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Stable isotopes differentiate bottlenose dolphins off west-central Florida

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ABSTRACT

Distinguishing discrete population units among continuously distributed coastal small cetaceans is challenging and crucial to conservation. We evaluated the utility of stable isotopes in assessing group membership in bottlenose dolphins (*Tursiops truncatus*) off west-central Florida by analyzing carbon, nitrogen, and sulfur isotope values ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) of tooth collagen from stranded dolphins. Individuals derived from three putative general population units: Sarasota Bay (SB), nearshore Gulf of Mexico (GULF), and offshore waters (OFF). Animals of known history (SB) served to ground truth the approach against animals of unknown history from the Gulf of Mexico (GULF, OFF). Dolphin groups differed significantly for each isotope. Average $\delta^{13}C$ values from SB dolphins (−10.6‰) utilizing sea grass ecosystems differed from those of GULF (−11.9‰) and OFF (−11.9‰). Average $\delta^{15}N$ values of GULF (12.7‰) and OFF (13.2‰) were higher than those of SB dolphins (11.9‰), consistent with differences in prey trophic levels. $\delta^{34}S$ values showed definitive differences among SB (7.1‰), GULF (11.3‰), and OFF (16.5‰) dolphins. This is the first application of isotopes to population assignment of bottlenose dolphins in the Gulf of Mexico and results suggest that isotopes may provide a powerful tool in the conservation of small cetaceans.

Key words: bottlenose dolphins, *Tursiops truncatus*, stable isotopes, population assignment, group membership, strandings, Florida, Gulf of Mexico.

Several species of small cetaceans (*e.g.*, bottlenose dolphins, *Tursiops* spp.; humpback dolphins, *Sousa* spp.; Tucuxi, *Sotalia* spp.; finless porpoises, *Neophocaena phocaenoides*; Irrawaddy dolphins, *Orcaella brevirostris*; and Franciscana dolphins *Pontoporia blainvillei*) occur with near continuous coastal distributions (Rice 1998, Jefferson *et al.* 2008). Distinguishing between populations within this mosaic of overlapping continuous distributions is crucial to the conservation and management of these animals. This is particularly true in light of increasing anthropogenic impacts (*e.g.*, fishery interactions, habitat degradation, and pollution) that may not influence all populations equally, with increased mortality rates having greater impact on the viability of small populations (Marmontel *et al.* 1997, D'Agrosa *et al.* 2000, Dawson *et al.* 2001). Therefore, the need to understand dolphin population structure is paramount, yet correctly assigning population identity remains a formidable task, particularly when supporting data are incomplete or lacking (*e.g.*, decomposed stranded individuals). When properly documented, however, such information allows for the long-term evaluation of human-induced impacts on demography (*e.g.*, Wells *et al.* 2005, 2008).

Common bottlenose dolphins (*Tursiops truncatus*) occur continuously along the Atlantic and Gulf of Mexico coasts of the United States (Shane *et al.* 1986; Wells and Scott 1999, 2002), but populations that display high site fidelity comprise a mosaic of localized or resident groups in bays and estuaries (Irvine *et al.* 1981, Wells *et al.* 1987, Gubbins 2002, Irwin and Würsig 2004). Although morphological and genetic (*e.g.*, Hersh and Duffield 1990; Mead and Potter 1990, 1995; Hoelzel *et al.* 1998) differences have been reported between coastal and offshore ecotypes of bottlenose dolphins, our ability to distinguish among nearshore populations is considerably less robust. In the Gulf of Mexico and off west-central Florida in particular, resident populations of bottlenose dolphins have been identified through long-term studies (Wells *et al.* 1980, 1987, 2005; Irvine *et al.* 1981; Scott *et al.* 1990; Wells 1991, 2003). The Sarasota Bay (SB) resident dolphin community has been observed across five consecutive generations spanning nearly four decades (Wells 1991, Wells *et al.* 2005). The demographic, ranging, social, and genetic characteristics of this community suggest limited social interactions and exchange with adjacent communities or transient animals (Duffield and Wells 1991, 2002; Sellas *et al.* 2005; Fazioli *et al.* 2006). The genetic composition of dolphins in the nearshore and offshore waters of the Gulf of Mexico is poorly known. Off west-central Florida, where the continental shelf may be 200 km wide, bottlenose dolphins are the predominant cetacean species in waters <20 m deep, with a few sightings made in water depths of 50–100 m (Griffin and Griffin 2003). Although the distribution of the offshore ecotype of the bottlenose dolphin is not known for this area, along the Atlantic seaboard these dolphins are often sighted in waters >34 m deep and at least 34 km from shore, typically beyond the continental shelf (Torres *et al.* 2003). Therefore, differentiating among populations or stocks, particularly from stranded animals, remains a challenge and additional methods to discriminate among populations are needed.

Recently, stable isotope values of teeth have been used to differentiate between dolphin populations in the northwestern Atlantic (Walker and Macko 1999, Walker *et al.* 1999, Knoff *et al.* 2008). This approach is based on the principle that the isotopic composition of a predator reflects that of its prey (Ostrom and Fry 1993,

Lajtha and Michener 1994). We also recognize that the isotope values of primary producers are transferred to each trophic level in the food web (Harrigan *et al.* 1989). Therefore, habitat type and prey distribution are expected to directly influence the isotopic signatures of the predator. In west-central Florida, the prey of bottlenose dolphins inhabiting estuarine sea grass habitats (*e.g.*, SB, Charlotte Harbor, and Tampa Bay) and phytoplankton-dominated habitats of the adjacent Gulf of Mexico are distinct (Barros and Odell 1990, Barros and Wells 1998, Barros, unpublished data). In addition, sea grass and offshore phytoplankton can differ in carbon, nitrogen, and sulfur isotope values ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$, respectively) (Peterson and Fry 1987, Connolly *et al.* 2004). Thus, west-central Florida offers a unique setting where we can demonstrate the application of isotope techniques to differentiate putative dolphin populations.

We collected teeth from dolphins believed to be from three different population units (Sarasota Bay, SB; nearshore Gulf of Mexico, GULF; and offshore, OFF), hereafter referred to as groups due to lack of genetic confirmation, that stranded in west-central Florida. The availability of long-term data on dolphin distribution, ranging patterns, and behavioral ecology from Sarasota Bay (Wells 1991, 2003; Wells *et al.* 2005) in contrast to the unknown history of Gulf nearshore and offshore individuals offers a unique opportunity to ground-truth the use of stable isotope proxies for understanding population membership. The goal of this study was to test the hypothesis that long-term feeding patterns, as recorded by the stable isotope composition of teeth from stranded animals, could distinguish various groups of dolphins occurring off west-central Florida. When used in conjunction with genetic and biological data, these ecological attributes may be useful in population delineation.

METHODS

Dolphin Group Membership

Teeth collected from 82 dolphins stranded along the coast of west-central Florida during 1977–2007 were included in this study. Dolphin group membership was based on the following criteria:

(1) Resident dolphins from Sarasota Bay (SB; $n = 39$) were identified by distinct individual markings on their dorsal fins (Wells *et al.* 1987) or the presence of a freeze brand applied during brief capture-release and health assessment operations (Scott *et al.* 1990, Wells 1991). These animals had a documented long-term history of estuarine residency (Table 1).

(2) Gulf of Mexico dolphins (GULF; $n = 36$) were animals found stranded along the Gulf of Mexico beaches of barrier islands along west-central Florida from Clearwater (27°58′N, 82°48′W) to Charlotte Harbor (26°50′N, 82°10′W). The GULF dolphins did not belong to either the SB community (*e.g.*, not recognized from existing photoidentification catalogs) and did not have the double-hemoglobin electrophoretic profile characteristic of offshore bottlenose dolphins (Duffield *et al.* 1983, Hersh and Duffield 1990). GULF dolphins are often sighted in waters less than 20 m deep, but their possible movements into deeper waters are less well understood.

(3) Dolphins of the offshore ecotype (OFF; $n = 7$) were primarily identified by their hemoglobin profile (Duffield *et al.* 1983, Hersh and Duffield 1990). All inshore individuals (GULF and SB resident dolphins) have the

Table 1. Mote Marine Laboratory (MML) field number, Dolphin Biology Research Institute (DBRI) identifications, number of sightings, and period over which Sarasota Bay (SB) bottlenose dolphin sightings were collected.

MML field no.	DBRI ID	No. of sightings	Period
MML-8607	C17-1	30	1984–1986
MML-8713	FB77	159	1975–1987
MML-8813	FB23	127	1990–1996
MML-8817	FB42	44	1984–1988
MML-9104	FB22	98	1987–1991
MML-9108	FB45	62	1976–1988
MML-9115	FB31	216	1980–1991
MML-9118	FB21	59	1988–1991
MML-9212	FB67	261	1976–1992
MML-9215	FB50	109	1988–1992
MML-9221	FB103	73	1988–1992
MML-9225	FB37	133	1983–1992
MML-9226	FB52	103	1984–1992
MML-9401	FB19	243	1976–1993
MML-9509	FB98	161	1980–1995
MML-9512	FB112	208	1980–1995
MML-9514	FB41	203	1976–1995
MML-9625	FB57	323	1970–1996
MML-9804	FB29	414	1976–1998
MML-9913	FB38	547	1980–1999
MML-0016	BRD2	28	1996–2000
MML-0018	DIPT	40	1983–2000
MML-0111	FB96	242	1980–2001
MML-0112	FB216	101	1998–2001
MML-0114	IKNO	106	1993–2001
MML-0208	FB212	179	1999–2002
MML-0229	FB111	437	1983–2002
MML-0309	FB59	568	1976–2003
MML-0313	1536?	62	1999–2003
MML-0324	FB1	467	1991–2003
MML-0412	FB119	536	1991–2004
MML-0413	FB101	494	1990–2004
MML-0416	BRDO	56	1989–2003
MML-0526	FB177	99	2002–2005
MML-0535	FB99	176	1989–2005
MML-0609	JOSE	153	1996–2006
MML-0611	FB75	582	1982–2006
MML-0614	FB6	568	1984–2006
MML-0619	FB100	359	1989–2006

single-hemoglobin electrophoretic profile as opposed to the double-banding profile shown by offshore bottlenose dolphins. Because blood was not available for two of the OFF animals, skull morphology (Hersh and Duffield 1990; Mead and Potter 1990, 1995; Turner and Worthy 2003), stomach contents, and parasitic load (Barros and Odell 1990, Mead and Potter 1990) were used to distinguish them from nearshore individuals.

Only animals 2 years or older (approximate age at weaning; Wells and Scott 1999) were included for analysis to minimize the maternal influence on isotope values (Knoff *et al.* 2008). Animals with no estimated age were included in this study when their total length was at least 200 cm (approximate size at 2 years of age; Read *et al.* 1993, Knoff *et al.* 2008). Associated data on stomach contents were used to confirm prey preference by habitat. Stomach content data were available for 4 (of 7) OFF, 23 (of 39) SB, and 34 (of 36) GULF animals included in this study.

Stable Isotope Analyses

For isotope analyses, teeth were fractured into small pieces (no separation of enamel, cementum, and dentine was performed) and ground to a fine powder using a ball and capsule amalgamator (Cresant Industries). The bone powder was demineralized with 1-N HCl for 24 h at 4°C. To isolate collagen from the noncollagenous proteins, the demineralized bone was centrifuged. The resulting collagen pellet was rinsed with an excess of ultra-pure deionized water, freeze-dried, and lipid extracted. Aliquots (*ca.* 1.0 mg) of collagen were analyzed for stable carbon and nitrogen isotopic composition using an elemental analyzer (either a Carlo-Erba or Eurovector) interfaced to a mass spectrometer (Prism or Isoprime, Elementar Instruments). For sulfur isotope analysis, aliquots of the collagen (8 mg) from each tooth were combusted in an elemental analyzer (Costech Analytical) interfaced to a mass spectrometer (Thermo-Finnigan DeltaPlus XP). Isotope values are expressed as follows:

$$\delta X = \left\{ \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right\} \times 1,000,$$

where X represents ^{13}C, ^{15}N, or ^{34}S and R represents $^{13}C/^{12}C$, $^{15}N/^{14}N$, and $^{34}S/^{32}S$, respectively. In-house standards used for $\delta^{13}C$ and $\delta^{15}N$ were calibrated against V-PDB and air, respectively. NBS127 (21.1‰) and IAEA-SO-6 (−34.05‰) were used to normalize $\delta^{34}S$ data to V-CDT; precision was estimated at ±0.2‰ for carbon, nitrogen, and sulfur isotope values.

Statistical Analysis

Differences in carbon, nitrogen, or sulfur isotope values among dolphin groups were assessed by one-way analysis of variance (ANOVA) followed by *post hoc* pairwise comparisons (Bonferroni) to determine differences between groups. Bonferroni's adjustment was used for *post hoc* tests because it is more conservative and controls the familywise type II error rate. Alpha was set at 5% and all significance tests were performed in SYSTAT (version 12).

Results

Analysis of variance indicated significant differences among groups for all three isotopes ($\delta^{13}C$: $F_{2,79} = 13.134$, $P < 0.001$; $\delta^{15}N$: $F_{2,79} = 7.186$, $P = 0.013$; $\delta^{34}S$: $F_{2,26} = 49.812$, $P < 0.000$). Results from pairwise comparisons indicated that teeth from the GULF and OFF groups had lower $\delta^{13}C$ values relative to teeth from SB animals (Fig. 1A; $P < 0.001$ and $P = 0.023$ for SB *vs.* GULF and SB *vs.* OFF,

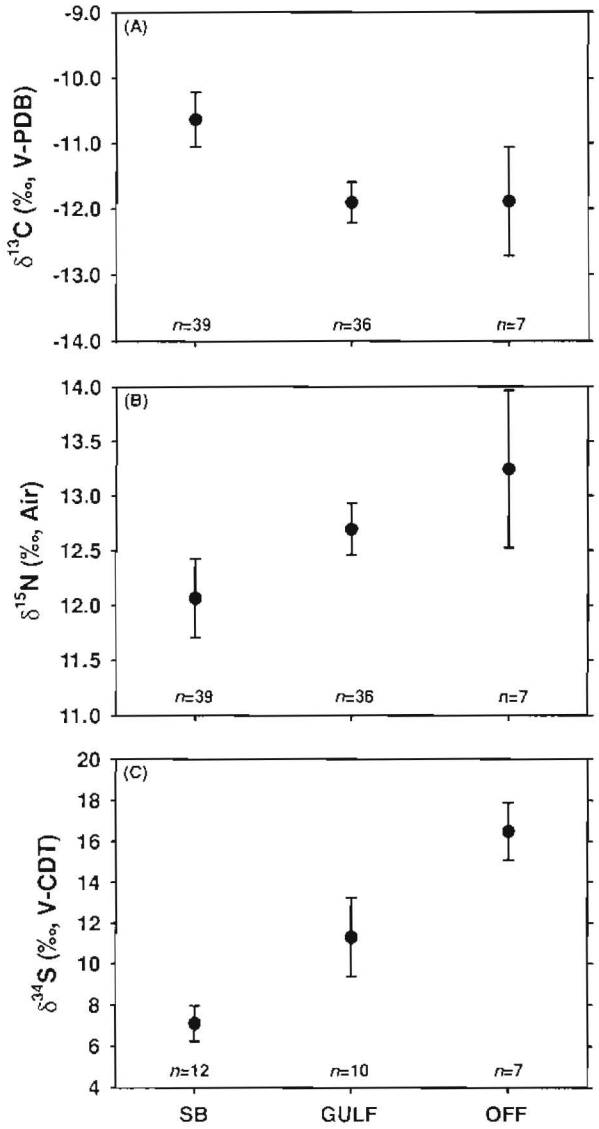

Figure 1. Average (A) carbon, (B) nitrogen, and (C) sulfur isotope value (with 95% confidence intervals) of teeth collected from stranded bottlenose dolphins from the central west coast of Florida, identified as originating from Sarasota Bay (SB), nearshore Gulf of Mexico (GULF), and offshore waters (OFF). Pairwise comparisons (Bonferroni) indicated significantly ($P < 0.05$) higher C values for SB dolphins (A), lower n values for SB dolphins (B), and different S values for all dolphin groups, with non-overlapping ranges.

respectively). For δ^{15}N values, pairwise comparisons showed that teeth of the SB dolphins were lower than those of the GULF and OFF groups (Fig. 1B; $P = 0.013$ and $P = 0.008$ for SB *vs.* GULF and SB *vs.* OFF, respectively). Dolphin teeth from all three groups differ in δ^{34}S values ($P < 0.000$ for SB *vs.* GULF, SB *vs.* OFF, and

GULF *vs.* OFF) (Fig. 1C). An intergroup comparison shows a decreasing trend in $\delta^{34}S$ with OFF > GULF > SB. Additionally, the $\delta^{34}S$ values of teeth from the OFF group were significantly higher than from the GULF animals, with non-overlapping ranges (7.2‰–12.7‰ and 13.8‰–18.4‰ for GULF and OFF, respectively).

Discussion

Carbon Isotope Values

The unique carbon isotope composition of sea grass provides a natural tracer of organisms that inhabit nearshore habitats dominated by sea grass (Peterson and Fry 1987, Harrigan *et al.* 1989). Whereas the $\delta^{13}C$ values of terrestrial C_3 plants that dominate in west-central Florida and phytoplankton-derived material from open waters of the Gulf of Mexico are typically less than −19‰, most sea grass carbon isotope values are greater than −13‰ (Smith and Epstein 1971, Eadie and Jeffery 1973, Jeffrey *et al.* 1983, Macko *et al.* 1984, Hemminga and Mateo 1996, Wang *et al.* 2004). The carbon isotope signature of sea grass is passed along to all trophic levels in the food web (Harrigan *et al.* 1989). Sea grass is a significant ecological feature of SB (Tomasko *et al.* 2005) and the average $\delta^{13}C$ values of teeth from SB resident dolphins (−10.6‰) are consistent with a sea grass-based food web. These findings corroborate the observations of dolphin feeding behavior over or near sea grass beds and the dominance of sea grass-associated prey fish (*e.g.*, pinfish, *Lagodon rhomboides*) inferred from stomach content analyses (Barros and Wells 1998).

In contrast to SB, $\delta^{13}C$ values of teeth from the GULF and OFF groups were significantly lower, consistent with a decrease in the influence of a sea grass-dependent food web. The distinction in $\delta^{13}C$ of dolphin teeth between SB and the GULF and OFF groups is also consistent with the observation that the stomach contents of SB dolphins differ from that of animals from adjacent Gulf nearshore waters (Barros and Wells 1998; Barros, unpublished data). However, $\delta^{13}C$ values of GULF and OFF dolphins do not show a large departure from those of SB (1.3‰) and are higher than what we would expect for a phytoplankton-dominated or *Sargassum*-based system ($\delta^{13}C$ of *Sargassum*-associated consumers = −16‰ to −18‰) (Rooker *et al.* 2006).

The $\delta^{13}C$ values of GULF and OFF dolphins appear more closely related to values reported for the phytoplankton *Trichodesmium* (*e.g.*, average $\delta^{13}C$ values of −12.9‰ for the northwest Caribbean Sea and southeast North Atlantic Ocean and −13.9‰ for the western Gulf of Mexico (Carpenter *et al.* 1997; Holl *et al.* 2007). Warm, nutrient-poor waters with high light intensity, such as those that can exist in deeper waters of the Gulf of Mexico, prompt widespread blooms of *Trichodesmium* (Capone *et al.* 1997). During such periods, high growth rates or CO_2 limitation may depress carbon isotope fractionation during photosynthesis and result in high $\delta^{13}C$ values for *Trichodesmium*. This is because carbon isotope values of autotrophs are influenced by fractionation during photosynthesis (*e.g.*, discrimination against ^{13}C during incorporation of carbon into the primary producer) (Farquhar *et al.* 1982, 1989; Laws *et al.* 1997). Fractionation occurs during the diffusion of CO_2 across the cell and enzymatic fixation of CO_2 (Farquhar *et al.* 1982, 1989). Fractionation associated with diffusion (*ca.* 4‰) is small relative to that imparted by the enzyme (−28‰) (Farquhar *et al.* 1982, 1989). Thus, phytoplankton $\delta^{13}C$ values can be quite high when the influence of diffusion in controlling photosynthetic fractionation increases. This can occur when concentration of aqueous CO_2 is low (*e.g.*, rapid

phytoplankton growth). Thus, it is possible that *Trichodesmium* influences the $\delta^{13}C$ composition of offshore and Gulf waters.

Nitrogen Isotope Values

The elevated $\delta^{15}N$ values of some OFF and GULF individuals relative to SB could reflect differences in trophic level or variation in the isotopic composition of nitrogen at the base of the food web (Ostrom *et al.* 1997). Our preliminary observations of stomach contents suggest that dolphins from the GULF and OFF ecotypes consume more prey from higher trophic levels (*e.g.*, GULF: squid of the neritic family Loliginidae, schooling fish; and OFF: octopods, benthic sharks, and jacks of the family Carangidae) than dolphins from SB (*e.g.*, pinfish, *L. rhomboides*; pigfish, *Orthopristis chrysoptera*; and mullet, *Mugil* spp.; Barros and Wells 1998). However, nutrients may also play a role in defining nitrogen isotope values of dolphin teeth. With the exception of a few locations (*e.g.*, small creeks in Little Sarasota Bay), sea grass within SB exhibits $\delta^{15}N$ values of *ca.* 0‰–3‰ (Dillon and Chanton 2008).

Phytoplankton $\delta^{15}N$ values vary with the source of inorganic nitrogen (*e.g.*, NH_4 or NO_3). If *Trichodesmium* contributes to the base of the food web in the Gulf and offshore waters, the low nitrogen isotope values of this organism would be expected to depress N isotope values throughout the food web (Holl *et al.* 2007). In this case, the high $\delta^{15}N$ values of OFF and GULF dolphins must reflect high trophic level prey that offset the influence of *Trichodesmium*. Alternatively, the typical value of open ocean marine nitrate of 6‰ suggests that consumers from more open waters such as offshore dolphins would have higher $\delta^{15}N$ values than those from estuaries dominated by sea grass (Fogel and Cifuentes 1993, Sigman *et al.* 1997).

Sulfur Isotope Values

While $\delta^{13}C$ and $\delta^{15}N$ values differ statistically among SB dolphins from the GULF and OFF animals, $\delta^{34}S$ values uniquely distinguish all three groups, as the ranges in values are non-overlapping. This is likely a consequence of the biogeochemical cycling of sulfur that produces distinct values between the open ocean and coastal environments. Marine sulfate is characterized by a $\delta^{34}S$ value of 21‰ and is subsequently reduced to hydrogen sulfide in the anaerobic sediments characteristic of coastal environments (Bottcher *et al.* 2006). This process results in a large decrease in the $\delta^{34}S$ of hydrogen sulfide (up to 70‰) relative to sulfate (Brunner and Bernasconi 2005). Oxidation of hydrogen sulfide and sulfide minerals within the zone of plant rhizospheres largely maintains the low $\delta^{34}S$ value in the newly formed secondary sulfate, which is taken up by rooted plants (*e.g.*, sea grasses) and subsequently transferred to consumers (Fry *et al.* 1982, 1988; Oakes and Connolly 2004). Because the subsequent shift in $\delta^{34}S$ between consumers and their diet is small (0‰–2‰; McCutchan *et al.* 2003), this accounts for the low $\delta^{34}S$ of dolphins in coastal marine environments. In contrast, the $\delta^{34}S$ values of individuals from offshore environments (*e.g.*, OFF dolphins) are influenced by the large well-mixed pool of sulfate in the open ocean (21‰). Deriving from a mixture of offshore and coastal sulfur sources, $\delta^{34}S$ values of GULF individuals are intermediate.

Isotopes as Tools for Population Differentiation

Although bottlenose dolphins from west-central Florida have the ability to move between habitats, our analysis reveals that isotope data, particularly $\delta^{34}S$ values, can

be used to differentiate individuals frequenting estuarine *vs.* nearshore (*e.g.*, GULF), and offshore habitats in the Gulf of Mexico. It is not surprising that sulfur isotopes provide excellent discrimination among the three dolphin groups from west-central Florida. The elevated and uniform $\delta^{34}S$ value of marine sulfate sharply contrasts the low $\delta^{34}S$ of the mixed and biogeochemically dynamic nearshore waters. This distinct isotopic transition imparts a high level of discrimination in the isotopic signatures of animals exploiting near- and offshore food webs, a particularly important attribute when considering a tissue such as teeth, which integrate over a long period (lifetime of the organism). In contrast, isotope values for carbon and nitrogen can change over time, particularly at the base of the food web, as a function of many processes. This is not to say that carbon and nitrogen isotopes are not good source indicators. The observation that some individuals from SB had extremely high $\delta^{13}C$ values (*ca.* $-10‰$) strongly suggests that they derive dietary resources from a habitat dominated by sea grass. Although increasing sample size will help constrain the distribution of potential $\delta^{34}S$ values for each population, bottlenose dolphin with $\delta^{34}S$ values more than $16‰$, the maximum value observed for GULF individuals, reliably classifies members of the OFF group.

This is the first study using stable isotopes to differentiate groups of small cetaceans in the Gulf of Mexico and adjacent nearshore waters. As teeth are easy to collect from stranded animals and are independent of carcass decomposition, this new tool will assist in elucidating the origin of stranded dolphins. While the technique has yet to be validated for distinguishing among specific discrete population units in regions of common habitat and prey availability, it appears to be a powerful means of distinguishing among groups of dolphins along an inshore-offshore gradient. Thus, it can be an important tool for determining if stranded animals originated in inshore *vs.* coastal *vs.* offshore waters—important distinctions for investigating unusual mortality events, for example. Building on these gross trophic distinctions and incorporation of genetic and other tests may allow more specific population assignment. In addition, it will be helpful in retrospective analysis of museum specimens of unknown origin. In most cases, the use of extensive osteological collections in ecological studies is hindered by the inability to properly assign population origin. Our results confirm the importance of sea grass beds as foraging grounds for estuarine bottlenose dolphins, a habitat also used by sympatric West Indian manatees (*Trichechus manatus latirostris*). The preservation and conservation of these habitats is recommended to ensure the population viability of both of these species of marine mammals in Florida waters.

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