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Unique seasonal forage bases within a local population of bottlenose dolphin (*Tursiops truncatus*)

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ABSTRACT

Using photo-identification data, bottlenose dolphin (*Tursiops truncatus*) populations can be differentiated based on their use of particular estuaries or coastal habitats. Questions remain, however, about the validity of such fine-scale population partitioning and whether the resulting assemblages utilize unique forage bases. To address the issue of forage base use, stable isotopes of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulfur ($\delta^{34}S$) were analyzed from skin tissues ($n = 74$) of bottlenose dolphins sampled seasonally along the coast and in three estuaries near Charleston, South Carolina. Autumn values of $\delta^{34}S$, $\delta^{15}N$, and $\delta^{13}C$ and summer values of $\delta^{34}S$ indicated that dolphins sampled from these four assemblages utilized unique forage bases, despite limited sample sizes. Likewise, autumn and spring differences in $\delta^{15}N$ and $\delta^{13}C$ values were evident in the North Edisto River, and in $\delta^{34}S$ from dolphins sampled from all three estuarine assemblages; no seasonal differences were identified in the coastal assemblage. Results demonstrate the importance of

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considering spatial and temporal variation in forage base when developing local management plans for bottlenose dolphin and highlight the discriminatory power of $\delta^{34}S$ for estuarine and coastal marine mammals. These results also suggest that stable isotopes could be developed as a complementary tool for photo-identification based partitioning of bottlenose dolphin populations.

Key words: stable isotopes, foraging ecology, estuaries, coastal, marine mammals, seasonal variation.

An alteration in forage base can influence levels of essential nutrients, such as fatty acids (Iverson *et al*. 1997), and levels of harmful contaminants, such as PCBs (Ross *et al*. 2000), in a consumer's tissues. These dynamics are especially important to track in top predators, including many species of marine mammals, which accumulate contaminants (Fisk *et al*. 2001, Houde *et al*. 2006) and essential fatty acids (Thiemann *et al*. 2008) commensurate with trophic level. Higher trophic level species can influence the structure and function of underlying marine communities (Estes *et al*. 1998, Daskalov 2002, Halpern *et al*. 2006).

Bottlenose dolphins (*Tursiops truncatus*) feed near the top of marine and estuarine food webs (Borrell *et al*. 2006) and exhibit varying degrees of site fidelity to estuarine, coastal, and offshore waters (Waring *et al*. 2007, Bearzi *et al*. 2009). Although some dolphins are known to travel great distances, long-term repeated observation of recognizable individuals indicates that others exhibit limited geographic movement (Scott *et al*. 1990, Zolman 2002, Rogers *et al*. 2004, Speakman *et al*. 2006). The home range of estuarine and coastal dolphins is likely smaller than that of their offshore conspecifics (Cockcroft and Ross 1990), and in combination with the dolphin's high trophic position may result in considerable localized pressure on prey resources (Wilson *et al*. 1997, Defran and Weller 1999). Thus, if bottlenose dolphins exhibit habitat partitioning, where individuals are found to be associated with specific coastal areas or estuaries, one might expect utilization of unique forage bases by localized assemblages of dolphins.

Stable isotopes of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulfur ($\delta^{34}S$) provide a time-integrated perspective of an individual's forage resources that overcome many of the limitations of conventional methods, such as stomach content analysis (Hebert *et al*. 2008, Rodgers and Wing 2008). In general, carbon isotopes are commonly used as indicators of carbon production pathways (*i.e.*, C_3 *vs.* C_4 plants) and for elucidating freshwater *vs*. marine habitat use (Peterson and Fry 1987). Sulfur isotopes provide an additional tracer for separating terrestrial/freshwater *vs*. marine sources in an ecosystem and have recently been used to differentiate bottlenose dolphins from estuary, inshore, and offshore habitats (Barros *et al*. 2010); marine values of $\delta^{34}S$ are ~21%, whereas freshwater $\delta^{34}S$ values are ~2%–8% (Fry *et al*. 1982, Peterson and Howarth 1987, Hebert *et al*. 2008). For nitrogen, a ^{15}N enrichment of 2%–4% between aquatic producers and consumers appears typical (Peterson and Howarth 1987, Michener and Schell 1994) providing an estimate of trophic position (Minagawa and Wada 1984).

Recently, stable isotope values in skin tissue of marine mammals have been used to assess feeding variation among social groups or clans (Marcoux *et al*. 2007, Witteveen *et al*. 2009), between sexes (Tucker *et al*. 2007) and species (Gross *et al*. 2009), and across seasons (de Stephanis *et al*. 2008) and ages (Knoff *et al*. 2008). Stable isotope analysis has even indicated segregation of dolphin species that were not apparent

from visual observations (Gross *et al*. 2009). Skin tissue is continuously renewed in marine mammals thus providing an estimate of recent diet that is dependent on the skin renewal time of the species. The use of skin tissue for stable isotope analysis avoids concerns about long turnover times and tissue routing, common to other tissue types, such as liver and muscle (MacAvoy *et al*. 2005). Importantly, skin tissue can be sampled nonlethally and is of particular value for species like marine mammals (Smith *et al*. 1996), where large samples sizes are often impractical.

Photo-identification (photo-id) studies of bottlenose dolphins within the estuarine and coastal areas of South Carolina have revealed complex temporal and spatial patterns of occurrence, where some individuals restrict their habitat usage to one area while others range among multiple locales (Speakman *et al*. 2006). We tested the hypothesis that different localized assemblages of bottlenose dolphins exploit unique forage bases, as reflected in $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values in dolphin skin tissues. We sampled seasonally to address questions regarding the temporal movement patterns of bottlenose dolphins. We hypothesized that the isotopic values of coastal dolphins would remain consistent throughout the year, but that estuarine dolphins would show seasonal shifts in forage base use that reflect the seasonal variability in prey composition observed from the stomach contents of stranded bottlenose dolphins (Gannon and Waples 2004, Pate 2008).

Material and Methods

From March 2002 to February 2003, skin samples ($n = 74$) were collected *via* remote biopsy (Lambertsen 1987) from free-ranging dolphins in three estuaries, Charleston Harbor (CHS), Stono River Estuary (SRE) and North Edisto River (NER), and in coastal waters (CST) near Charleston, South Carolina (SC) (Fig. 1). Remote biopsies were typically collected from the area below the dorsal fin and above the lateral midline using nontethered remote biopsy darts (Lambertsen 1987), fired from a modified 0.22 caliber rifle. Sampling tips were 1.0 cm in diameter by 2.5 cm long. The floating dart with the intact biopsy sample was retrieved within ~30 s for processing. Skin samples (epidermis and dermis) were immediately subsectioned from the blubber (hypodermis) using precleaned forceps and scalpel with a sterile blade, rinsed several times with distilled water, placed in a precleaned Teflon vial and immediately stored in a liquid nitrogen (LN_2) vapor cooler. In the laboratory, samples were stored at $-80^{\circ}C$ until processed.

Photo-id sighting data and techniques are outlined in Speakman *et al*. (2006). Briefly, 136 boat-based photo-id surveys were carried out between January 1997 and May 2003 near Charleston, SC (Speakman *et al*. 2006). Surveys were conducted by a crew of 2–3 observers on small (5–7 m) outboard motor powered boats following established routes through the estuaries and along the coast at approximately 8–16 km/h. Dolphin dorsal fins were videotaped and/or photographed and used to establish a catalog of distinctively marked fins. The proportion of sightings in each of the four locations (CHS, SRE, NER, and CST) was calculated for each dolphin, and each dolphin was then assigned to a geographically defined assemblage. A minimum association of 50% for assignment to an assemblage was used, although for many of the dolphins the association was greater than 75% (Table 1). If no sighting history existed for a dolphin, it was assigned to an assemblage based on where the sample was collected (Table 1). Sampled individuals ranged in age from juvenile (>2 yr old) to

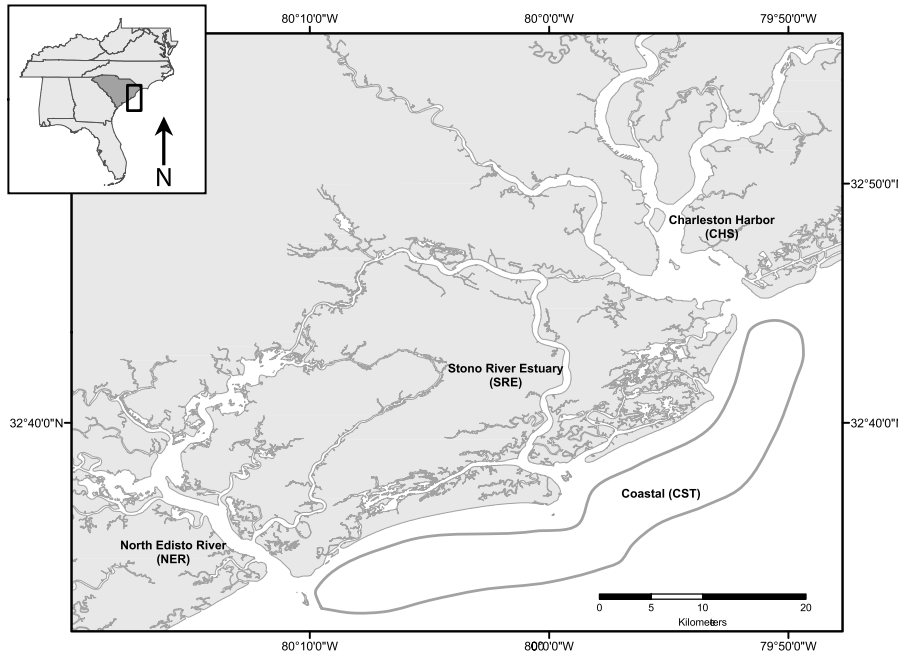

Figure 1. Sampling locations, one coastal (CST) and three estuarine (CHS, SRE, NER) near Charleston, South Carolina, where bottlenose dolphin skin tissues were collected. Coastal samples were obtained from the designated area, while estuarine samples were collected from the harbor and rivers shown, as well as their associated small creeks and harbor/river mouths.

adults, based on sighting history. The sex of each individual dolphin was determined by genetic analysis of the biopsy samples.

Frozen skin samples were freeze dried for 48 h, homogenized, and subsampled (~1 μg) into tin capsules for analysis of $\delta^{15}N$ and $\delta^{13}C$ by a Thermo-Finnigan Delta^{Plus} IRMS at the Institute of Ecology Stable Isotope Laboratory, University of Georgia, and subsampled (~6 mg) into tin capsules for analysis of $\delta^{34}S$ by a Thermo-Electron Delta^{Plus} Advantage IRMS at the Colorado Plateau Stable Isotope

Table 1. Sighting criteria used to classify sampled bottlenose dolphin into one of four sampling locations (coastal CST; estuarine CHS, SER, and NER) near Charleston, South Carolina, from March 2002 to February 2003. Adjusted sighting potentials (ASP) reflect the proportion of an individual's sightings that occurred within the designated area, adjusted for differences in survey effort (Speakman *et al*. 2006).

	CST	CHS	SER	NER
Total number of individuals assigned	25	21	13	15
Criteria 1: ≥75% ASP with 5+ sightings	1	2	3	8
Criteria 2: ≥50–74% ASP with 5+ sightings	15	14	5	1
Criteria 3: ≥75% ASP with 2–4 sightings	3	0	0	0
Criteria 4: ≥50% ASP with 2–4 sightings	0	0	0	2
Criteria 5: Sample collection location only	6	5	5	4

Laboratory, Northern Arizona University. Skin samples were not lipid extracted prior to isotope analysis as the C:N ratio was 3.5 ± 0.02 (mean ± SE) across all samples (Post *et al*. 2007). Stable isotope results are expressed in standard delta (δ) notation (Peterson and Fry 1987) relative to conventional reference material: PeeDee Belemnite, atmospheric nitrogen, and Canyon Diablo Triolite. Replicate standard analyses yielded a precision of ±0.04‰ (δ^{13}C), ±0.30‰ (δ^{15}N), and ±0.25‰ (δ^{34}S), respectively.

Stable isotope data were normally distributed based on probability plots. Consequently no data transformations were performed. Dolphins were grouped according to age (*sensu* Schroeder 1990; juvenile, subadult, and adult) based on sighting history and similar to Knoff *et al*. (2008). We ruled out the possible effects of sex (δ^{13}C $F_{1,70} = 3.84$, $P = 0.06$; δ^{15}N $F_{1,70} = 0.16$, $P = 0.70$; δ^{34}S $F_{1,70} = 1.28$, $P = 0.26$) and age class (juvenile, subadult, adult) on δ^{13}C ($F_{2,70} = 0.72$, $P = 0.39$), δ^{15}N ($F_{2,70} = 0.04$, $P = 0.83$) and δ^{34}S ($F_{2,70} = 0.28$, $P = 0.59$) values, respectively, using an analysis of variance (ANOVA; *lm* in R). Therefore data were grouped for all following analyses.

Stable isotope values were grouped into three-month seasons: spring (March–May), summer (June–August), autumn (September–November), and winter (December–February). The three-month season was chosen to reflect known variation in prey composition in the stomach contents of bottlenose dolphins in the region (Gannon and Waples 2004, Pate 2008). To compare seasonal isotopic values within and among assemblages, an ANOVA was performed for each of the stable isotopes (C, N, and S) using assemblage and season as independent variables. For each season, assemblages were compared using a Bonferroni pair-wise comparison with a Bonferroni correction of the *P*-value ($n = 3$ dependent variables, P significant < 0.017; Quinn and Keough 2002). Statistical analyses were conducted using program R (R Development Core Team 2009).

Results

Significant seasonal differences in the dolphin isotopic values were observed both within and among the four assemblages (δ^{13}C: $F_{5,58} = 2.85$, $P = 0.0022$; δ^{15}N: $F_{5,58} = 4.01$, $P < 0.0001$; δ^{34}S: $F_{5,58} = 8.07$, $P < 0.0001$).

Seasonal differences within each assemblage were most evident from the δ^{34}S values of the three estuarine dolphin assemblages (Fig. 2C); whereas only the NER assemblage exhibited seasonal differences in all three isotopes. Specifically, NER dolphins sampled in the autumn were significantly enriched in ^{13}C and significantly depleted in ^{34}S and ^{15}N relative to NER dolphins sampled in spring (Fig. 2A–C). The significant seasonal differences in dolphin skin δ^{34}S values indicated that in the SRE, autumn δ^{34}S values were lower relative to spring values, and in the CHS, winter δ^{34}S values were lower relative to spring and summer values (Fig. 2C). As predicted, based on pair-wise comparisons, seasonal differences in δ^{13}C, δ^{15}N, and δ^{34}S isotopic values were not evident for the coastal dolphins (Fig. 2A–C).

When comparing among season, evidence for bottlenose dolphin assemblages exploiting isotopically distinct seasonal forage bases was most notable from the δ^{34}S, δ^{15}N, and δ^{13}C values observed in autumn and from the δ^{34}S values observed in the summer (Fig. 3). Alternatively, δ^{34}S, δ^{15}N, and δ^{13}C values of the four assemblages sampled from the spring season, indicate a similar forage base being exploited, as minimal variability around the isotopic values is exhibited (Fig. 3). The significant

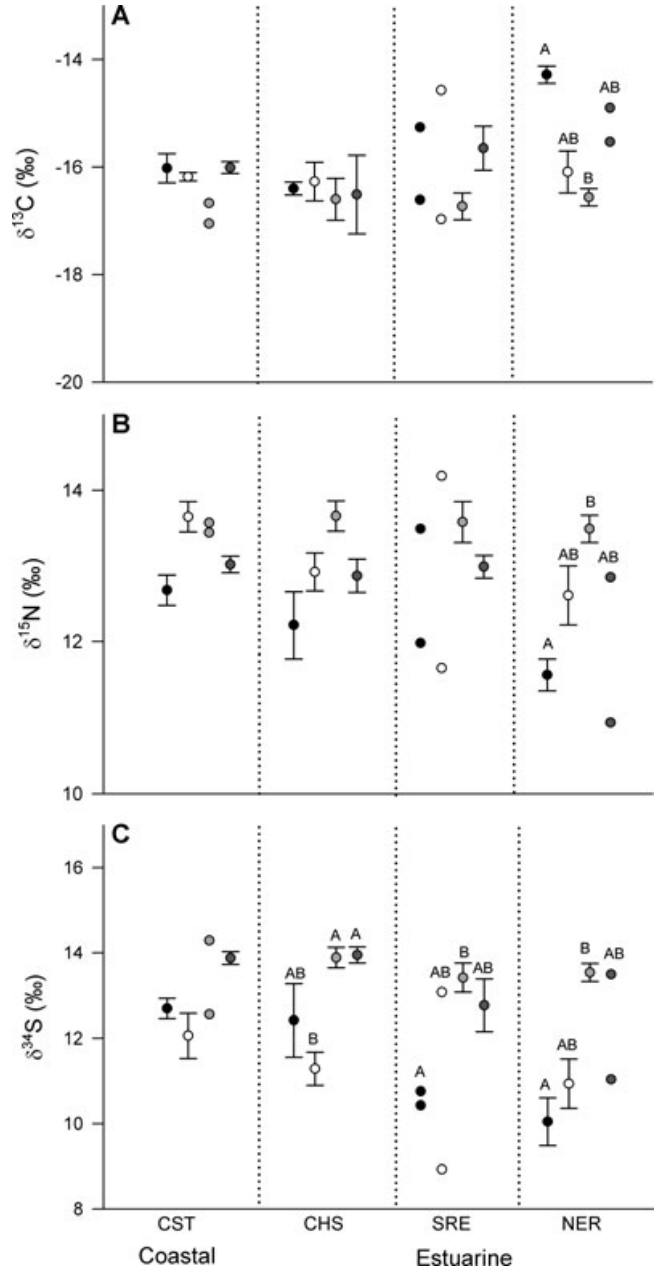

Figure 2. Seasonal (A) $\delta^{13}C$, (B) $\delta^{15}N$, and (C) $\delta^{34}S$ values (mean ‰ ± SE; black, autumn; white, winter; light gray, spring; dark gray, summer) of bottlenose dolphin from the four sampled assemblages. Letter displayed above mean values depict only the seasonal differences that were significant within each assemblage, respectively, based on pair-wise comparisons. Letters are not displayed in cases where no statistically significant seasonal differences within each assemblage were observed.

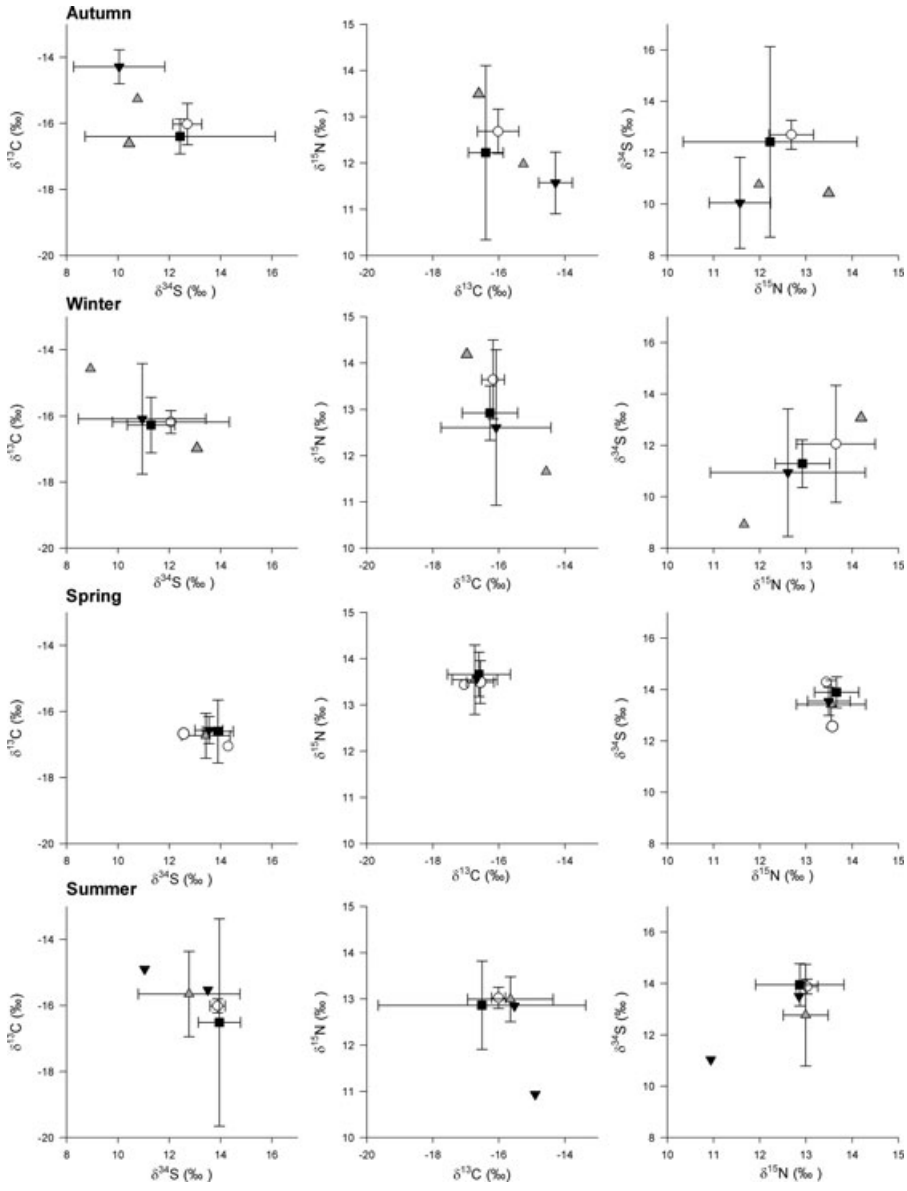

Figure 3. $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ (‰ mean ± 95% CI) values of bottlenose dolphin depicting seasonal differences among assemblages (coastal, CST ○; and estuarine, CHS ■, SRE Δ; NER ▼).

isotopic differences between assemblages in autumn, however, were largely driven by the NER assemblage. Specifically, the NER assemblage was significantly depleted in ^{13}C relative to the CST and CHS assemblages; significantly depleted in ^{15}N relative to the coastal assemblage and depleted in ^{34}S relative to the other three assemblages

Table 2. Stable isotope values (n = individuals sampled; ‰ mean ± SE) of bottlenose dolphin skin tissue samples collected seasonally from four locations, one coastal (CST) and three estuarine (CHS, SRE, NER) in South Carolina. For $n = 2$, both values are presented.[1]

Season	Location	n	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Autumn	CST	8	-16.02 ± 0.27^{A}	12.68 ± 0.20^{A}	12.70 ± 0.24^{A}
	CHS	3	-16.40 ± 0.12^{A}	12.22 ± 0.44^{AB}	12.42 ± 0.86^{AB}
	SRE	2	$-15.26; -16.61^{AB}$	$11.98; 13.49^{AB}$	$10.43; 10.76^{B}$
	NER	4	-14.28 ± 0.16^{B}	11.57 ± 0.21^{B}	10.05 ± 0.56^{C}
Winter	CST	3	-16.18 ± 0.08	13.65 ± 0.20	12.06 ± 0.53
	CHS	8	-16.27 ± 0.36	12.92 ± 0.25	11.29 ± 0.39
	SRE	2	$-14.57; -16.97$	11.66; 14.19	8.93; 13.08
	NER	3	-16.09 ± 0.39	12.61 ± 0.39	10.94 ± 0.58
Spring	CST	2	$-16.67; -17.05$	13.44; 13.57	12.56; 14.29
	CHS	7	-16.60 ± 0.39	13.66 ± 0.20	13.89 ± 0.24
	SRE	5	-16.73 ± 0.25	13.58 ± 0.27	13.42 ± 0.34
	NER	6	-16.56 ± 0.16	13.49 ± 0.18	13.54 ± 0.21
Summer	CST	12	-16.01 ± 0.11	13.02 ± 0.11	13.88 ± 0.15^{A}
	CHS	3	-16.51 ± 0.73	12.87 ± 0.22	13.95 ± 0.19^{A}
	SRE	4	-15.65 ± 0.41	12.99 ± 0.15	12.77 ± 0.62^{AB}
	NER	2	$-14.90; -15.53$	10.94; 12.85	$11.04; 13.50^{B}$

[1]Letters displayed adjacent to a given mean indicate significant results of pair-wise tests for seasonal comparisons among assemblage, given each isotope. Shared letters among assemblages and where letters do not appear indicate lack of statistical difference among assemblage within the given season, and for the given isotope but do not indicate differences across season.

(Table 2). Likewise, ^{34}S of NER dolphins sampled in the summer were significantly depleted relative to the CST and CHS dolphin assemblages, However seasonal differences were not limited to the NER assemblage. Significant $\delta^{34}S$ differences among the dolphin assemblages in autumn also included those between the CST and the SRE assemblages, where SRE was depleted in ^{34}S relative to CST (Table 2; Fig. 3).

Discussion

This study provides evidence for small-scale differentiation in forage base use by bottlenose dolphin. The analysis of stable isotopes in skin tissue provides evidence that bottlenose dolphins within the Charleston region, differentiated into unique assemblages by photo-id, utilize unique forage bases despite close geographical proximity (<50 km). Differences in forage base use assessed by stable isotopes have been reported for bottlenose dolphin assemblages over a larger geographic range than reported here (Borrell *et al*. 2006, Barros *et al*. 2010). Similar aggregations of feeding assemblages have also been identified using stable isotopes in other marine mammals including humpback whales (*Megaptera novaeangliae*) (Witteveen *et al*. 2009), long-finned pilot whales (*Globicephala melas*) (de Stephanis *et al*. 2008), and sperm whales (*Physeter macrocephalus*) (Marcoux *et al*. 2007).

Significant differences in stable isotope values among dolphin assemblages and seasons were identified despite the limitation of small sample size, particularly for assemblage-season combinations that had only one or two samples, and issues related

to the characteristics of the dolphins and the tissue samples. Large sample sizes for marine mammals are often logistically difficult or impossible to achieve; however, the following factors lend confidence to the conclusions drawn from this study: (1) isotopic trends identified from this study persisted when sample sizes were relatively large (*e.g.*, significant $\delta^{34}S$ differences between CST ($n = 8$) and NER ($n = 4$) in autumn), and (2) significant differences between assemblages were evident even under conservative pair-wise comparisons with Bonferroni adjusted *P*-values. Significant differences in stable isotopes were seen among dolphin assemblages despite the fact that skin tissue may be representative of two seasons (based on a skin turnover time of 73 d, Hicks *et al*. 1985) and that a number of the dolphins sampled here had been observed in multiple locations based on sighting histories. A larger sample size with more complete sighting histories would likely provide more specific details regarding assemblages and individual forage base use, and could potentially be used to establish assemblage-based stable isotope characteristics, and as suggested by Newsome *et al*. (2010), could be employed as a tool for assessing population structure.

Most dolphins exhibited $\delta^{34}S$ values $>10‰$, which are considered to be indicative of a predominately marine forage source (Lott *et al*. 2003, Hebert *et al.* 2008). The dolphins sampled along the coast had the most enriched $\delta^{34}S$ signatures across all seasons, with the exception of spring, relative to those measured for estuarine dolphins, and also exhibited no seasonal variation in any isotope value. These results indicate that the coastal dolphins utilized a similar, more marine forage base throughout the year. Values of $\delta^{34}S$ in estuarine dolphins were generally higher ($\sim 13‰$) in the spring and summer relative to the $\delta^{34}S$ values in the autumn and winter ($\sim 10‰$), suggesting a potential shift from a more marine forage base in warmer months to one influenced by estuarine resources in the cooler months. Separation of diet between estuarine and coastal dolphins has been demonstrated based on stomach contents of stranded animals (Gannon and Waples 2004) and stable isotopes of tooth collagen (Barros *et al*. 2010), consistent with the stable isotope results of our study. As well, shifts in forage base with season have been reported for estuarine bottlenose dolphins (Pate 2008) and other marine mammals (de Stephanis *et al*. 2008). However, the relative importance of estuarine forage base among estuarine dolphins varied with location and season in this study. The NER dolphins had the lowest $\delta^{34}S$ values in both autumn and winter, perhaps indicating a stronger and more consistent use of estuarine forage base than dolphins sampled in CHS and SRE; CHS dolphins had a more marine $\delta^{34}S$ value in the autumn and SRE in the winter, although sample size were low for the SRE autumn and winter collections.

During spring and summer, dolphins sampled in all four areas utilized a forage base with similar isotopic values, one of a more marine origin, based on higher $\delta^{34}S$, than observed for the estuarine dolphins in the autumn and winter. There was also less variation in the stable isotope values, particularly sulfur, during the spring, suggesting more selective prey consumption. This is somewhat counterintuitive, given the high diversity and abundance of prey generally available in estuaries during spring and summer (*i.e.*, Tuckey and Dehaven 2006, Sheaves *et al*. 2010). However, it is possible that either a single or combination of several, commonly preferred prey item(s) is more heavily targeted by dolphins during this time of year. Stomach contents of stranded bottlenose dolphins on the eastern seaboard of the United States exhibited preferences for particular fish species, although many different species of fish were found (Gannon and Waples 2004, Pate 2008).

It should be recognized that differences in stable isotope values between dolphin assemblages do not necessarily imply that these dolphins are feeding on different

prey species, or that dolphins with similar stable isotope values are feeding on the same items. Whether this variation in stable isotopes values is due to the selection of different prey species or selection of the same species of prey that have different isotope values would require additional data on prey species. The latter is an especially plausible explanation for variation observed among the more geographically disparate assemblages as a number of bottlenose dolphin studies on stomach content have identified similar prey items (Gannon and Waples 2004, Pate 2008). Regardless, these dolphins, at times, are utilizing forage bases with different isotope values within a relatively narrow geographic area.

In general, enriched $\delta^{13}C$ values were associated with depleted $\delta^{34}S$ values across all dolphins, supporting the link between enriched $\delta^{13}C$ and an estuarine forage base; enriched $\delta^{13}C$ values are commonly observed in estuaries (Peterson and Howarth 1987, Peterson and Fry 1987, Kwak and Zedler 1997). This is consistent with the results of Lusseau and Wing (2006), who found that inshore bottlenose dolphins inhabiting a New Zealand fjord utilized inshore and benthic prey resources, as opposed to pelagic marine based on $\delta^{13}C$. However, for certain seasons and estuaries depleted $\delta^{13}C$ in estuarine dolphins were not consistent with enriched $\delta^{34}S$ values (*e.g.*, dolphins sampled in SRE in winter). Interpreting $\delta^{13}C$ values in estuarine organisms can often be difficult because a mixture of terrestrial ($\sim$27‰) and salt-marsh ($\sim$13‰) organic matter can yield a $\delta^{13}C$ value similar to marine phytoplankton ($\sim$21‰) (Peterson and Fry 1987). Although $\delta^{13}C$ in marine mammals may provide some insights into the relative importance of estuary *vs*. marine forage use, additional tracers such as $\delta^{34}S$ are warranted or necessary.

Values of $\delta^{15}N$ did not vary among assemblage-season combinations with the exception of $\delta^{15}N$ values of NER dolphins sampled in autumn compared with values of the CST. The lower $\delta^{15}N$ in the autumn NER dolphins may reflect differences in the nitrogen signal of the system, greater dependence on a freshwater/estuarine forage base, and/or feeding on lower trophic level prey. Spatial variation of $\delta^{15}N$ at the base of food webs is often observed and can influence values throughout the food web (Vander Zanden and Rasmussen 2001). For example, proximity to urban areas can often elevate $\delta^{15}N$ values (Heaton 1986); the NER was the farthest from Charleston and that could explain the lower $\delta^{15}N$ values observed in the dolphins sampled at that location. Additionally, base-lining $\delta^{15}N$ values of the dolphins to a species that has a known feeding behavior, often a filter-feeding species such as a mussel or clam (Vander Zanden and Rasmussen 1999), would indicate whether the trophic positions were different between dolphins sampled at the different locations. Thus caution is warranted on assigning trophic positions based on $\delta^{15}N$ to the dolphins in this study.

The separation of the photo-id based spatial assemblages by stable isotopes supports previous evidence of bottlenose dolphin site fidelity in the SC population (Speakman *et al*. 2006), and suggests that stable isotopes may be an effective tool for validating assemblages based on photo-id data. However larger sample sizes would be required for stronger inference on separating assemblages based on stable isotopes alone. Nonetheless these stable isotope results provide unique insights, in particular temporal variation, into the forage base used by bottlenose dolphins, suggesting fine-scale forage base use by an important and abundant marine mammal. Critical to the conclusions of this work was the addition of $\delta^{34}S$ data, particularly in estuarine and coastal systems, where multiple carbon sources can confound the use of two stable isotopes (Fry *et al*. 1982), and the use of skin tissue, which provided a critical estimate of the seasonal forage base of the dolphins without the confounding issue of long turnover time. These results demonstrates the utility of stable isotopes to discriminate

between forage base utilization of localized assemblages of bottlenose dolphins and thereby emphasize the need to consider spatial and temporal variation in habitat and forage base resource utilization when managing for localized assemblages.

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