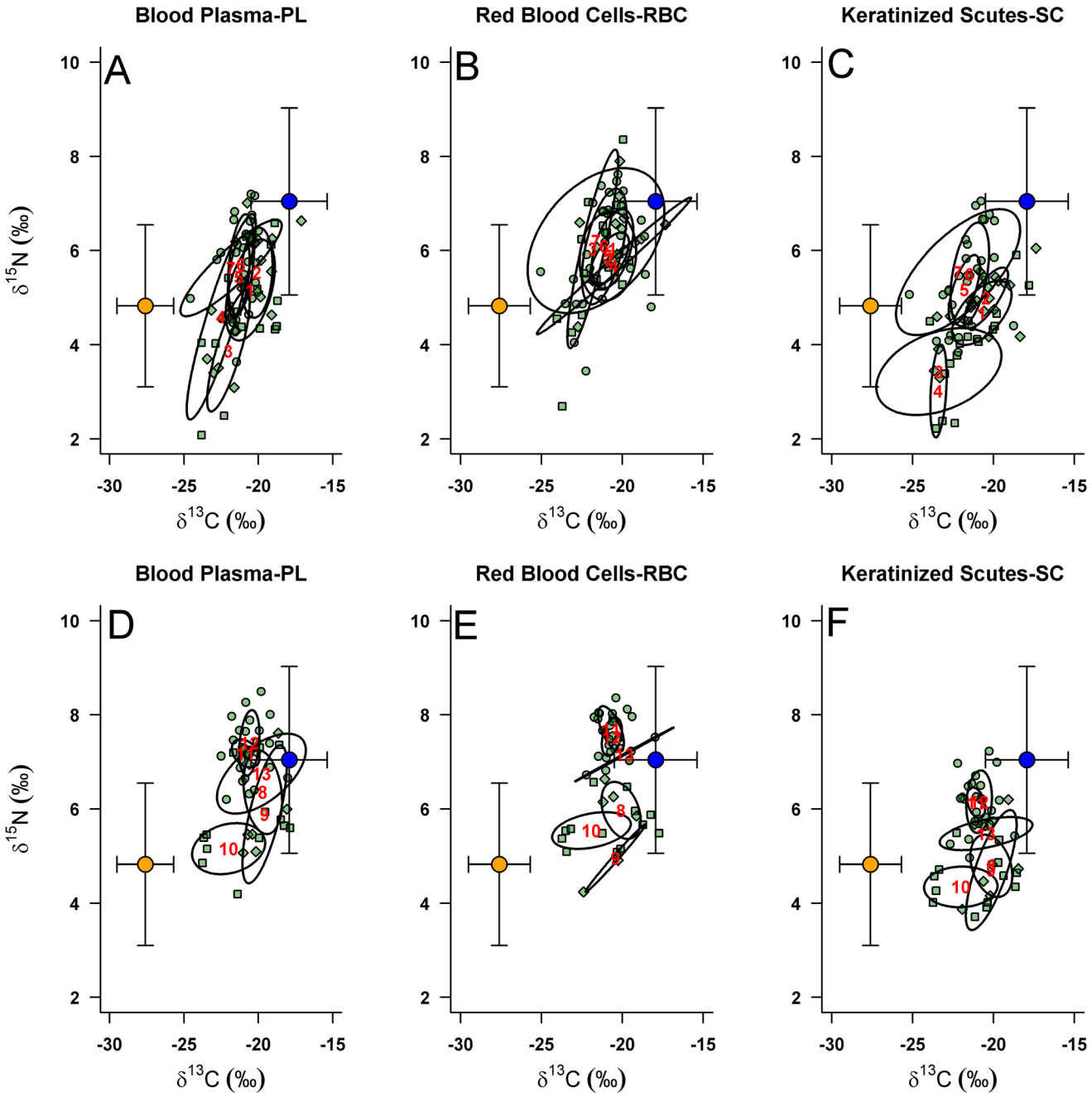

Figure 4. Stable isotope bi-plots ($\delta^{13}C$ and $\delta^{15}N$) of standard ellipse area (SEAc) calculated for *A. mississippiensis* sub-populations. Lines enclose SEAc for each sub-population and mean values are represented by the sub-population number (1-13) as defined in Table 4. Panels A–C corresponds to 2010 and 2012 captures (sub-populations 1-7). Panels D–F corresponds to 2011 captures (sub-populations 8-13). Filled circles are mean isotope values for freshwater prey (orange) and estuarine prey (blue). Open light green circles (adults), diamonds (sub-adult), and squares (juveniles) are stable isotope values of individual *A. mississippiensis* adjusted for tissue-specific isotopic discrimination. Error bars are ±1 SD.

followed similar patterns of overlap in SEAc-$_{PL}$, however, there were some key differences (Table 8). For nearly half of the pair-wise comparisons (42%) the proportional overlap of sub-populations' SEAc-$_{RBC}$ was greater than overlap of SEAc-$_{PL}$. Similar to SEAc-$_{PL}$, overlap in SEAc-$_{RBC}$ among sub-populations was relatively high between sexes within the same size class captured in the same habitat in the same years. Likewise, overlap in SEAc-$_{RBC}$ among sub-populations of the same sex within the same size class from different years was relatively high (Table 8). Proportional overlap of SEAc-$_{RBC}$ calculated for different size classes regardless of sex or capture habitat was greater compared to overlap of SEAc-$_{PL}$, however, this pattern seemed to be predominantly driven by the large area of SEAc-$_{RBC}$ estimated for adults (both sexes) captured in freshwater habitats during years 2010 and 2012 (Fig. 4B). There were also more cases of low overlap (<5%) between sub-population's SEAc-$_{RBC}$ as compared to SEAc-$_{PL}$ (70 vs 52 pair-wise comparisons).

Overlap in sub-population's SEAc-$_{SC}$ (Figs. 4C, 4F) followed similar patterns to both SEAc-$_{PL}$ and SEAc-$_{RBC}$ comparisons (Table 8). Proportional overlap in SEAc-$_{SC}$ among sub-populations was highest for different sexes within the same size class captured in similar habitats in the same years. Overlap of SEAc-$_{SC}$ was also high for sub-populations of the same sex captured in similar habitat types in different years. Overlap of SEAc-$_{SC}$ was lowest for sub-populations within the same size class captured in different habitats and for sub-populations of different size classes (regardless of year or sex). In general, overlap of sub-population's SEAc-$_{SC}$, was less than that of SEAc-$_{PL}$ and SEAc-$_{RBC}$, with 73% and 67% of the pair-wise overlap calculations being less in magnitude than overlap of sub-population's SEAc-$_{PL}$ and SEAc-$_{RBC}$, respectively. Comparisons of SEAc-$_{SC}$ demonstrated the lowest overlap of any tissue. In total, I found that proportional overlap among sub-population's SEAc-$_{SC}$ was less than 5% for 71 pair-wise overlap calculations relative to 70 for SEAc-$_{RBC}$ and 52 for SEAc-$_{PL}$.

Within each sub-population I calculated overlap in SEAc for tissues differing in turnover rates. Thus, greater overlap indicates greater temporal stability in a sub-population's isotopic niche and trophic interactions (Jackson et al., 2011). Proportional overlap of SEAc-$_{PL}$ with SEAc-$_{RBC}$ (calculated as area of overlap divided by SEAc-$_{PL}$), representing short and intermediate isotopic niches, ranged from 0% to 96% (mean = 42±31%, Table 9). Overlap of SEAc-$_{PL}$ with SEAc-$_{RBC}$ was highest (81–96%) for adults (both sexes) captured in either habitat in 2010 and 2012, moderately high for juvenile and sub-adult males captured in estuarine habitats in 2011 (45%), and both sexes of juvenile/sub-adults captured in freshwater habitats in 2011 (40%,). There was no overlap of SEAc-$_{PL}$ with SEAc-$_{RBC}$ for juvenile and sub-adult males captured in freshwater in 2010 and 2012, but overlap was low (8–33%) for all other sub-populations.

The proportion of SEAc-$_{RBC}$ overlapping with SEAc-$_{PL}$ maintained a somewhat different pattern across sub-populations (Table 9). Mean proportional overlap of SEAc-$_{RBC}$ with SEAc-$_{PL}$ was 51±31%. Complete overlap of SEAc-$_{RBC}$ with SEAc-$_{PL}$ was found for adult females captured in estuarine habitats and adult males captured in freshwater habitats during 2011. Conversely, no overlap was found for juvenile and sub-adult males captured in freshwater habitats in years 2010 and 2012). I found high proportional overlap of SEAc-$_{RBC}$ with SEAc-$_{PL}$ (47–74%) for juvenile and sub-adults (both sexes) captured in 2011 and estuarine captures in years 2010 and 2012 and adult females captured in estuarine habitats in years 2010 and 2012 (Table 9). I found low proportional overlap of SEAc-$_{RBC}$ with SEAc-$_{PL}$ for juvenile and sub-adult females captured in freshwater habitats in 2010 and 2012; adult males captured in estuarine habitats in all years; and adults (both sexes) captured in freshwater habitats in 2010 and 2012.

Mean proportional overlap of SEAc-$_{PL}$ and SEAc-$_{SC}$ over the shortest and longest time periods studied here was 37±36% (Table 9). Proportional overlap of SEAc-$_{PL}$ and SEAc-$_{SC}$ was highest for adults (both sexes) captured in freshwater habitats and adult females captured in estuarine habitats during 2010 and 2012. Moderate degrees of overlap

Table 8. Proportional overlap of isotopic niche space (SEAc's) among *A. mississippiensis* sub-populations. Values above the center 1:1 diagonal are calculated as the overlap area (‰^{2}) divided by SEAc of the group number indicated in the group row (top row). Values below the center diagonal are calculated as the overlap area divided by the SEAc of the group indicated by the number in the group column (first column). For example, the value in the second cell of the first row (0.89) is the proportion of group 2's SEAc which overlaps with group 1's SEAc. The value in the second cell of the first column (0.57) is the proportion of group 1's SEAc which overlaps with group 2's SEAc. Letters E and F denote capture habitat type: estuarine-E and freshwater-F.

	Plasma-PL												
	2010 & 2012							2011					
	Juv./sub.				Adult			Juv./sub.			Adult		
	E		F		E		F	E		F	E		F
	♂	♀	♂	♀	♂	♀	♂+♀	♂	♀	♂+♀	♂	♀	♂
Sub. pop #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	1.00	0.89	0.33	0.21	0.72	0.56	0.24	0.29	0.57	0.49	0.00	0.00	0.04
2	0.57	1.00	0.05	0.08	0.27	0.31	0.24	0.37	0.56	0.27	0.00	0.00	0.09
3	0.23	0.06	1.00	0.15	0.16	0.11	0.00	0.00	0.06	0.10	0.00	0.00	0.00
4	0.30	0.19	0.31	1.00	0.92	0.81	0.40	0.15	0.04	0.50	0.00	0.00	0.15
5	0.27	0.15	0.09	0.24	1.00	0.63	0.22	0.01	0.04	0.30	0.00	0.00	0.05
6	0.31	0.27	0.09	0.32	0.95	1.00	0.30	0.14	0.10	0.32	0.00	0.01	0.16
7	0.25	0.40	0.00	0.30	0.61	0.57	1.00	0.40	0.28	0.52	0.00	0.00	0.22
8	0.21	0.41	0.00	0.08	0.03	0.18	0.28	1.00	0.49	0.00	0.28	0.19	0.37
9	0.56	0.86	0.08	0.03	0.11	0.17	0.26	0.67	1.00	0.24	0.00	0.00	0.29
10	0.40	0.35	0.12	0.29	0.66	0.48	0.42	0.00	0.20	1.00	0.00	0.00	0.00
11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	1.00	0.41	0.07
12	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.08	0.00	0.00	0.72	1.00	0.08
13	0.05	0.18	0.00	0.14	0.17	0.37	0.28	0.67	0.38	0.00	0.54	0.38	1.00

Table 8. Continued.

	Redblood-RBC												
	2010 & 2012							2011					
	Juv./sub.				Adult			Juv./sub.			Adult		
	E		F		E		F	E		F	E		F
	♂	♀	♂	♀	♂	♀	♂+♀	♂	♀	♂+♀	♂	♀	♂
Sub.pop #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	1.00	0.97	0.14	0.29	0.56	0.53	0.16	0.71	0.00	0.43	0.00	0.00	0.00
2	0.53	1.00	0.08	0.25	0.33	0.33	0.09	0.46	0.00	0.36	0.00	0.00	0.00
3	0.23	0.23	1.00	0.20	0.38	0.51	0.21	0.07	0.00	0.38	0.52	0.82	0.28
4	0.26	0.42	0.11	1.00	0.21	0.17	0.11	0.28	0.00	0.29	0.00	0.00	0.00
5	0.95	1.00	0.41	0.40	1.00	0.79	0.27	0.91	0.11	0.66	0.00	0.00	0.00
6	0.78	0.87	0.47	0.29	0.68	1.00	0.24	0.51	0.00	0.52	0.04	0.09	0.33
7	0.99	1.00	0.82	0.78	0.99	0.99	1.00	0.99	0.21	0.99	0.52	0.80	0.85
8	0.51	0.59	0.03	0.22	0.38	0.25	0.12	1.00	0.05	0.20	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01	1.00	0.00	0.00	0.00	0.00
10	0.39	0.60	0.22	0.30	0.35	0.33	0.15	0.26	0.00	1.00	0.00	0.00	0.00
11	0.00	0.00	0.10	0.00	0.00	0.01	0.03	0.00	0.00	0.00	1.00	0.70	0.00
12	0.00	0.00	0.12	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.54	1.00	0.00
13	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	1.00

Table 8. Continued.

	Keratinized Scutes												
	2010 & 2012							2011					
	Juv./sub.				Adult			Juv./sub.			Adult		
	E		F		E		F	E		F	E		F
	♂	♀	♂	♀	♂	♀	♂+♀	♂	♀	♂+♀	♂	♀	♂
Sub. pop #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	1.00	0.80	0.06	0.00	0.16	0.19	0.05	0.72	0.52	0.52	0.00	0.00	0.06
2	0.46	1.00	0.00	0.00	0.03	0.13	0.05	0.47	0.32	0.14	0.00	0.00	0.20
3	0.18	0.00	1.00	0.78	0.00	0.00	0.00	0.00	0.28	0.42	0.00	0.00	0.00
4	0.00	0.00	0.11	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0.08	0.03	0.00	0.00	1.00	0.43	0.14	0.04	0.00	0.09	0.04	0.14	0.45
6	0.22	0.27	0.00	0.00	0.94	1.00	0.29	0.18	0.02	0.13	0.99	0.77	0.44
7	0.18	0.36	0.00	0.00	0.97	0.93	1.00	0.24	0.15	0.24	0.99	0.99	0.73
8	0.58	0.67	0.00	0.00	0.07	0.13	0.05	1.00	0.46	0.17	0.00	0.00	0.14
9	0.67	0.74	0.11	0.00	0.00	0.02	0.05	0.72	1.00	0.34	0.00	0.00	0.32
10	0.49	0.24	0.12	0.00	0.17	0.11	0.06	0.21	0.25	1.00	0.00	0.00	0.00
11	0.00	0.00	0.00	0.00	0.01	0.14	0.05	0.00	0.00	0.00	1.00	0.33	0.00
12	0.00	0.00	0.00	0.00	0.14	0.34	0.14	0.00	0.00	0.00	0.99	1.00	0.08
13	0.05	0.30	0.00	0.00	0.45	0.32	0.17	0.15	0.21	0.00	0.00	0.13	1.00

were found for juvenile and sub-adults (both sexes) and adult males captured in estuarine habitats during 2010 and 2012 and juvenile and sub-adult females captured in estuarine habitats during 2011. Sub-populations that maintained zero overlap in SEAc-$_{PL}$ and SEAc-$_{SC}$ included juveniles, sub-adult, and adult males captured in estuarine habitats during 2011. I found low overlap of SEAc-$_{PL}$ and SEAc-$_{SC}$ for adult females captured in estuarine habitats and adult males captured in freshwater habitats during 2010, juvenile and sub-adult females captured during 2010 and 2012, and juvenile and sub-adults (both sexes) captured in freshwater habitats during 2011. The amount of SEAc-$_{SC}$ overlap with SEAc-$_{PL}$ followed similar patterns, however, proportion of SEAc-$_{SC}$ was slightly higher compared to SEAc-$_{PL}$ due to the smaller size of SEAc-$_{SC}$ (Table 9).

The least proportional overlap was found for SEAc-$_{RBC}$ and SEAc-$_{SC}$, with 8 of the 13 sub-populations estimated to maintain no overlap (Table 9). Overlap of SEAc-$_{RBC}$ and SEAc-$_{SC}$ was highest for juvenile and sub-adult females captured in estuarine habitats during 2011 (with 77% of SEAc-$_{RBC}$ and 10% of SEAc-$_{SC}$ encompassed by the overlap area). Adult females captured in estuarine habitats and adults (both sexes) captured in freshwater habitats during 2010 and 2012 maintained high overlap between SEAc-$_{RBC}$ and SEAc-$_{SC.}$ This corresponded to 75% and 84% of SEAc-$_{SC}$ bounded by the overlap region. I found much lower overlap of SEAc-$_{RBC}$ and SEAc-$_{SC}$ for juvenile and sub-adult males captured in freshwater habitats during 2010 and 2012 (5% of SEAc-$_{RBC}$ and 2% of SEAc-$_{SC}$), and adult males captured in estuarine habitats during 2010 and 2012 (19% of SEAc-$_{RBC}$ and 58% of SEAc-$_{SC}$).

Relationship between niche specialization index (ε) and isotopic niche (SEAc)

To examine the potential for the size of the isotopic niche space (SEAc) to influence niche specialization index (ε) values estimated for GRWMA sub-populations, I performed correlation tests and linear regressions to assess the form and strength of the associations of mean ε values and variation in ε with isotopic niche size (SEAc). Size of the isotopic niche is theorized to represent the breadth of the trophic or ecologic niche, as such ε and SEAc are expected to be negatively correlated (i.e., smaller isotopic niche = increased dietary specialization and larger isotopic niche = increased dietary generalization). Mean ε_{RBC} was negatively correlated to SEAc-$_{RBC}$ (P = 0.009, γ = -0.56), however, the correlations of PL and SC data were non-significant. Linear regression analysis was not significant (P = 0.20) and seemed to be strongly influenced by the large size of sub-population 7's SEAc-$_{PL}$ (18.72‰^{2}). This was confirmed by removing sub-population 7's data from the regression analysis and obtaining a significant result (intercept = 0.41, β = -0.03, P = 0.03, R^2 = 0.33). Furthermore, variation in ε should be positively correlated to the size of SEAc, because variation in ε represents variability in niche specialization among individuals within a group, and the size of SEAc for a particular group represents the breath of their trophic interactions. Variation of ε_{PL} was positively correlated to SEAc-$_{PL}$ (P = 0.04, γ = 0.46), while RBC and SC correlation tests were non-significant. The linear relationship of the variation in ε_{PL} and SEAc-$_{PL}$ was also significant (intercept = 0.06, β = -0.02, P = 0.03, R^2 = 0.46). Lack of correlation between SEAc and ε for PL and SC data and lack of association of ε standard deviation and SEAc for RBC and SC data indicates that size of isotopic niche may not inform the breadth of the trophic or ecologic niches.

DISCUSSION

To what extent does the use of estuarine prey vary by size class, sex, and capture habitat?

Short-term food habits over the previous two weeks to one month, as inferred by SCA, provided evidence that all *A. mississippiensis* size classes inhabiting GRWMA consume estuarine prey to some degree; however, importance of estuarine prey to diet increased through ontogeny (Table 1, Fig. 2A). Prey composition and relative importance of certain prey taxa in the diet also changed through ontogeny. Freshwater insects were the most important prey taxa in diets of juvenile and sub-adult size classes, while being a minor component of adult size class's diet. Conversely, estuarine

Table 9. Proportional overlap of isotopic niche space (SEAc) among tissue types for *A. mississippiensis* sub-populations. Each overlap proportion is presented as the area of overlap (e.g., PL-RBC) divided by the SEAc calculated for each tissue in a pair, indicated by the tissue abbreviation in the denominator.

					Proportional Overlap of SEAc					
Sub.pop #	**Year**	**Size-class**	**Habitat**	**Sex**	**PL-RBC/PL**	**PL-RBC/RBC**	**PL-SC/PL**	**PL-SC/SC**	**RBC-SC/RBC**	**RBC-SC/SC**
1	2010/2012	Juv./sub.	E	♂	0.29	0.47	0.51	0.79	0.00	0.00
2	-	-	-	♀	0.33	0.62	0.39	0.69	0.00	0.00
3	-	-	F	♂	0.00	0.00	0.73	0.24	0.05	0.02
4	-	-	-	♀	0.13	0.34	0.11	0.54	0.00	0.00
5	-	Adult	E	♂	0.81	0.29	0.58	0.62	0.19	0.58
6	-	-	-	♀	0.87	0.53	0.94	0.68	0.62	0.75
7	-	-	F	♀+♂	0.96	0.26	1.00	0.43	0.53	0.84
8	2011	Juv./sub.	E	♂	0.45	0.74	0.00	0.00	0.00	0.00
9	-	-	-	♀	0.08	0.69	0.39	0.45	0.77	0.10
10	-	-	F	♀+♂	0.40	0.58	0.07	0.09	0.00	0.00
11	-	Adult	E	♂	0.11	0.09	0.00	0.00	0.00	0.00
12	-	-	-	♀	0.51	1.00	0.03	0.03	0.00	0.00
13	-	-	F	♀+♂	0.51	1.00	0.03	0.03	0.00	0.00
mean					0.42	0.51	0.37	0.35	0.17	0.18
SD					0.31	0.31	0.36	0.30	0.28	0.32

baitfish incrementally increased in importance with increasing size class, becoming the principal prey in the diet of adult individuals. Short-term food habits of *A. mississippiensis* size classes in estuarine and freshwater habitats aligned closely with findings reported by previous studies performed on other coastal populations (Louisiana-Gabrey, 2010; Sapelo Island, Georgia-Nifong et al., 2015; Cape Canaveral, Florida-Boggs et al., 2016), although there are a few key differences in particular taxa of prey consumed by the GRWMA sub-populations. Specifically, estuarine crustaceans (i.e., crabs, shrimp, etc.) were important components of sub-adult and adult size class diets; however, few of these prey species were found in diets of individuals within the same size classes inhabiting GRWMA (Table 1). Rather, estuarine fish were major components of sub-adult and adult diets at GRWMA and of little importance in the diets of most other coastal populations studied thus far. Diet composition of adult individuals at GRWMA was similar to the composition reported for adult individuals sampled in Shark River Estuary (SRE) of the Florida Everglades (i.e., high prevalence of estuarine baitfish, Rosenblatt et al., 2015a). Diets of juveniles were similar among all Atlantic coast populations and chiefly comprised of freshwater insects, fish, and amphibians. I hypothesize that variation in diet composition among coastal *A. mississippiensis* populations is driven by local differences in prey availability and catchability within the particular habits studied. To validate this hypothesis, however, detailed information on prey diversity and abundances, as well as catchability, is required.

Although sample sizes were too small to draw any meaningful cross-sex comparisons within size classes, I found differences in diet composition of males and females when data were combined across size classes (Fig. 2B). Interestingly, short-term food habits of females indicated a stronger reliance on estuarine prey than males. In other coastal populations, females (particularly adults) often displayed a bimodal pattern in their use of estuarine prey (i.e., some individuals heavily relied on estuarine prey while others relied more on freshwater prey). Nifong et al. (2015) hypothesized that the driver for this pattern was the reproductive status of the individual. Reproductively active females constructed and guarded nests within freshwater wetlands and rarely ventured into estuarine habitats. At GRWMA, I observed female *A. mississippiensis* constructing nests, guarding hatchlings, and excavating/maintaining nursery ponds along the periphery of the estuarine Guana Lake impoundment (above high water line), essentially creating freshwater refuges in close proximity to estuarine foraging grounds. Salinity in these excavated areas was low, ranging from zero to 5 ppt (JCN, pers. obs.), and potentially provided nesting females and their progeny with enough low salinity water for survival while permitting straightforward access to estuarine foraging grounds.

Capture habitat strongly affected the inferred short-term food habits of *A. mississippiensis* at GRWMA. Not surprisingly, freshwater prey occurred more often in stomachs of individuals captured in freshwater habitats, while estuarine prey occurred more often in individuals captured in estuarine habitats (Fig. 2C). Perhaps the most interesting insight this comparison yielded was that it verified individuals forage in both habitat types independent of their capture location (i.e., stomach contents of some individuals contained both estuarine and freshwater prey). Because freshwater wetlands within GRWMA are essentially isolated from estuarine habitats, recovery of estuarine prey in the stomach contents of individuals captured in freshwater locations (or vice versa) confirms cross-ecosystem travel over the previous two weeks to one month. Similar dual-use of estuarine and freshwater prey has been documented in other coastal *A. mississippiensis* populations (Nifong et al., 2015; Rosenblatt et al., 2015).

Median SIAR predicted that proportional contributions of estuarine prey to the diet was greater than SCA measures (i.e., %IRI) for juvenile and sub-adult size classes and less than SCA measures for adults. Despite this result, the majority of %IRI's calculated for estuarine prey fell within 95% BCI's of the SIAR-based

proportional contribution estimates for estuarine prey. One possible explanation for discrepancies in the inferred trophic interactions yielded by 'snap-shot' (SCA) and time-integrated (SIA) data is SCA was performed only on 35% of the total number of individuals used in SIAR simulations. To examine this matter further, I repeated SIAR simulations using only isotopic data from individuals from which I collected stomach contents. Predicted proportional contributions of estuarine prey to juvenile diet included in the SCA subset were greater than predictions based on all data for juvenile and sub-adults captured in freshwater habitats and less than those captured in estuarine habitats. Furthermore, subset predictions were similar to full dataset predictions for sub-adult and adult size classes. Thus, differences in sampling effort for SCA and SIAR analyses may not be the primary driver of the differences. Alternatively, the time-integrated nature of stable isotope data may have contributed to these discrepancies. Because stomach contents represent prey consumed over the previous two-weeks to one month and stable isotope values represent integration of trophic interactions over the time period of tissue generation, SCA does not provide information on trophic interactions over the majority of time (88–97%) over which stable isotope values are integrated. Another potential confounding factor in SIAR predictions was use of a broad collection of potential prey for calculation of end-member (i.e., resource groups) means and standard deviations used in SIAR simulations. Isotopic values of prey captured in each habitat varied substantially among species and may not represent isotopic values of prey consumed by certain individuals. For example, mean $\delta^{13}C$ values of estuarine prey species ranged from -15.1‰ to -22.8‰, while the majority of estuarine prey taxa recovered from stomach contents were estuarine baitfish species with $\delta^{13}C$ values closer to -16‰ (Table 3). In the future, use of a smaller subset of potential prey to calculate mixing model end-member parameters may better represent *A. mississippiensis* trophic interactions that give rise to isotopic variation in their tissues.

SIAR results based on scute keratin-SC, representing integration of long-term dietary choices (>1 year), indicated juvenile/sub-adult sub-populations used freshwater prey more extensively over longer time periods compared to intermediate (RBC) and short (PL) time periods (particularly those individuals captured in freshwater areas). The similarly of GRWMA juvenile and sub-adult individuals that used estuarine prey was predicted by SIAR (~50% diet), rather than a gradual increase from juveniles to sub-adults reported for other coastal populations and indicated by SCA in this study (Nifong et al., 2015; Boggs et al., 2016). Adult sub-populations were consistently predicted to use estuarine prey extensively (median proportion of estuarine prey ranged from 0.51 to 0.72). Although I did not capture a sufficient number of adult individuals in freshwater habitats to draw cross-sex comparisons captured in estuarine habitats, inferred trophic interactions within each sampling period was highly similar in both adult sex classes (i.e., years 2010 and 2012 vs 2011).

In other populations of *A. mississippiensis*, use of estuarine habitat and prey resources is known to be affected by body size, sex, environmental factors (e.g., salinity, temperature, season), and individual-level specialization in foraging and movement patterns (Rosenblatt and Heithaus, 2011; Rosenblatt et al., 2013b, 2015; Fujisaki et al., 2014; Nifong et al., 2015; Boggs et al., 2016; JCN, unpublished). Increased reliance on estuarine prey by *A. mississippiensis* throughout ontogeny at GRWMA may result from interactive effects of multiple biological and ecological factors. First, in all coastal populations studied thus far, larger body size is associated with greater prevalence of estuarine prey in the diet, as well as increased occupancy of estuarine habitats (Nifong et al., 2015; Boggs et al., 2016; Fujisaki et al., 2016). As in all crocodilians, increasing body size of *A. mississippiensis* reduces the surface area-to-volume ratio, resulting in decreased rates of water loss when exposed to high salinities (Mazzotti and Dunson, 1989). Thus, larger body size of sub-adult and adult individuals likely enables them to occupy estuarine habitats for longer time periods and forage across ecosystems more frequently. Furthermore, large body size increases mobility,

enabling large individuals to move long distances. During a mark-recapture study in coastal Louisiana sub-adult and sexually mature adults traveled long-distances (0.3 to 90 km) over periods ranging from 29 to 3,336 days (9.1 years, Lance et al., 2011). The maximum overland distance *A. mississippiensis* could travel when transitioning from freshwater to estuarine habitats at GRWMA is approximately 1.5 km (straight-line distance). Second, the majority of freshwater habitats within GRWMA are ephemeral and do not support large populations of aquatic prey for extended time periods (Frazel, 2009). Conversely, the estuarine Guana Lake sustains aquatic prey year-round. One potential consequence of this mismatch in prey availability is increased competition for limited prey within freshwater habitats, which may force larger sub-adult and adult *A. mississippiensis* with higher metabolic demands to forage into estuarine habitats where prey are more plentiful. Lastly, crocodilians including *A. mississippiensis* are highly social, as well as territorial. As *A. mississippiensis* transition to sexually mature adults, territorial behaviors such as aggression toward conspecifics increase and subordinate individuals are often excluded from dominant individuals' territories (Lance, 1989; Vliet, 1989). Within the context of this study, dominant female and male *A. mississippiensis* may monopolize high quality nesting and refuge areas such as semi-permanent freshwater ponds, thus relegating subordinates to lower quality ephemeral wetlands.

Is *A. mississippiensis* a dietary specialist or generalist at the sub-population level?

Historically, *A. mississippiensis* has been considered an opportunistic generalist predator due to the wide range of prey species identified in dietary studies and primary reliance on sit-and-wait hunting techniques (Wolfe et al., 1987; Nifong et al., 2014). However, recent evidence suggests certain populations can display significant variation in individual-level specialization, particularly within coastal populations that have access to estuarine habitats and prey (Rosenblatt et al., 2015).

Within coastal *A. mississippiensis* populations, variation in individual-level dietary specialization manifests itself by producing behavioral types ranging from individuals that readily exploit estuarine prey through cross-ecosystem foraging to those that strictly rely on freshwater prey and habitat resources (Fujisaki et al. 2014; Nifong et al., 2015; Rosenblatt et al., 2013b, 2015). Although the majority of *A. mississippiensis* captured at GRWMA appeared to specialize on estuarine prey, foraging patterns of certain sub-populations deviated from the norm. For example, juvenile and sub-adult sub-populations were dietary generalists as evidenced by the low value and variation in ε estimated for short (ε_{PL}) and intermediate (ε_{RBC}) time scales (Table 7). Conversely, ε values calculated for adult sub-populations at GRWMA were relatively high and more variable, indicating greater individual-level variation in dietary preferences. Nifong et al. (2015) provided the only comparative data on dietary niche for *A. mississippiensis* sub-populations (i.e., size classes and sexes) within a population inhabiting Sapelo Island, Georgia using SC isotopic values. Although the range in mean specialization index values calculated for Sapelo Island sub-populations (ε = 0.10–0.87) exceeded the range of ε found for GRWMA sub-populations (ε = 0.12–0.45), variation in ε was lower for Sapelo Island sub-populations. Wide variation in ε values determined for GRWMA sub-populations suggests individual-level variation in dietary niche specialization is more pervasive within this population relative to the Sapelo Island population.

The broad range and high variation in niche specialization indices calculated for *A. mississippiensis* sub-populations in this study combined with findings from other populations (Nifong et al., 2015; Rosenblatt et al., 2015) suggest that individual-level variation in use of estuarine prey is a ubiquitous characteristic of coastal inhabiting populations. However, biologic and ecologic mechanisms driving the prevalence of individual-level variation in foraging tactics by coastal *A. mississippiensis* populations requires further investigation (Araújo et al., 2011; Rosenblatt et al., 2015). Implications of high variation in individual-level dietary specialization suggest there may not be a one-size-fits-all strategy for managing coastal *A. mississippiensis* populations.

A broader more inclusive approach is warranted, one that takes into account the full range of dietary and habitat requirements of all behavioral types present in a specific population.

WHAT IS THE AMOUNT OF OVERLAP IN THE ISOTOPIC NICHES OF *A. MISSISSIPPIENSIS* SUB-POPULATIONS?

Here I report the first estimates of isotopic niche size (SEAc) and proportional isotopic niche overlap for *A. mississippiensis*. Overlap in SEAc among or within sub-populations among tissue types can serve as a relative measure of the degree of similarity in trophic interactions (dietary niche) of sub-populations or assessment of temporal stability in trophic interactions of a single sub-population using cross-tissue comparisons (Jackson et al., 2011). The only other study on crocodilians that has reported size and overlap of isotopic niches (Bayesian standard ellipse areas SEA_B in this case) was performed by Marques et al. (2013) on size classes of *Caiman latirostris* (Broad-snouted Caiman) in freshwater habitats located on two silviculture farms in Brazil. They reported that isotopic niches of *C. latirostris* size classes (juveniles and adults) did not overlap and significantly differed in location within δ-space. In terms of the size of the isotopic niche, most *A. mississippiensis* sub-populations at GRWMA maintained larger niche spaces than those reported for *C. latirostris*. Differences in the size of isotopic niches among *A. mississippiensis* and *C. latirostris* may be due to the location-specific isotopic mixing space or δ-space available for these consumers. δ-space is ultimately constrained by the isotopic signatures of basal resources (e.g., autotrophs, microbes, etc.) and trophic discrimination of isotope values within the food chain. Thus, differences in isotopic signatures of resident basal resources could explain inter-species differences in isotopic niche size and overlap among sub-populations.

In general, overlap in isotopic niches of *A. mississippiensis* sub-populations at GRWMA was highest for sexes in the same size class captured in similar habitats regardless of year captured or tissue sampled. I observed lower overlap in isotopic niches for sub-populations of different size classes and sub-populations of individuals captured in different sampling periods (i.e., years 2010 and 2012 vs 2011). I determined the largest isotopic niche area ($SEAc\text{-}_{RBC}$ = 18.72‰^{2}) for adults (both sexes) captured in freshwater habitats during years 2010 and 2012 based on intermediate time-scale RBC data. The large size of sub-population 7's $SEAc\text{-}_{RBC}$ resulted in an increased average overlap among all sub-populations. Specifically, proportional overlap of sub-population's $SEAc\text{-}_{RBC}$'s was greater than 42% and 67% of the pair-wise proportional overlap calculations for $SEAc\text{-}_{PL}$ and $SEAc\text{-}_{SC}$, respectfully. Large size of sub-population 7's $SEAc\text{-}_{RBC}$ was a consequence of the high variability in $\delta^{13}C$ values among individuals in this sub-population.

Predictions from the two end-member mixing model analyses (SIAR) were similar to the estimated proportional contribution of estuarine prey to *A. mississippiensis* sub-population group's diets. However, the isotopic niche (SEAc) occupied by many sub-populations showed little to no overlap with one another. Lack of overlap in sub-population's isotopic niches despite similarities in SIAR estimates is likely due to differential use of particular prey taxa whose isotopic compositions deviate from end-member isotopic values used in the SIAR model calculated by averaging isotopic values of multiple prey species from each habitat. Future applications of isotopic mixing models should use more narrowly defined end-members (i.e., estuarine crustaceans, estuarine baitfish, freshwater insects, etc.) or weighted mean values for end-members based on some measure of dietary importance for prey taxa determined by SCA. This will enable further discernment in dietary choices that govern size, shape, and location of the isotopic niche area. Either of these refinement techniques should increase the congruency of isotopic niche overlap with mixing model results.

TO WHAT EXTENT DOES USE OF ESTUARINE PREY AND THE ISOTOPIC NICHE OF *A. MISSISSIPPIENSIS* SUB-POPULATIONS VARY TEMPORALLY?

The SIAR-predicted proportional contributions of habitat-specific prey to diets of most sub-populations were similar for each of the three tissues used here, indicating relatively high temporal stability in sub-populations' trophic

interactions over the past 250 to 1,100 days. The greatest temporal stability was demonstrated by sub-populations consisting of adults, and juvenile, and sub-adult size classes. Inferred contributions of habitat-specific prey to diets of adults (both sexes) captured in estuarine and freshwater habitats in all time periods were similar across tissues, with the predicted proportion of estuarine prey to the diet of these sub-populations ranging no more than 10% across tissues. Correspondingly, the proportional contribution of estuarine prey to diets of juveniles and sub-adults captured in estuarine habitats in all years and those captured in freshwater habitats during 2011 were similar across tissues (ranging no more than 6%). Juveniles and sub-adults captured in freshwater habitats in 2010 and 2012 demonstrated the least similarity in inferred trophic interactions based on isotopic values of different tissues, with the proportional contribution of estuarine prey estimated at the longest time scale (SC isotopic data); 15–23% lower than predications based on intermediate-(RBC) and short-term (PL) data. I hypothesize that high temporal stability in inferred trophic interactions of adult sub-populations is driven by specialization in microhabitat use or specialization on specific prey taxa. Lack of temporal stability in trophic interactions of juvenile/sub-adults captured in freshwater habitats during 2010 and 2012 is congruent with 'snap-shot' SCA findings; freshwater prey are important in juvenile and sub-adult diets. Increase in estimated proportional contribution of estuarine prey to this sub-population's diet based on integration of trophic interactions over short (PL isotopic data) and intermediate (RBC isotopic data) time-scales suggests that individuals at GRWMA begin use of cross-ecosystem foraging behaviors at relatively small body sizes (<100 cm TL). Based on keratinized scute isotopic data collected from *A. mississippiensis* inhabiting Sapelo Island, Georgia, Nifong et al. (2015) postulated there is a size constraint which limits use of high-salinity habitats, and individuals <79 cm TL may suffer greater physiological stress compared to larger individuals. However, salinity of the estuarine habitats surrounding Sapelo Island is higher (24–35 ppt) and more stable than water salinity in Guana Lake. Lower salinities in the estuarine habitats at GRWMA may lower the size threshold which permits individuals to use estuarine resources without suffering detrimental physiological effects.

Use of estuarine habitats was predicted for nearly all size classes, sexes, and tissues to be higher in 2011 than years 2010 and 2012. This apparent increase in use of estuarine prey was potentially driven, at least in-part, by effects of decreased rainfall and subsequent drought conditions experienced in 2011 on prey availability in freshwater wetlands and *A. mississippiensis* foraging and movement patterns. Decreased rainfall in 2011 affected water conditions in Guana Lake by raising water salinity to >30 ppt for over 120 days and appeared to reduce *A. mississippiensis* consumption rates. Reduced food intake by individuals likely contributed to the elevated $\delta^{15}N$ values measured in 2011 sub-populations' tissues which in-turn would have produced higher estimates for the proportional contribution of estuarine prey from SIAR simulations because estuarine prey maintained higher $\delta^{15}N$ values than freshwater prey. Reduced food intake by individuals in 2011 is substantiated by SCA data, wherein the average size-corrected prey mass recovered from stomach contents was 6.5 times lower than prey mass recovered from stomach contents captured in 2012. Effects of water salinity on *A. mississippiensis* feeding were characterized by Laurén (1985) who demonstrated spontaneous feeding by juvenile *A. mississippiensis* ceased after one week when exposed to salinities greater than 10 ppt; fasting continued for three weeks until individuals returned to freshwater. In addition, $\delta^{15}N$ values for *A. mississippiensis* tissues may be seasonally variable; wherein $\delta^{15}N$ values are elevated in spring resulting from enrichment of ^{15}N (higher $\delta^{15}N$ values) produced by catabolism of endogenous proteins while fasting during periods of cooler temperatures in the fall and winter (Lance, 2003; Woodborne et al., 2012). This pattern was found during the study of a mass mortality event of *Crocodylus niloticus* in the Olifants River of South Africa. Woodborne et al. (2012) reported an oscillating pattern of rising and falling $\delta^{15}N$ values

in a nominal time-series of isotopic signatures by serial sampling inert keratinized claw tissue. They postulated this pattern could be explained by reduced prey consumption and catabolism of endogenous proteins while over-wintering. Starvation and/or food limitation are known to increase $\delta^{15}N$ discrimination (i.e., $\Delta^{15}N_{tissue\text{-}diet}$, the change in $\delta^{15}N$ from diet to tissue) during incorporation of food resources into animal tissues (McCutchan et al., 2003). The higher $\delta^{15}N$ values measured in *A. mississippiensis* tissues captured in 2011 may have been a consequence of seasonally elevated $\delta^{15}N$ values combined with effect of prolonged starvation/fasting on N-discrimination rather than increased consumption of estuarine prey with higher $\delta^{15}N$ values. Additionally, all upland freshwater habitats were greatly reduced in size in 2011. Most were completely dry except for artificially maintained wetlands and areas of wetlands that contained alligator den and hole complexes (JCN, pers. obs.). Reduced water levels in freshwater wetlands in 2011 may have affected *A. mississippiensis* foraging patterns by reducing abundance of resident freshwater prey, forcing individuals that usually rely on these taxa to fast or venture into estuarine habitats to locate adequate prey. In conclusion, increased $\delta^{15}N$ values and subsequent higher estimates of proportional contribution of estuarine prey to diets of 2011 sub-populations are likely consequences of combined effects of seasonal $\delta^{15}N$ variation, physiological effects of food limitation on $\delta^{15}N$ values, and an actual increase in use of estuarine prey.

In other populations, salinity significantly alters *A. mississippiensis* movement patterns associated with exploitation of estuarine habitats and prey species. In SRE in the Florida Everglades, adult *A. mississippiensis* used downstream estuarine habitats twice as much during lower salinity wet seasons compared to higher salinity dry seasons (Rosenblatt and Heithaus, 2011; Rosenblatt et al., 2013b). Similarly, in a population on Sapelo Island, Georgia, movement data garnered from deployments of GPS telemetry units (JCN, unpublished) demonstrated that increasing water salinity significantly decreases duration of cross-ecosystem forays into estuarine habitats. Moreover, higher water salinity has been found to increase rates of movement by *A. mississippiensis* while occupying estuarine habitats (Fujisaki et al., 2014; JCN, unpublished). Further evidence supporting negative effects of increasing water salinity on *A. mississippiensis* use of estuarine habitats comes again from SRE. Fujisaki et al. (2016), using relative abundance data from count surveys, observed a significant decrease in density of all *A. mississippiensis* size classes in the estuarine downstream zone of SRE during higher salinity dry seasons compared to lower salinity wet seasons.

Taken together, these findings indicate that extreme weather events such as drought conditions experienced in 2011 at GRWMA are capable of influencing the use of estuarine prey by *A. mississippiensis*. Given that frequency and intensity of extreme weather events are expected to increase as global climate change progresses (IPCC, 2014), this information should be useful to conservation managers developing conservation strategies for coastal *A. mississippiensis* populations. For example, if managers wish to encourage reciprocal cross-ecosystem movements of *A. mississippiensis* from freshwater to estuarine habits during drought conditions then efforts should be made to increase the availability of freshwater refuges by pumping groundwater into wetlands or diverting surficial flow. In conclusion, increased frequency and intensity of extreme weather events combined with expected rise in sea levels may reduce the ecological benefits, such as translocation of nutrients between habitats and increased habitat heterogeneity associated with *A. mississippiensis* habitat engineering behavior (Rosenblatt et al., 2013a; Nifong et al., 2015).

For all sub-populations, temporal stability in trophic interactions was highest for short and intermediate time scales. Although overlap of $SEAc\text{-}_{PL}$ with $SEAc\text{-}_{RBC}$ and $SEAc\text{-}_{SC}$ ranged from 0% to 100%, each sub-population shared on average 42% of their $SEAc\text{-}_{PL}$ with $SEAc\text{-}_{RBC}$ compared to 32% of their $SEAc\text{-}_{PL}$ with $SEAc\text{-}_{SC}$. The least amount of overlap in isotopic niches was found between intermediate and long time-scales; each group

shared 51% of SEAc-$_{RBC}$ with SEAc-$_{PL}$ and only 17% with SEAc-$_{SC}$. Furthermore, eight of the 13 sub-populations maintained 0% overlap between SEAc-$_{RBC}$ and SEAc-$_{SC}$. Sub-populations which displayed the highest temporal stability were adult individuals (both sexes) captured in 2010–2012. Specifically, these sub-populations demonstrated considerable overlap (>40%) among SEAc's calculated for all tissue types. Temporal stability was lower for adults captured in 2011 and lowest for juvenile and sub-adults captured in freshwater habitats (all years) which maintained almost no overlap between SEAc-$_{SC}$ and either SEAc-$_{PL}$ or SEAc-$_{RBC}$. The isotopic niches of juveniles and sub-adults in estuarine habitats were more stable than those in freshwater habitats; however, this pattern was only evident for comparisons made at short and intermediate time-scales. RBC isotope values for all groups behaved somewhat differently than those measured for PL and SC. For the majority of sub-populations, SEAc-$_{RBC}$ were truncated and shifted upwards along the y-axis. Turnover of $\delta^{13}C$ and $\delta^{15}N$ in *A. mississippiensis* red blood cells proceeds at different rates, with $\delta^{15}N$ taking nearly twice as long as $\delta^{13}C$ and even surpassing turnover time of these elements in scute keratin-SC. Rosenblatt and Heithaus (2013) hypothesized that differential turnover rates in RBCs could be partially explained by physiological differences in use of these elements. N is an essential component of the long-lived hemoglobin molecules in RBCs (Cline and Waldmann, 1962; Dessauer, 1970), while C is exchanged more readily as CO_2 during respiration (Jensen et al., 1998). Differences in turnover rates could confound inferences drawn from temporal comparisons of isotopic niche space defined by RBC data. Taking this into account, comparisons of short- and long-term isotopic niches (SEAc-$_{PL}$ and SEAc-$_{SC}$) may be a more informative comparison of temporal diet stability.

The only other study that has assessed temporal diet stability of *A. mississippiensis* based on stable isotope data (Rosenblatt et al., 2015) found that most sub-populations examined were temporally stable across short and intermediate time-scales as inferred by strong correlations of $\delta^{13}C$ values from blood plasma-PL and red blood cells-RBC. The single outlier from this study was the same population studied here, whose PL and RBC $\delta^{13}C$ values were weakly correlated. The discrepancy with the findings reported here are likely because all data were combined for GRWMA in the previous study, thus the effects of size class, sex, year, and capture habitat on isotopic variation were not taken into account.

All inferences based on stable isotope ratios measured in *A. mississippiensis* tissues used in this study are the product of integrated dietary choices over the previous ~250 to 1,100 days depending on the element and tissue. Hence, there was no isotopic information resulting from integration of very short-term dietary interactions (2 weeks to 1 month) to compare isotopic data to SCA results characterizing recent food habits. Future isotopic studies on *A. mississippiensis* and other crocodilians may benefit from finding easily sampled tissues with faster turnover rates to enable comparisons of SCA and SIA data, as well as examination of stabilities of short-term dietary interactions though time.

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