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Sources of stable isotope variation among stranded Western Atlantic dolphins (*Tursiops truncatus*) in North Carolina

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Trophic shifts among Western Atlantic dolphins (*Tursiops truncatus*) as a function of sex and life stage are of interest to conservation biologists tasked with maintaining the species.

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For decades now the use of stable isotope ratios to assess various aspects of community ecology among marine mammals has been effective when traditional methods of observation or gut content analysis are not feasible. An added benefit of stable isotope analysis is that tissues of stranded animals, routinely collected as part of necropsies, can be used. In this paper we use a suite of isotopes from stranded dolphins to assess differences in trophic ecology at different life stages (calves, juveniles, and adults) as well as between sexes.

Samples for stable isotope analyses can be relatively decomposed (Payo-Payo *et al.* 2013) or can consist of inorganic tissue, such as teeth and bone (DeNiro 1987). Stable isotopes of the organic carbon collected from dolphin teeth or skin have been used to characterize offshore and coastal populations (Walker *et al.* 1999, Olin *et al.* 2012, Wilson *et al.* 2013), age and sex related feeding patterns (Nino-Torres *et al.* 2006, Knoff *et al.* 2008, Rossman *et al.* 2015), and historical changes in trophic status (Walker *et al.* 1999). For example, coastal populations tend to have lower $\delta^{13}\text{C}$ than offshore populations (Walker *et al.* 1999, Botta *et al.* 2012), sometimes indicating the incorporation of sea grass carbon (Clementz and Koch 2001). Marine mammals that incorporate freshwater organic material in estuaries may be substantially ^{13}C depleted relative to more coastal animals (Michener and Kaufman 2007). Nitrogen isotopes can be useful as well. In coastal Florida, nitrogen isotope signatures among dolphins were low in coastal populations relative to those offshore (Barros *et al.* 2010). This could reflect the difference in prey trophic level (Barros *et al.* 2010) or a difference in $\delta^{15}\text{N}$ at the base of the food web. For example, marine nitrate is typically around 6‰ while nitrogen

fixers and fertilizers are 0%-2%, so the food webs differ in the primary producer $\delta^{15}\text{N}$ (reviewed in Michener and Kaufman 2007). In contrast to Barros *et al.* (2010), Olin *et al.* (2012) and Walker *et al.* (1999) report offshore populations with the same, or slightly lower, $\delta^{15}\text{N}$ relative to coastal or estuarine populations. The degree of ^{15}N enrichment or depletion among estuarine populations depends on season, suggesting seasonal shifts in nitrogen sources (Olin *et al.* 2012). However, offshore populations did not show seasonal $\delta^{15}\text{N}$ fluctuations, and were consistently low (Olin *et al.* 2012).

Sulfur isotopes have also revealed large and significant differences among dolphin populations in the Sarasota Bay, Florida, estuary (7.1%), offshore (16.5%), or Gulf of Mexico populations (11.3%) (Barros *et al.* 2010, Nino-Torres *et al.* 2006). Differences were also reported by Knoff (2004) who found North Carolina dolphin $\delta^{34}\text{S}$ means to be 11.4% for estuaries, 13.5% for coastal, and 16.8% for offshore populations. Marine sulfate is largely responsible for the ^{34}S enriched offshore animals, whereas estuary animals are influenced by ^{34}S depleted sulfate related to oxidation of marsh sulfides. Similar findings were obtained by Olin *et al.* (2012), who observed some estuarine *T. truncatus* to be ^{34}S depleted relative to offshore animals near Charleston, South Carolina, although there was both seasonal and geographical variation in $\delta^{34}\text{S}$ within different estuaries.

In addition to the isotopes of organic material, the inorganic carbon (C) and oxygen (O) from tooth carbonate has been used to examine nutrient sources among modern and fossil marine mammals (Clementz and Koch 2001, Clementz *et al.* 2006). Carbon in marine mammal tooth and bone is derived from a mixture of diet and the carbonate of surrounding water, while the oxygen

is derived from body water (Longinelli, 1984, Huertas *et al.* 1995). For marine mammals with relatively constant body temperature, carbonate-carbon in teeth can reflect food sources (Tieszen and Fagre 1993), and carbonate-oxygen can yield information on the influence of freshwater in coastal environments (Clementz and Koch 2001). For example, Clementz *et al.* (2006) used carbonate $\delta^{13}\text{C}$ to determine the importance of C3 vegetation vs. marine primary producers for fossil ungulates and *Archaeocetes*.

In this paper we examine whether a suite of isotopes can collectively yield information assessing differences in trophic ecology among dolphins at different life stages (calves, juveniles, and adults), as well as between sexes. Whereas most studies have examined tooth/bone or skin tissue, here we report the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ values of skin and gum tissue, as well as tooth inorganic $\delta^{13}\text{C} + \delta^{18}\text{O}$ and tooth organic $\delta^{13}\text{C} + \delta^{15}\text{N}$, for 20 stranded bottlenose dolphins from the North Carolina coast (Fig. 1). Although a full suite of isotopes was examined for most individuals, skin $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ could not be collected from three and $\delta^{34}\text{S}$ could not be collected from one (see N in Table 1). We also compare lipid and nonlipid extracted skin and gum/connective tissues for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Altogether, 173 individual isotope measurements were compiled from 20 stranded animals.

Dolphin tissue was collected from animals stranded between February and May 1997 along the North Carolina coast. Strandings comprised males and females and dolphin length ranged from 163 to 271 cm. To test for effects of relative age, we stratified the sample into three age/maturation classes using length as a proxy. Calves (<180 cm, Fernandez and Hohn 1998) obtain the

majority of their nutrition from nursing rather than obtaining prey independently and have been previously shown to have isotopic values different from older animals (Knoff *et al.* 2008). Estimated total length (TL) at sexual maturation for female bottlenose dolphins is about 230 cm (Mead and Potter 1990, Fernandez and Hohn 1998), so this length was used as an estimate for when the animals were considered adults. While bottlenose dolphins are sexually dimorphic in length at maturation (Read *et al.* 1993, Fernandez and Hohn 1998), the sample size was too small to stratify by length and sex. Dolphins from 180 to 230 cm were considered juveniles. Using these length strata, dolphins in this study included 2 calves, 12 juveniles, and 6 adults. The group consisted of 8 males, 11 females, and 1 unknown sex.

The preparation for isotope analysis followed that of Cortese (2000) and Walker and Macko (1999). Each tooth was cleaned of attached connective tissue (portions of which were saved for analysis), enamel, and pulp by abrasion. The tooth was then crushed with a stainless steel mortar and pestle until it was a fine powder. For $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of tooth organic material, carbonate was removed by placing 30 mg of each powdered tooth into a glass vial and immersing in 1 mL of 30% HCL for 5 d. Samples were then rinsed in deionized water and dried at 45°C. Between 3 and 4 mg of the dried, acidified tooth powder was then analyzed using a Carlo Erba NA 1500 NCS elemental analyzer (EA) coupled to a Micromass Optima (Manchester, U.K.) isotope ratio mass spectrometer. Skin and gum tissue (collected while cleaning the teeth above) were dried at 60°C, lipids were extracted by refluxing in dichloromethane for 35 min (Knoff *et al.* 2002) and ground to powder with mortar and pestle. Approximately 1 mg of

tissue was used for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis and 5 mg was used for $\delta^{34}\text{S}$. The instruments used were the same as for the tooth organic material.

For the carbonate $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, 20-25 mg of powdered tooth material was placed in stainless steel or glass cups and reacted in a common acid bath with 100% phosphoric acid (H_3PO_4) at 90°C under vacuum which was coupled to the mass spectrometer. Water was cryogenically removed and resulting carbon dioxide was then introduced into a VG PRISM stable isotope mass spectrometer for isotope analysis. Machine precision was typically better than 0.05%. Oxygen and carbon isotope values are reported relative to Vienna Pee Dee Belemnite limestone (VPDB), nitrogen relative to AIR (Ambient Inhalable Reservoir), and sulfur relative to Canyon Diablo Troilite (CDT).

Several different statistical tests or techniques were used to examine the data. For two-group paired comparisons, nonparametric Wilcoxon tests or t -tests were used. The strength of linear correlations (paired) were tested using a Pearson product-moment correlation coefficient, which does not assume normality. Principle Components Analysis (PCA) was used for analyzing covariation among many variables and was an excellent way to examine how the different isotopes among the 20 individual dolphins correlated. The PCA can be thought of as a way of linearly transforming the data as it is arranged in three-dimensional space. Since the results of the PCA are a way of organizing the data and were only to be used to examine relationships, as opposed to generating predictive models, the data can be nonparametric, not needing to be transformed (Jolliffe 2002). A cluster analysis was run to determine whether the total sample formed more than one cluster of isotopically

distinct C sources. When that result was positive, we conducted a recursive partition analysis also referred to as a Classification and Regression Tree (CART), which is an iterative process that splits data on a single covariate resulting in groups or "nodes" that minimize the misclassification rate of observations, and thus predict the class to which an observation belongs (Sutton 2005). The CART analysis requires a learning sample with known class membership. As we did not have the known membership of the stranded dolphins, results from the cluster analysis were used as initial class assignments. Following convention, the α -level used for statistical significance was 0.05 for all tests.

Lipid-extracted dolphin skin tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ranged from -19.0% to -15.2% and 13.6% to 18.2%, respectively (Table 1). Lipid-extracted dolphin gum tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ranged from -18.8% to -10.4% and 15.6% to 19.8% respectively (Table 1). Lipid-extracted skin tissue was significantly ^{13}C enriched ($-16.5\% \pm 0.9\%$) relative to unlipid-extracted tissue ($-17.7\% \pm 1.1\%$) (paired t -test, $t = 4.45$, $P = 0.0005$, $n = 16$). In contrast, lipid-extracted skin was ^{15}N depleted ($15.9\% \pm 1.2\%$) relative to unlipid-extracted skin ($16.2\% \pm 1.2\%$) ($t = -2.49$, $P = 0.025$, $n = 16$). Paired t -tests also showed that lipid-extracted gum tissue was significantly ^{13}C enriched ($-14.6\% \pm 2.2\%$) compared to unlipid-extracted gum tissue ($-16.9\% \pm 1.9\%$) ($t = 6.65$, $P = 0.0001$, $n = 18$) while gum $\delta^{15}\text{N}$ was lower in lipid-extracted ($17.6\% \pm 1.0\%$) vs. unlipid-extracted tissue ($18.1\% \pm 1.1\%$) ($t = -2.51$, $P = 0.02$, $n = 18$). Lipid-extracted skin and gum tissue values were used for statistical comparisons with the tooth isotope values. Tooth organic $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ranged from -14.8% to -11.8% and 15.5% to 21.6%, respectively (Table 1).

Some isotope variation exists among the different tissues of individual animals, particularly in ^{13}C (Table 1). Gum tissue was generally enriched in ^{13}C relative to skin tissue and both exhibited greater variation than that previously observed in teeth. Differences in isotope ratios among the tissues of individual animals could reflect chemical composition differences among tissues or differential rates of tissue turnover. Skin tissue turnover is generally the most rapid (≤ 73 d Hicks *et al.* 1985, Browning *et al.* 2014), followed by muscle (or gum) and, lastly, teeth (Browning *et al.* 2014).

Some stable isotope values were correlated (highest was $r = -0.49$, Pearson product-moment correlation coefficient) with dolphin body length, although none of the correlations were statistically significant. Gum, carbonate and tooth organic $\delta^{13}\text{C}$ were all positively correlated with length. The $\delta^{15}\text{N}$ of skin, gum, and tooth were all negatively correlated with length ($r = -0.45$, $n = 17$; -0.49 , $n = 20$ and -0.42 , $n = 20$, respectively). This trend was also observed by Knoff *et al.* (2008) in tooth organic material (dentin) and supported by results from the PCA in the current study (see below). This suggested either a shift to lower trophic status, a shift to a part of the food web with ^{15}N depleted inorganic nitrogen sources, or (most likely) the turnover of nitrogen derived from the mother (Knoff *et al.* 2008). Two of the calves had the highest skin $\delta^{15}\text{N}$ of the group, which strongly suggests that body N reflected the ^{15}N elevated signature of the mother. Marine nitrate $\delta^{15}\text{N}$ is generally higher (6%) than coastal or estuarine sources, so the shift does not seem to be associated with larger animals moving further offshore to feed.

The evidence for dietary shifts between calf and juvenile

(Knoff *et al.* 2008) seems to occur at approximately 183 cm (growth during the first year), after which juveniles obtain most of their nutrition independent from reliance on nursing (Wells and Scott 1990). Dolphins in this study ranged from 163 to 271 cm, from calves to adults, and seem to confirm the diet shift observed by Knoff *et al.* (2008).

Gum $\delta^{34}\text{S}$ values ranged from 12.0% to 19.1%, however all but one individual was below 15% (Table 1). These $\delta^{34}\text{S}$ values suggests that most of the animals were not deriving a majority of their sulfur from marine sulfate (which has a value of 20%). Additionally, there was no correlation between $\delta^{34}\text{S}$ and length (hence, age class) or sex (Table 1). Barros *et al.* (2010) reported $\delta^{34}\text{S}$ of tooth collagen from *T. truncatus* offshore of Sarasota Bay to average 16.5%, while those in the Bay (estuarine) averaged 7.1% and nearshore Gulf of Mexico populations averaged 11.3%. The dolphins reported here have gum $\delta^{34}\text{S}$ values which are higher relative to collagen from the nearshore Gulf of Mexico animals and depleted relative to collagen from offshore of Sarasota Bay dolphins (Barros *et al.* 2010). These differences could arise from $\delta^{34}\text{S}$ differences at the base of the food web between Sarasota Bay and estuarine North Carolina, reflecting salt marsh sulfur or other common sources of $\delta^{34}\text{S}$ variability (Michener and Kaufman 2007). In the only North Carolina dolphin $\delta^{34}\text{S}$ data we are aware of, Knoff (2004) reported skin $\delta^{34}\text{S}$ values from offshore North Carolina dolphins similar to those of Barros *et al.* (2010) (16.8%). Knoff (2004) also reported skin $\delta^{34}\text{S}$ values for North Carolina estuarine dolphins to be $11.4\% \pm 0.17\%$ ($n = 44$) and coastal to be $13.5\% \pm 0.08\%$ ($n = 183$). The $\delta^{34}\text{S}$ values reported here have a mean similar to Knoff's (2004) coastal values, but with a larger

standard deviation (Table 1).

Tooth carbonate $\delta^{13}\text{C}$ ranged from -14.1‰ to -9.9‰ and $\delta^{18}\text{O}$ from -4.3‰ to -0.1‰ (Table 1). Dolphins living in environments influenced by freshwater should reflect the lower $\delta^{18}\text{O}$ of that water relative to ocean water. Because the $\delta^{18}\text{O}$ of freshwater flowing into estuaries is variable, coastal populations tend to have larger variation than marine populations (Clementz and Koch 2001). Differences among age classes could indicate differential habitat use. Among all dolphins, the mean $\delta^{18}\text{O}$ was $-3.0\% \pm 1.2\%$ and while a single individual had a value of -0.1‰, all the other animals were below -1.2‰. For a population spanning the ocean to estuarine continuum, it may seem reasonable to hypothesize that $\delta^{34}\text{S}$ and $\delta^{18}\text{O}$ would correlate since areas with more freshwater would be ^{34}S and ^{18}O depleted relative to ocean water. However, there was no correlation observed between these values ($r = 0.009$). The range in $\delta^{18}\text{O}$ observed (-4.3‰-0.1‰, VPDB) was close to the range found in coastal areas of the Atlantic (-3.1‰ to -1.3‰, SMOW) (Khim and Krantz 1996). This suggests all dolphins were reflecting coastal, not offshore, waters.

There was a significant difference between males (8) and females (11) in $\delta^{18}\text{O}$ values (t -test, $t = 1.85$ assuming unequal variances, $P = 0.0455$, statistical power >95%). Females clustered between -4.3‰ and -3‰ (all but two). Males range from -4.3‰ to 0‰. The clustering of females with more negative values suggests that they tended towards more estuarine habitat, since those environments tend to be ^{18}O depleted relative to marine waters. The larger range among males suggests that they are wider ranging, perhaps into marine waters with $\delta^{18}\text{O}$ values closer to 0‰. If this was the case however, the males ranging into more open ocean waters did not result in greater ^{34}S enrichment

relative to females (as noted above). None of the other isotopes showed a difference with sex, similar to other findings (long-beaked common dolphin, *Delphinus capensis*; Nino-Torres *et al.* 2006).

Carbonate $\delta^{13}\text{C}$ is a good indicator of diet (Cerling and Harris 1999). There is an approximate $14.1\% \pm 0.5\%$ enrichment between enamel bioapatite and diet for ruminant mammals and a 13.3 offset for nonruminant mammals (Cerling and Harris 1999; Passey *et al.* 2005), although the cause of the enrichment remains poorly understood. A database of carbonate $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, compiled from dolphin teeth taken from coastal and offshore animals, could potentially assist with an analysis of small data sets such as this one. The diets of offshore and coastal dolphins are known to be different (Mead and Potter 1995), and differences may occur among coastal and estuarine stocks. There was a 4.2% range in tooth carbonate $\delta^{13}\text{C}$ among the dolphins in this study. Subtracting 14% from their signatures would place their carbon sources between -24% and -28%, which are several parts per thousand lower than would be expected for typical marine predators (if marine plankton is approximately -20%). Unfortunately, there are few data with which to contrast the carbonate isotope values.

A PCA identified three components that together explained 63.8% of the data set variance. The first component explained 28.6% of the variance, was positively correlated with organic N from teeth, skin and gum tissue (Table 2), and was negatively correlated with length. This component essentially identifies the calves as distinct from the remaining dolphins, with elevated $\delta^{15}\text{N}$ from the mother as discussed earlier. The second component (22.5%) was correlated with organic carbon from teeth

and gum, as well as carbonate (Table 2). It also was positively correlated with length, although with a relatively low r (0.54). The third component explained 12.7% of the variance and was correlated with sulfur. Neither gum nor tooth organic nitrogen and carbon isotope ratios were correlated in the PCA (Table 2). Given the close positive correlation of these two isotope ratios with trophic status, the absence of a correlation points to a source of either C or N obscuring the trophic signal. If dolphins were from the same population and feeding on the same prey items, there would be a positive correlation between the N and C isotopes.

Olin *et al.* (2012) observed that coastal dolphin skin showed variable $\delta^{15}\text{N}$ depending on where in the Charleston, South Carolina, estuary they originated. In fact, it was the different $\delta^{15}\text{N}$ values that supported the hypothesis that the dolphins in the estuary formed distinct stocks. Although the dolphins reported here appear to be estuarine or coastal (based on the $\delta^{34}\text{S}$ values), they could originate from areas in the estuary with $\delta^{13}\text{C}$ signals different enough to confound the ideal 3:1 $\delta^{15}\text{N}:\delta^{13}\text{C}$ trophic level increase associated with predators consuming the same prey (Michener and Kaufman 2007). The gum $\delta^{13}\text{C}$ standard deviation was 2.2‰ yet the gum $\delta^{15}\text{N}$ standard deviation was only 1.1‰, strongly suggesting that the deviation in $\delta^{13}\text{C}$ is not a result of trophic enrichment (which should result in a much larger $\delta^{15}\text{N}$ deviation), but is a result of different C sources within the near shore environment. However, it should be noted that processes and transformations leading to fixation for N and C are quite different, so interpreting the standard deviation difference this way is just one possibility. Another possibility is that nitrogen from the mother, which turns over more slowly

than carbon, could cause smaller individuals to retain ^{15}N even when the protein source has shifted to prey (Knoff *et al.* 2008).

The cluster analysis was run on isotope results from gum and tooth samples; skin samples were excluded because missing values for three animals would have reduced the overall sample size. Calves (3) were also excluded since their isotopes do not reflect prey species $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, but are heavily influenced by the isotope composition of their mother. Two distinct clusters were identified (Fig. 2, Table 3). The clusters were characterized by large and significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, but not $\delta^{34}\text{S}$ or $\delta^{18}\text{O}$ (Table 3). The CART analysis, using results from the cluster analysis to define "source populations," identified three nodes in two splits, both on tooth inorganic $\delta^{13}\text{C}$, with a resultant $r = 0.80$ ($n = 17$). Node 1 contained all samples with inorganic $\delta^{13}\text{C}$ values $< -11.1\%$ ($n = 7$). Mean isotope values between that node and samples with inorganic $\delta^{13}\text{C}$ values $\geq -11.1\%$ (representing the other two nodes) showed significant differences for gum $\delta^{13}\text{C}$ ($P = 0.0157$) and $\delta^{15}\text{N}$ ($P = 0.0495$), and tooth organic $\delta^{13}\text{C}$ ($P = 0.0002$) (t -tests assuming unequal variances). These differences suggest a coastal vs. estuarine separation among the dolphins. More ^{13}C enriched carbon signatures would occur with coastal animals, whereas more ^{13}C depleted values may be associated with estuaries (particularly where freshwater rivers enter) (Michener and Kaufman 2007). Node 1 was significantly enriched in ^{13}C for all tissues examined by the analysis, suggesting the dolphins may have been more coastal rather than estuarine. Although all but one of the stranded dolphins were found on coastal barrier islands (not in the estuary, Fig. 1), the CART analysis suggested both estuarine and coastal dolphins among those

sampled. It is possible, of course, that the dolphins could have drifted from the estuary to the barrier islands where they were found. Another possible reason for this is related to the time of collection. Strandings occurred between February and May, when estuarine water temperatures were low and estuarine dolphins are thought to move to more coastal environments, following prey (Goodman *et al.* 2007).

Taken as a whole, these data suggest that multiple isotope analysis on individual dolphins can provide information on trophic ecology from several angles, and can help characterize ontogenetic changes.

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Figure 1. Stranding locations for all dolphin based on the

necropsy reports. Clusters are statistical groupings although not all individuals could be assigned a cluster. The clusters refer to those identified in the present study.

Figure 2. Gum, tooth organic and tooth carbonate $\delta^{13}\text{C}$ for cluster 1 (red) and cluster 2 (blue), as assigned from this study. Open circles are the calves, which were assigned to a cluster using parameters derived while excluding calves. Cluster 1 animals tend to be ^{13}C enriched, which suggests costal carbon sources, versus relatively ^{13}C depleted cluster 2 animals, which suggests more estuarine C sources.

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Table 1. Means, standard deviations, and δn for isotope signatures. Correlations of length (TL) vs. isotopes for all dolphins. Skin and gum tissue are lipid-extracted. r -values are Pearson product moment correlation coefficients.

	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{18}\text{O}$	$\delta^{34}\text{S}$
Skin	-16.5 ± 0.9 ($n = 17$)	15.9 ± 1.2 ($n = 17$)	—	—
correlation with TL	-0.17	-0.45		
Gum	-14.8 ± 2.2 ($n = 20$)	17.7 ± 1.1 ($n = 20$)	—	13.9 ± 1.5 ($n = 19$)
correlation with TL	0.44	-0.49		-0.07
Tooth organic	-13.1 ± 0.9 ($n = 20$)	19.1 ± 1.6 ($n = 20$)	—	—
correlation with TL	0.26	-0.43		
Tooth carbonate	-11.5 ± 1.2 ($n = 20$)	—	-3.0 ± 1.2 ($n = 20$)	—
correlation with TL	0.41		0.17	

Table 2. Principle component analysis correlation matrix. PC1 was highly correlated with organic nitrogen. PC1 also was negatively associated with length. PC2 was correlated with organic C from gum and tooth but not skin. PC2 was also positively associated with length.

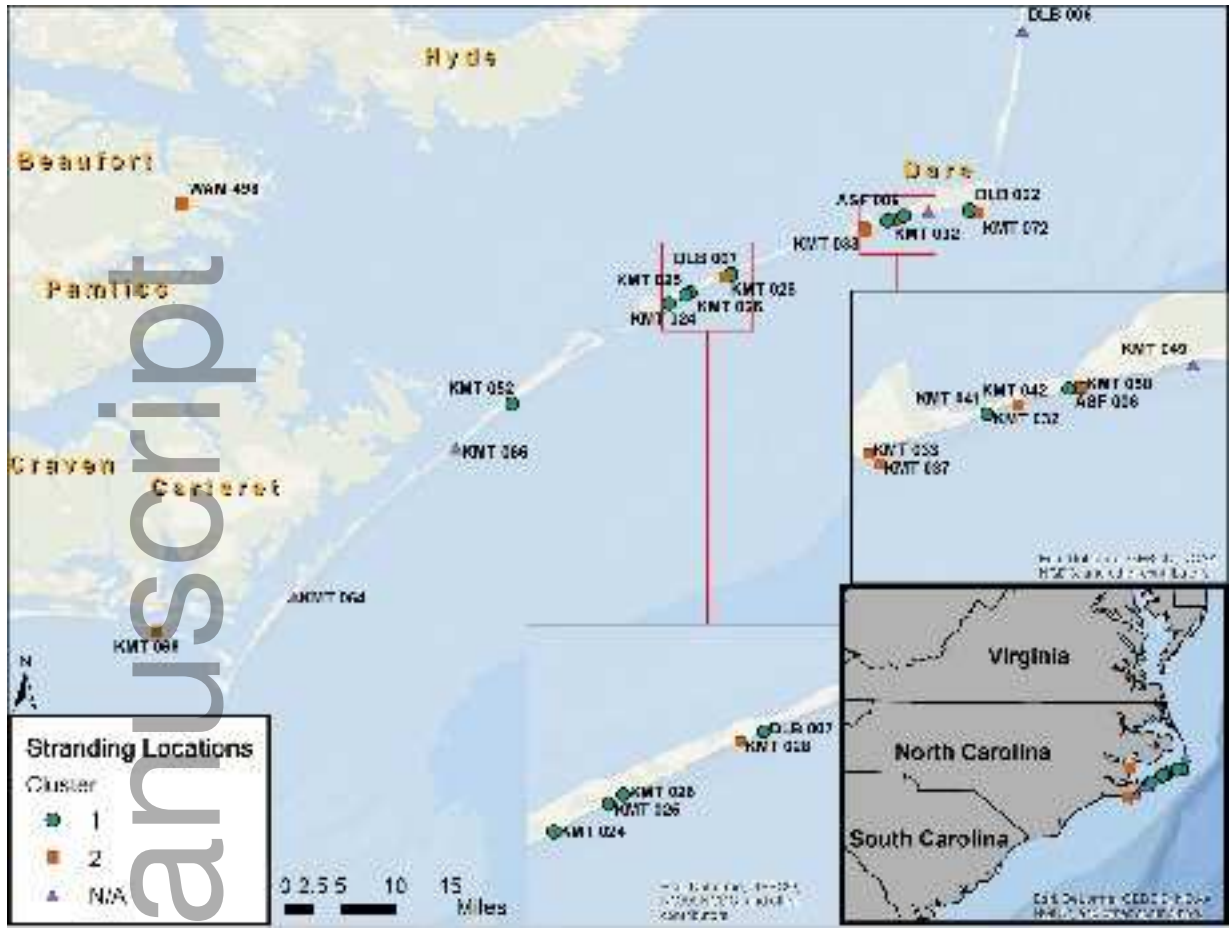
	PC1 (28.6%)	PC2 (22.5%)	PC3 (12.7%)

Skin $\delta^{13}\text{C}$	0.50173	0.29741	0.25257
Gum $\delta^{13}\text{C}$	-0.27929	0.65104	0.2102
Skin $\delta^{15}\text{N}$	0.89582	0.11561	-0.0054
Gum $\delta^{15}\text{N}$	0.73871	0.13299	-0.31315
Gum $\delta^{34}\text{S}$	-0.03316	-0.30472	0.88531
Tooth carbonate C	0.03069	0.69598	0.39246
Tooth carbonate O	-0.14105	0.23057	-0.07059
Tooth organic C	0.1475	0.87627	-0.22287
Tooth organic N	0.8163	0.09525	0.27434
Length	-0.68674	0.54626	0.02266

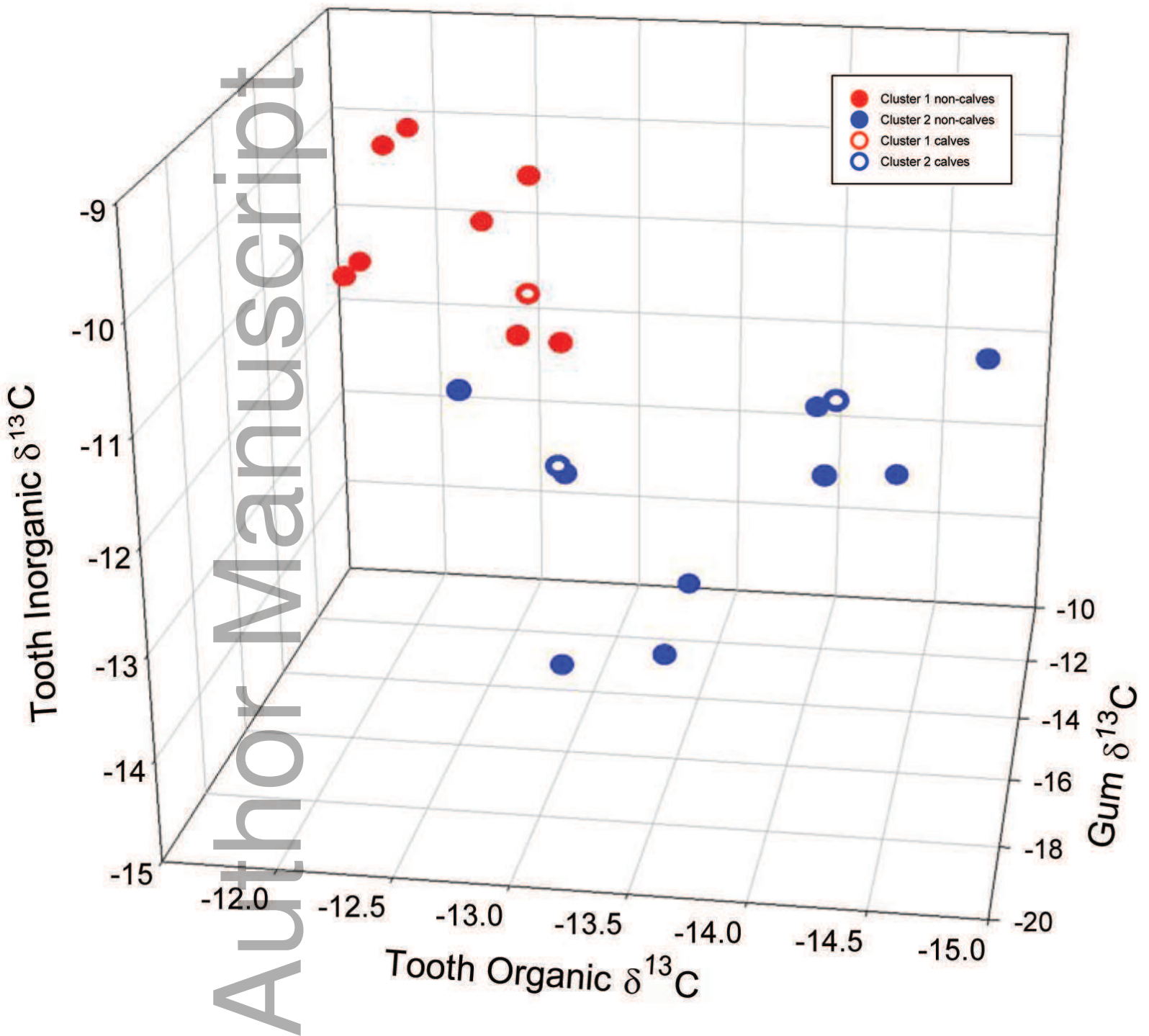
Note: All values >0.5 or <-0.5 are in bold.

Table 3. Clusters's means and standard deviations for isotope signatures. Different letters between clusters indicate significant differences shown by t -tests ($P \leq 0.05$). Cluster 1 $n = 8$. Cluster 2 $n = 9$.

Tissue and isotope ratio	Cluster 1	Cluster 2
gum $\delta^{13}\text{C}$ (‰)	-13.4 ± 2.0 A	-15.7 ± 1.9 B
gum $\delta^{15}\text{N}$ (‰)	17.8 ± 0.9 A	17.1 ± 0.7 B
gum $\delta^{34}\text{S}$ (‰)	13.8 ± 0.6	14.1 ± 2.1
tooth inorganic $\delta^{13}\text{C}$ (‰)	-10.5 ± 0.5 A	-12.5 ± 1.2 B
tooth inorganic $\delta^{18}\text{O}$ (‰)	-2.6 ± 1.4	-3.1 ± 1.0
tooth organic $\delta^{13}\text{C}$ (‰)	-12.3 ± 0.5 A	-13.7 ± 0.7 B
tooth organic $\delta^{15}\text{N}$ (‰)	19.3 ± 1.3	18.5 ± 1.8



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