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Fine-scale population structure of estuarine bottlenose dolphins (*Tursiops truncatus*) assessed using stable isotope ratios and fatty acid signature analyses

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Abstract Stable isotope ratios and fatty acid signature analyses were employed to examine the fine-scale population structure of a year-round resident population of 600–800 bottlenose dolphins (*Tursiops truncatus*) in the Indian River Lagoon (IRL), Florida. The IRL, a 250-km-long estuary running along the central east coast of Florida (28.0°N, 80.6°W), is comprised of the northern and southern IRL, Mosquito Lagoon (ML), Banana River (BR), and St. Lucie Estuary. Samples of skin and blubber were collected from dead stranded ($n = 61$, 1994–2004) and live dolphins ($n = 153$, 2002–2007, 2010, 2011) from throughout the IRL and surrounding environs. Using stable isotopes (SI), dolphins could be assigned to a ML subpopulation, a St. Lucie Estuary subpopulation, and an IRL subpopulation. Fatty acid signature analysis (FASA) allowed for finer resolution, detecting ML and BR subpopulations, a separation of northern and southern IRL subpopulations, and a St. Lucie Estuary subpopulation. Differences between sexes were detectable within subpopulations using FASA, but not using SI. This may indicate that males and females are foraging in similar locations at a similar trophic level (detected using SI), but are varying in the types or proportions of specific prey (indicated by FASA). The combination of these complementary analyses results in a powerful tool for assessing fine-scale population substructure.

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Introduction

Many studies on small cetaceans have described the social structure of populations, but until recently few have examined the ecological forces that shape that structure (e.g., Heithaus and Dill 2002; Fernandez et al. 2011; Kiszka et al. 2012). The distribution of food resources is one of the most important factors affecting bottlenose dolphin movement patterns, and multiple studies have concluded that these animals are primarily tracking prey movements (e.g., Shane et al. 1986; Hanson and Defran 1993; Hart 1997). Stable isotope analysis has been used as an important tool to describe the general ecology of many marine mammal species (e.g., Fernandez et al. 2011; Rioux et al. 2012; Wilson et al. 2012, 2013). Several factors can influence the isotopic ratio of predators living in different marine regions, including differences due to oceanographic factors in a given area and variation in feeding habits of the prey species consumed. Isotope ratios are ultimately determined by the general type of food (i.e., original method of carbon fixation (δ^{13}C), number of trophic levels ($\delta^{15}N$), etc.) that has been incorporated into the animal over the past several weeks or months and can provide an overall portrait of an average diet.

While stable isotope analysis can provide information on trophic level and sources of primary production, fatty

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acid analysis can potentially give more detailed insight into a consumer's diet (Iverson et al. 2004), possibly even identifying which individual species were eaten (Budge et al. 2006). The use of fatty acid signatures to study predator feeding ecology relies upon (1) characteristic patterns of fatty acid composition for each potential prey species, (2) narrow limits on the biosynthesis of fatty acids by the consumer, and (3) the prevalence of large accessible storage depots of lipid in the consumer (Iverson et al. 2004). Fatty acid analysis has been utilized in many feeding ecology studies to attempt to discern dietary choices; however, to properly interpret fatty acid signatures, development of taxa-specific correction factors is needed, and these are not currently available for cetaceans (see Budge et al. 2006). Despite this limitation, fatty acid signature analysis (FASA) can be used to discriminate between different subpopulations of animals that are exhibiting different feeding habits (e.g., Iverson et al. 1997; Quérouil et al. 2013) and has been successfully used to infer spatial and temporal differences in diet both within and between a number of different species (e.g., Smith and Worthy 2006; Budge et al. 2006, Quérouil et al. 2013). Differences in fatty acid signatures can detect differences in feeding habits even in groups living in close proximity. Iverson et al. (1997) showed that Alaskan harbor seals (*Phoca vitulina*) could be assigned to different haul-out sites, based on FASA, with >95 % accuracy and that this was consistent with tracking data showing that these seals were feeding in different geographic areas.

The Indian River Lagoon (IRL) region, along the central east coast of Florida, is an ideal location for applying these methodologies to better understand habitat utilization and fine-scale population structure of bottlenose dolphins (*Tursiops truncatus*). This system is a semi-closed lagoon environment that supports a year-round resident population of bottlenose dolphins which have been extensively studied (Mazzoil et al. 2005, 2008a, 2011; Durden et al. 2011). It is estimated that the approximately 600–800 resident dolphins rarely, if ever, leave the lagoon (Mazzoil et al. 2008a, 2011; Durden et al. 2011) and that they show strong site fidelity to specific areas in either northern or southern portions of the system (Mazzoil et al. 2005, 2008a, 2011; Durden et al. 2011). A number of recent studies have noted significant health issues related to their distribution patterns, with northern IRL dolphins exhibiting significantly greater health issues than southern IRL (sIRL) animals (e.g., Bossart et al. 2003; Goldstein et al. 2006; Reif et al. 2006; Greig et al. 2007; Durden et al. 2007).

Geographic location has long been known to affect the carbon and nitrogen isotope ratios of both aquatic and terrestrial taxa (e.g., Boyce et al. 2001; Gerard and Muhling 2010; Guest et al. 2010). Despite long-held theories of large-scale movement and assimilation of nutrients in estuaries, recent evidence suggests that, in some estuaries, movements of nutrients occur at a much finer scale than previously considered, in some cases over meters, and that a much more limited exchange occurs (Guest and Connolly 2004; Adams and Paperno 2012). Recent isotope studies on invertebrates and fish (e.g., Guest and Connolly 2004; Adams and Paperno 2012; Fletcher-Odom 2012) have indicated significant differences in isotope ratios over limited geographic areas. These data, in conjunction with recent data for captive bottlenose dolphins that indicate isotopic turnover rates of 2–3 weeks (Browning et al. 2014), suggest that there is potential for assessing both recent feeding ecology and habitat usage.

The IRL is made up of four major basins (Fig. 1) which are known to vary in the relative abundance of seagrasses and macroalgae, which in turn will be reflected in the carbon signature of consumers (i.e., Adams and Paperno 2012), including resident dolphins. Several important prey species for IRL bottlenose dolphins (Barros and Odell 1990), such as white mullet (*Mugil curema*), pinfish (*Lagodon rhomboides*), and spotted seatrout (*Cynoscion nebulosus*), exhibit significant regional differences in isotopic ratios and fatty acid signatures—over distances of as little as 15–25 km separation (e.g., Adams and Paperno 2012; Fletcher-Odom 2012; Worthy and Worthy unpublished data). Regional differences in prey signatures, combined with previously described site fidelity of dolphins, should translate into regional differentiation of dolphin signatures using isotopes and/or fatty acids.

Given the potential of these chemical tracer methodologies, the primary objective of the current study was to investigate the efficacy of using stable isotope and fatty acid signature methodologies to investigate fine-scale population structure in cetacean populations. Locally, the goal was to better understand regional site fidelity of putative subpopulations of bottlenose dolphins within the IRL to interpret potential threats to the health of this system.

Materials and methods

Sample collection

Skin and blubber samples were collected in January 2002 from free-swimming dolphins using biopsy darts (NMFS permit number 770-1339-02) fired from a modified 0.22-caliber rifle affixed with a video camera to record the targeted dolphin's identification and its reaction to sampling. The biopsy dart consisted of a thin-walled, hollow arrow shaft with a collecting head (2 cm long and 1 cm in diameter), sharpened on the anterior end, and equipped with an internal barbed shaft to hold the sample. The free-floating dart was recovered from the water immediately after sampling. The sample (0.7–0.9 g) typically extended

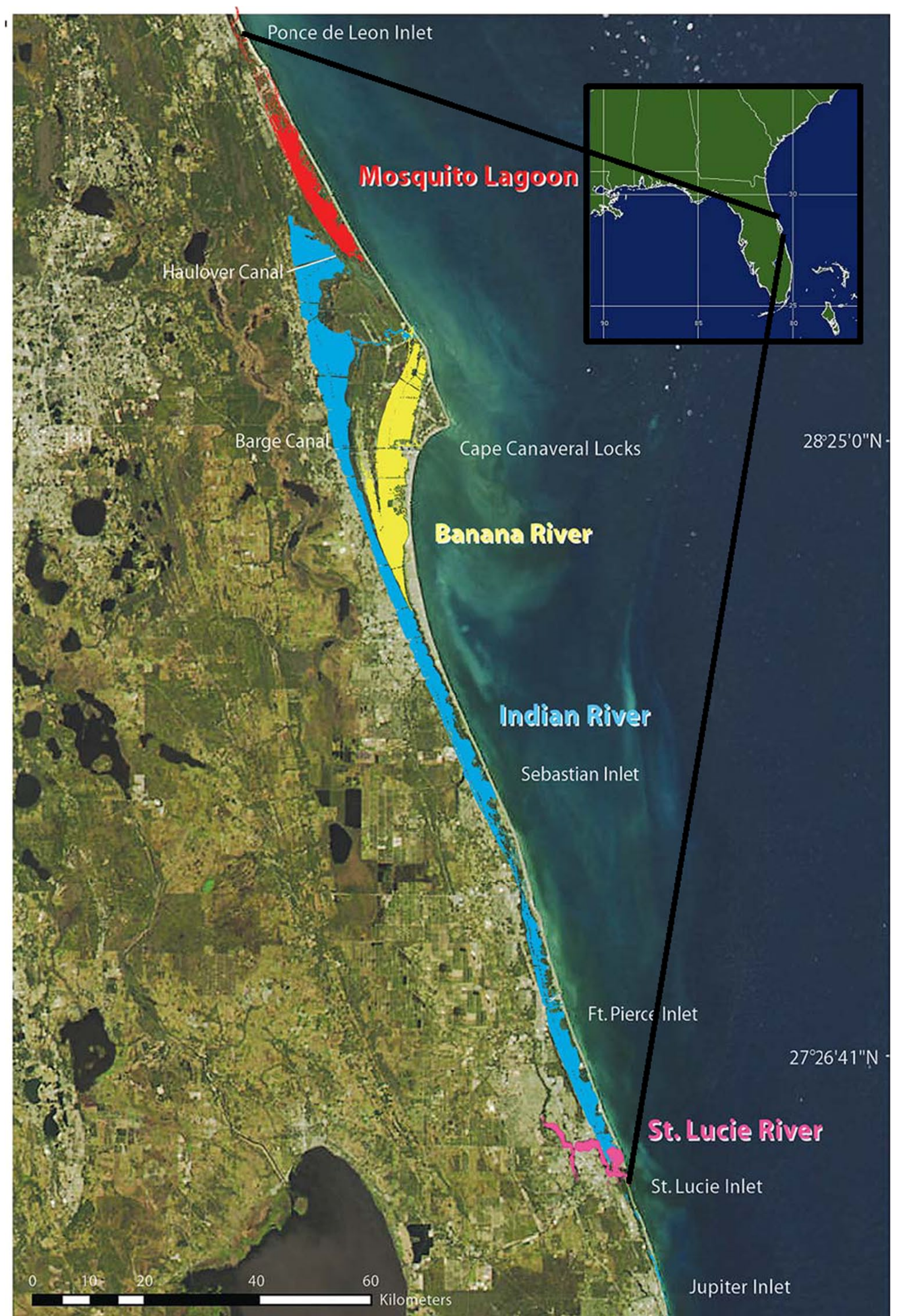

Fig. 1 The Indian River Lagoon (IRL) located on the east coast of Florida was split into six segments (Mazzoil et al. 2008a). Segments sampled in the present study were Mosquito Lagoon (ML), Banana River (BR), northern IRL (nIRL) and southern IRL (sIRL) (separated at Sebastian Inlet), and the St. Lucie Estuary (SLE). In addition, samples were obtained from the Atlantic Ocean (AO) coast near ML

from the epidermis to the blubber/muscle interface and was stored at −80 °C until analyses could be performed.

Skin (2003–2007, 2010, 2011) and blubber samples (2003–2005) were also collected from dolphins that were live-captured as part of a comparative dolphin health and environmental risk assessment (HERA) study (NMFS permit numbers 998-1678-00) (see Bossart et al. 2006). Dolphins were encircled with a large-mesh seine net in water depths of approximately 2 m or less in June of 2003 through 2007, 2010, and 2011 in two separate areas in the IRL. The northern capture area (north of latitude 28°15′N) included portions of the northern Indian River Lagoon (nIRL), Banana River (BR), and Mosquito Lagoon (ML), while the southern capture area (south of latitude 27°25′N)

included the sIRL and the north and south forks of the St. Lucie River Estuary and the St. Lucie Inlet (collectively SLE) (Fig. 1). Wedge biopsies of skin and full blubber depth (approx. 10 g) were taken along the mid-lateral line below the dorsal fin (see Bossart et al. 2006). After a short period of observation, dolphins were roto-tagged or freeze-branded, removed from the net enclosure, and released. Samples were stored at −80 °C until further analyses.

In addition, skin samples were opportunistically collected from dead stranded dolphins during 1994–2004. Samples of skin (approx. 5 g) were collected and kept on ice until they were returned to the laboratory at which time they were placed in freezers and kept at −20 °C. Standard length of animals was measured and sex determined.

Standard length was used as a gross estimate of age based on growth equations (Stolen et al. 2002) and weaning age estimates (Mann et al. 2000). Mann et al. (2000) reported weaning ages ranging from 2.7 to 8 years with 67 % weaned by 4 years old. In the present study, dolphins were grouped into three age classes: calves, subadults, and adults. As a conservative estimate (due to the variability in growth rates between individuals), calves were considered to be less than 1 year old since it is anticipated that they would not be weaned and that the majority of their diet consisted of milk. Subadult animals were between 1 and 3.5 years and may have included nursing animals as well as those beginning to incorporate fish into their diet. Adults were considered to be those individuals that were older than 4 years old.

Stable isotope analysis

Approximately 10 mg of skin was put in a drying oven for 24 h to remove water. Since lipids are depleted in ^{13}C relative to lean tissue, all samples were lipid-extracted using petroleum ether prior to isotope analysis (Schlechtriem et al. 2003; Post et al. 2007). Lipid-extracted samples were again placed in a drying oven for 24 h to remove any remaining solvent. Dried, lipid-extracted samples were ground to a fine homogeneous powder using a Wig-L-Bug Amalgamator (Crescent Dental Manufacturing, model MSD). Aliquots (0.9–1.5 mg) were sealed into 5 mm by 9 mm tin capsules and sent to the Stable Isotope and Ecology Lab, University of Georgia, Athens, GA, for isotope analysis by mass spectrometry (Thermo Finnigan DELTA-plus and DELTA C). Data were expressed and reported as per mil (‰) using delta notation (δ):

$$X(\%) = \left(\left(R_{\text{sample}}/R_{\text{standard}}\right) - 1\right) \times 1000 \quad (1)$$

where X is ^{15}N or ^{13}C, and R is the corresponding ratio of ^{15}N/^{14}N or ^{13}C/^{12}C. Standard reference materials were carbon from PeeDee Belemnite limestone and atmospheric nitrogen gas. To assure quality control in sample analysis of stable isotope ratios, a known standard sample (bovine tissue) was run after every 12 unknown samples. Analytical errors for standard samples were ±0.01 ‰ (SD) for δ^{13}C and δ^{15}N.

Fatty acid analysis

Lipid analyses were performed following methodologies of Iverson et al. (1997) as modified by Samuel and Worthy (2004). Lipids were extracted from the inner layer (Smith and Worthy 2006) of blubber samples ($n = 65$) using a solution of 2:1 chloroform/methanol. Fatty acid methyl esters (FAMEs) were prepared from the extracted lipid by adding 0.5 N sulfuric acid in methanol and dichloromethane, and the resultant solution placed in the dark for 72–96 h. FAMEs were purified in hexane and analyzed using gas–liquid chromatography (PerkinElmer Autosystem XL) with appropriate software (Totalchrom version 6.3.1, PerkinElmer). Resultant chromatograms were calibrated by comparing to known standard mixtures (Nu-Chek Prep, Elysian MN) and secondary external reference standards to determine fatty acid composition. Fatty acids were converted to percent amount of total sample and standardized by dividing each detected fatty acid by the total percent amount of all identified fatty acids. The subset of total assessed fatty acids referred to as "extended dietary fatty acids" (Iverson et al. 2004) was used in statistical analyses.

Statistical analyses

Ecologists have long relied on hypothesis testing to include or exclude variables in models. More recently, measures such as the Akaike information criterion (AIC) and associated measures of model uncertainty have been used. These approaches provide a framework on which to base both model selection and inference from ecological data. The AIC provides an objective way of determining which model among a set of models is most parsimonious, without relying on α. AIC was used to identify which variables were important in the most significant models. Significance between regions was tested using t tests with the level of statistical significance set at $\alpha = 0.05$. Mean values presented in the text are ±SE (except where noted).

Stable isotope data were tested for normality using Shapiro–Wilks ($n < 50$) and Kolmogorov–Smirnov tests ($n > 50$) and tested for homogeneity of variance using Levene's test. Due to differences in sampling period and methodologies, samples collected from stranded IRL dolphins were evaluated separately from those of the live-captured dolphins, and statistical comparisons between the two were not undertaken due to small sample sizes. For stranded animals, differences among age class, sex, season, and year were explored for δ^{15}N and δ^{13}C using GLM-MANOVA.

For live-captured animals, differences in stable isotope values due to sampling location within the IRL, sex, and collection year were explored for using GLM-MANOVA with AIC. Data were analyzed in GLM because: transformations failed to improve the few non-normal data; visual inspection of normal Q–Q plots and histograms indicated normality; and general linear models are considered robust to deviations from normality (Field 2005). Regression analyses were done between stable isotope data and salinity, as well as between isotope data and water temperatures. Salinity and water temperatures from 2002 to 2011 were determined from historical data collected by the St. Johns Water Management District. All statistical analyses were conducted using SPSS 20.0 with a critical value of $\alpha = 0.05$ and plotted using SigmaPlot (Version 10.0, Systat Software). Values reported are mean ± SE.

Classification and regression tree (CART) analysis (S-Plus-Professional Edition, Version 6.2.1, Insightful Corporation) was used to analyze fatty acid data. CART trees are grown by repeatedly splitting the data via algorithms that partition the data into mutually exclusive groups (Breiman et al. 1984; De'ath and Fabricius 2000) ultimately dividing samples into a series of sequential dichotomous groups based on an individual fatty acid with the greatest deviance in concentration. CART is a nonparametric method that does not limit the number of variables due to small sample size, and variables need not be normally distributed, so that untransformed data may be analyzed with this method. Multi-response permutation procedure (MRPP) (PC-ORD 5), another nonparametric test, was also used to examine differences between or among groups and was used to verify CART findings.

Results

Stable isotope analysis

Stable isotope ratios were analyzed for 17 skin samples collected by biopsy dart from free-swimming dolphins (2002) and 136 skin samples collected from live-captured dolphins (2003–2007, 2010, 2011). Isotope data (δ^{15}N and δ^{13}C) were normally distributed for all geographic locations. Isotopic data for females were distributed normally for δ^{15}N (S–W = 0.966, $p = 0.336$); however, data for males were not (K–S = 0.119, $p = 0.002$). Neither female nor male data were distributed normally for δ^{13}C (females: S–W = 0.788, $p < 0.001$; males: K–S = 0.145, $p < 0.001$). Finally, sampling years 2006, 2007, 2010, and 2011 were normally distributed for both δ^{15}N and δ^{13}C (all p values ≥ 0.062), whereas sampling years 2003 and 2005 were not distributed normally for either δ^{15}N (2003: S–W = 0.920, $p = 0.014$; 2005: S–W = 0.840, $p = 0.010$) or δ^{13}C (2003: S–W = 0.893, $p = 0.003$; 2005: S–W = 0.869, $p = 0.027$). Sampling year 2004 was distributed normally for δ^{15}N ($p = 0.105$), but not for δ^{13}C (S–W = 0.887, $p = 0.007$).

Isotope data (δ^{15}N and δ^{13}C) for live dolphins were not significantly different for sex (Wilks' Lambda: $F_{2, 93} = 0.859$, $p = 0.427$) but were for location within the IRL (Fig. 1) (Wilks' Lambda: $F_{8, 186} = 8.491$, $p < 0.001$). ANOVA indicated that live dolphins sampled in SLE had significantly higher δ^{15}N values ($F_{3, 152} = 4.258$, $p < 0.01$) and significantly lower $\delta^{13}C$ values ($F_{3, 152} = 82.902$, $p < 0.001$) than any other IRL dolphins (Table 1; Fig. 2). Of the remaining groups, nIRL and BR were not significantly different for either isotope and were pooled (Table 1; Fig. 2). Dolphins sampled in the nIRL/BR and sIRL did not differ significantly, but ML dolphins had significantly higher δ^{13}C values (Table 1; Fig. 2).

Stable isotope ratios were analyzed for skin samples taken from dead, stranded dolphins collected between 1994 and 2004 ($n = 47$ adults and 14 calves/subadults) within the IRL (37 adults and 11 calves/subadults) and along the Atlantic Ocean coast (10 adults and 3 calves/subadults). Isotope data (δ^{15}N and δ^{13}C) were normally distributed for all age classes, both sexes, seasons, and all collection years. δ^{13}C values did not differ between age classes for stranded dolphins ($F_{2, 20} = 0.764$, $p = 0.479$), but δ^{15}N values did differ significantly across age classes ($F_{2, 20} = 7.780$, $p = 0.003$). Tukey's HSD post hoc tests revealed that skin from calves was most enriched in ^{15}N (14.5 ± 0.3 ‰),

Table 1 Isotopic values for skin of live dolphins sampled in different segments of the Indian River Lagoon, Florida (see Fig. 1)

	n	δ^{15}N (range)	δ^{13}C (range)
Mosquito Lagoon	15	11.5 ± 0.2 ‰ (10.0 to 13.1 ‰)	−14.4 ± 0.3 ‰ (−15.6 to −12.7 ‰)
Northern IRL/Banana River	59	11.8 ± 0.2 ‰ (9.7 to 14.0 ‰)	−15.2 ± 0.1 ‰ (−17.6 to −12.4 ‰)
Southern IRL	68	12.1 ± 0.1 ‰ (10.4 to 14.0 ‰)	−15.6 ± 0.1 ‰ (−18.1 to −14.2 ‰)
St. Lucie Estuary	11	12.8 ± 0.3 ‰ (10.5 to 13.8 ‰)	−20.3 ± 0.4 ‰ (−22.1 to −18.1 ‰)

Dolphins sampled in SLE had significantly higher δ^{15}N values ($p < 0.01$) and significantly lower δ^{13}C values ($p < 0.001$) than any other IRL dolphins. nIRL, BR, and sIRL dolphins were not significantly different for either isotope. ML dolphins had significantly higher δ^{13}C values ($p < 0.001$)

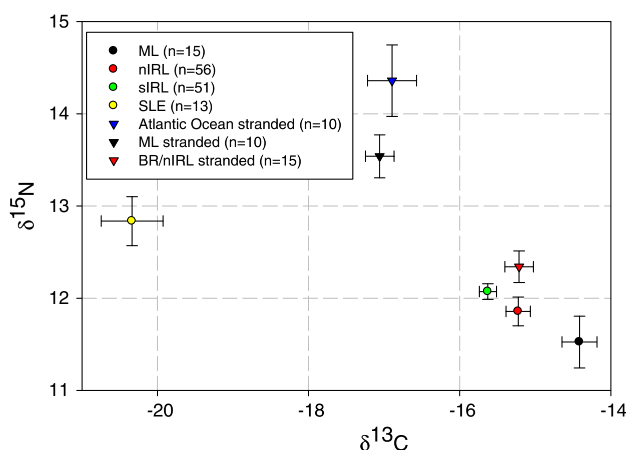

Fig. 2 Isotope ratios (mean ± SE) for remote biopsy sampled and live-captured dolphins sampled throughout the IRL (identified as being from Mosquito Lagoon (ML), Banana River (BR), northern IRL (nIRL), southern IRL (sIRL), and the St. Lucie Estuary (SLE)), as well as values (mean ± SE) for stranded dolphins (2000–2004). Dead stranded dolphins are grouped according to geographic area of recovery since actual region of residency is unknown. Stranded animals recovered in BR and northerly portions of the nIRL were combined. Animals were also recovered from ML and along the Atlantic Ocean coast near ML

followed by subadults (13.6 ± 0.3 ‰), with adults being most depleted (12.7 ± 0.1 ‰) ($p \leq 0.028$). Skin from calves was enriched in ^{15}N by an average of 1.80 ‰ compared with that of adults.

δ^{13}C values did not differ among sampling years for dead stranded animals ($F_{7,\,20} = 2.11$, $p = 0.09$); however, δ^{15}N values did ($F_{7,\,20} = 2.93$, $p < 0.03$) (Fig. 2). Nitrogen-15 values for 1998 were not significantly different from 1994 (Tukey's HSD, $p = 0.27$) or 1995 (Tukey's HSD, $p = 0.20$), but did differ significantly from all other sampling years (2000–2004) (Tukey's HSD, all p values ≤ 0.04). ^{15}N values from 1994 to 1999 were enriched (13.7 ± 0.4 ‰) relative to 2000–2001 (12.2 ± 0.2 ‰).

Dead adult stranded animals (collected during 2000–2004) were divided into three groups based on where they were recovered: Atlantic Ocean coast (AO) ($n = 10$), ML ($n = 10$), and nIRL/BR ($n = 15$) (Table 2). Dolphins stranded along the AO and in ML were significantly different with respect to δ^{13}C ($p < 0.0001$) and δ^{15}N ($p < 0.0001$) (Table 2). Dolphins collected in the nIRL/BR showed significantly enriched ^{13}C ($p < 0.0001$) and depleted ^{15}N ($p < 0.0001$) relative to dolphins collected in ML (Table 2).

Fatty acid signature analysis

CART analysis was performed on blubber samples collected from free-swimming (2002, $n = 22$) and live-captured dolphins (2003 through 2005, $n = 65$). Comparisons of collection years indicated a significant difference (CART overall misclassification ratio (MR) = 3/87, $p = 0.034$; MRPP, $p < 0.001$). Two samples collected in 2004 were misclassified as 2005, and one sample collected in 2005 was misclassified as 2004. Male and female dolphins showed significant differences with only two animals being misclassified ($p = 0.022$); the results of MRPP analysis were consistent ($p < 0.001$) with there being sex differences. CART and MRPP analyses resulted in the separation of dolphins into ML, nIRL, BR, sIRL, and SLE segments (CART overall MR = 4/87, $p < 0.046$; MRPP, $p < 0.001$) (Fig. 3). The majority of dolphins from the northern segments of the IRL separated from dolphins sampled in more southerly segments of the IRL using 18:2n-4. ML dolphins separated from other nIRL dolphins using 22:1n-11 (MR = 0/10) (Fig. 3). Within this northern group, dolphins sampled in the BR could be further distinguished with a MR = 2/10 (using 18:3n-3 and 18:4n-1). Nine sIRL dolphins that clustered with these northern dolphins, separated as a distinct group with increased levels of 16:1n-7 (MR = 0/8).

Dolphins from the southern regions could be subdivided into sIRL animals and SLE dolphins, along with a small group of misclassified nIRL dolphins (Fig. 3). Eight nIRL dolphins were misclassified in the southern cluster, six of which separated with 14 sIRL dolphins using 22:5n-3 (Fig. 3). Thirteen of these 14 latter dolphins were sampled in the winter season of 2002 and 11/14 were sampled in the northernmost portion of the south-central IRL. Six nIRL dolphins separated from this cluster using 20:3n-3, along with two misclassified sIRL dolphins. Two of these nIRL

Table 2 Dead, adult stranded animals (collected during 2000–2004) were divided into three groups based on where they were recovered: Atlantic Ocean coast (AO) ($n = 10$), ML ($n = 10$), and nIRL/BR ($n = 15$)

	n	δ^{15}N (range)	δ^{13}C (range)
Mosquito Lagoon	10	13.5 ± 0.2 ‰ (13.7 to 15.4 ‰)	−17.1 ± 0.2 ‰ (−18.1 to −16.1 ‰)
Northern IRL/Banana River	15	12.4 ± 0.2 ‰ (11.4 to 13.7 ‰)	−15.2 ± 0.2 ‰ (−16.9 to −13.7 ‰)
Atlantic Ocean coast	10	14.4 ± 0.5 ‰ (12.9 to 16.9 ‰)	−16.9 ± 0.3 ‰ (−19.4 to −16.0 ‰)

Dolphins stranded along the AO and in ML were significantly different with respect to δ^{13}C ($p < 0.0001$) and δ^{15}N ($p < 0.0001$). Dolphins collected in the nIRL/BR showed significantly enriched δ^{13}C ($p < 0.0001$) and depleted δ^{15}N ($p < 0.0001$) relative to dolphins collected in ML

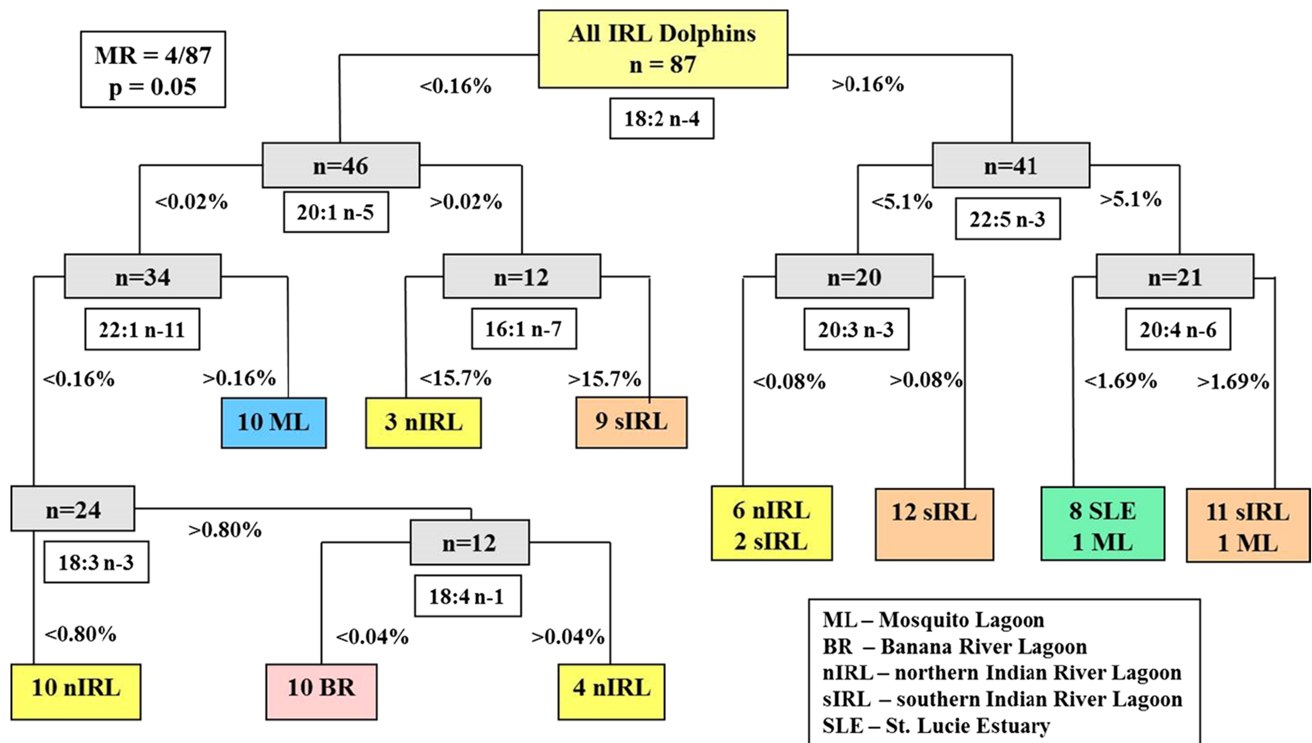

Fig. 3 CART analysis of live IRL bottlenose dolphins (overall misclassification ratio = 4/87, $p = 0.05$). Spilt points are identified with the specific fatty acid being used to split the dataset as well as the absolute concentrations of that fatty acid that determine the separation. Terminal nodes indicate the number of dolphins and subregion assigned to the node, as well as the number and actual subregion of any misclassified dolphins. Two sIRL and 2 ML dolphins were misclassified

dolphins (FB916 and FB918) were actually sampled in the far northern region of the IRL as a male–male pair in July 2003. FB918 was subsequently recaptured in the same area in June 2011. Dolphins identified as having been biopsy-sampled in the SLE were distinct (MR = 0/8) from other southern dolphins (MR = 0/11) by having low levels of 20:4n-6 (Fig. 3). Two ML dolphins that misclassified in this southern cluster (FB956 and FB984) were both sampled on the same date in June 2004 within 1 mile of each other.

Discussion

Recognizable subpopulations of bottlenose dolphins were distinguishable in the Indian River Lagoon using stable isotopes (SI) and fatty acids. Similar results have been documented in other studies, examining a variety of marine mammal species, but not on the same spatial scale (e.g., Lowther and Goldsworthy 2011; Bentaleb et al. 2011; Botta et al. 2011; Gibbs et al. 2011; Meissner et al. 2011; Wilson et al. 2012, 2013). Assignment of dolphins to home range areas using these data agrees with general observations of resident dolphin movements (Mazzoil et al. 2008a, 2011). Using SI, dolphins could be assigned to a ML subpopulation in the north and a St. Lucie Estuary subpopulation in the south, but stable isotope analyses were unable to separate northern and sIRL subpopulations (as defined by Mazzoil et al. 2008a, 2011). Application of FASA allowed for finer resolution of subpopulations with ML and BR subpopulations being identified in the north, a separation of northern and sIRL subpopulations, and a St. Lucie Estuary subpopulation in the south. Dolphins were grouped into their respective subpopulations regardless of sex, year, or season of sampling. Our FASA results are similar to those derived from mitochondrial and microsatellite analyses which indicated a distinct ML population and two distinct populations (genetic clusters) within the IRL (Richards et al. 2013).

It has been previously documented that bottlenose dolphins in the IRL have a high level of site fidelity (e.g., Mazzoil et al. 2005, 2008a, 2011; Durden et al. 2011). Regional differentiation of isotopic and fatty acid signatures noted in the present study could be a result of regional differences in feeding habits or be consistent with observed regional isotopic differentiation of likely prey (Adams and Paperno 2012). There is evidence of significant differences in the stable isotope and fatty acid signatures of white mullet (*Mugil curema*), pinfish (*Lagodon rhomboides*), and

spotted seatrout (*Cynoscion nebulosus*) over distances of 10–15 miles separation in the IRL (Adams and Paperno 2012; Fletcher-Odom 2012) which would support the regional differentiation of bottlenose dolphins. Our data are consistent with the observations of Mazzoil et al. (2008b) when they radio-tracked two male dolphins in the IRL and observed fidelity to relatively restricted regions. St. Lucie Estuary dolphins showed significantly different stable isotope and fatty acid signature values from other IRL dolphins due to their site fidelity to an area where there is a higher riverine influence. The St. Lucie estuary segment has a high influx of fresh water from rivers, lakes, and drainage canals. Freshwater sources generally are more depleted in ^{13}C compared with brackish or marine sources (e.g., Garcia et al. 2007), and this would be reflected in the primary producers. St. Lucie Estuary dolphins were also significantly more enriched in ^{15}N values than dolphins in the other segments. Southern parts of the IRL and the St. Lucie Estuary receive large amounts of runoff from flood control drainage canals, including from agricultural watersheds which introduce large amounts of pesticides, pollutants, and fertilizers (Mazzoil et al. 2008a). The input of these fertilizers most likely contributes to a higher nitrogen level in this segment which is then carried up the food chain.

Skin samples collected from dead stranded dolphins collected in the 1990s were significantly enriched in ^{15}N but not in ^{13}C relative to samples collected post-2000. These temporal differences were not associated with changes in salinity or water temperature nor did they correspond with any algal blooms or unusual mortality events. Additionally, since recent studies have shown that neither stage of decomposition (Payo–Payo et al. 2013) nor nutritional condition prior to death (Gómez-Campos et al. 2011) is reflected in the isotopic ratios of dead stranded dolphins, these values are presumably representative of live animals. Observed changes in isotopic signatures between the 1990s and recent years are consistent with changes in commercial fishing in response to the 1995 ban on commercial gill netting. Since the gill net ban, mullet stocks have increased significantly (Mahmoudi 2000) and thread herring have shown increasing recruitment throughout Florida from 1998 to 2002 (FFWCC 2006). Commercial landings of spot declined by 95 % after the gill net ban (McRae et al. 1997) and presumably population numbers have increased. Spotted sea trout, on the other hand, have not shown any significant changes in abundance over the same time period (Murphy et al. 2006). Collectively the results of the present study suggest that as the thread herring, spot, and mullet populations have increased, dolphins have likely switched their feeding preferences.

In the present study, stable isotope analysis did not detect significant differences between sexes, whereas fatty acid analysis did. This suggests that male and female dolphins in the IRL are likely feeding at the same trophic level and in the same vicinity (both indicated by SI), but are potentially choosing different prey species (indicated by fatty acid signature) or differing in the proportions of prey species they ingest. Differences in foraging habits between male and female common dolphins (*Delphinus delp*his) have been documented (e.g., Young and Cockcroft 1994; Chou et al. 1995), and it has been shown that mature females had a significantly higher proportion of cephalopods in their diet compared with mature males (Silva 1999). However, when examining stomach contents, Amir et al. (2005) found no significant differences in prey preferences between male and female Indo-Pacific bottlenose dolphins, whereas Cockcroft and Ross (1990) found sex-specific differences in prey choice. Walton et al. (2007) also observed no significant differences between sexes implying that their dolphins were feeding consistently on the same prey species. Walton et al. (2007) also failed to identify any inter-island group differences suggesting significant mixing of groups of dolphins consistent with genetic analyses of this same population.

Most dolphin groups retain a fission–fusion nature with an open social network where individuals move in and out due to the low energetic cost of locomotion (Randic et al. 2012). In mammals, female lifetime reproductive success is tied to access to resources such as food, while in males it is limited to access to receptive mates (Moeller 2012). This can result in males moving over larger areas than that of female dolphins, thereby giving males access to differing prey items. Mature females may also be restricted in their range and diving depths because of accompanying nursing calves and/or immatures (e.g., Barros and Odell 1990; Ringelstein et al. 2006).

Along the US Gulf coast, female dolphins with calves have been shown to inhabit a relatively limited core habitat area, whereas males encompass a much wider range incorporating several female areas (Barros and Odell 1990; Scott et al. 1990) allowing them to feed on different prey items from each habitat. Bottlenose dolphins feeding on different prey items, as well as on different size classes within each prey species, would exhibit differences in blubber fatty acid composition (Iverson et al. 1997).

Over the past 15 years, bottlenose dolphins in the IRL have exhibited increased numbers of strandings (Stolen et al. 2007), Unusual Mortality Events (Marine Mammal Commission 2002), and have shown increased accounts of pathological issues such as infections and inflammatory diseases (Bossart et al. 2003). The results of the stable isotope and fatty acid analysis in the present study indicate that these dolphins reside in relatively discrete portions of the IRL. It is clear that there have been significant changes in IRL over the past 20 years (e.g., Bossart et al. 2006), and our analyses suggest that there is potential in using dolphin

blubber/skin to monitor long- and short-term ecological changes that are occurring in the region. The results of the present study indicate that there may have been significant shifts in the feeding ecology and trophic interactions of many species in the Lagoon, including bottlenose dolphins. This suggestion reinforces the need for considering both spatial and temporal scales when assessing ecosystem processes from a trophic perspective. Although continuing analysis is needed to conclusively demonstrate any changes, the ability to compare fatty acid and isotope composition back to the early 1990s and ultimately to compare against stomach content analysis gives us a unique opportunity to retrospectively try to understand how the IRL has changed and how it will continue to change.

On a wider scale, isotopic and fatty acid signatures could potentially assign unknown dolphins to home Bay systems. Barros et al. (2010) and Gibbs et al. (2011) have distinguished coastal/bay resident dolphins from offshore populations using SI, and more recently, Wilson et al. (2012, 2013) found that they could distinguish different groups of dolphins residing in different Bays. Our present data suggest the potential for resolving even finer scale habit use. Application of isotopic and fatty acid signatures over a wider scale could ultimately help resolve questions relating to the broader population structure of dolphins, allow for better understanding of the roles of these dolphins as apex estuarine predators, and allow for a more effective management of the ecosystems in which they live.

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