© 2014. Published by The Company of Biologists Ltd | The Journal of Experimental Biology (2014) 217, 214-221 doi:10.1242/jeb.093963

RESEARCH ARTICLE

Isotope turnover rates and diet–tissue discrimination in skin of *ex situ* bottlenose dolphins (*Tursiops truncatus*)

Nicole E. Browning[1], Christopher Dold[2], Jack I-Fan[3] and Graham A. J. Worthy[1,*]

ABSTRACT

Diet–tissue discrimination factors ($\Delta^{15}N$ or $\Delta^{13}C$) and turnover times are thought to be influenced by a wide range of variables including metabolic rate, age, dietary quality, tissue sampled and the taxon being investigated. In the present study, skin samples were collected from *ex situ* dolphins that had consumed diets of known isotopic composition for a minimum of 8 weeks. Adult dolphins consuming a diet of low fat (5–6%) and high $\delta^{15}N$ value had significantly lower $\Delta^{15}N$ values than animals consuming a diet with high fat (13.9%) and low $\delta^{15}N$ value. Juvenile dolphins consuming a diet with low fat and an intermediate $\delta^{15}N$ value had significantly higher $\Delta^{15}N$ values than adults consuming the same diet. Calculated half-lives for $\delta^{15}N$ ranged from 14 to 23 days (17.2±1.3 days). Half-lives for $\delta^{13}C$ ranged from 11 to 23 days with a significant difference between low fat (13.9±4.8 days) and high fat diets (22.0±0.5 days). Overall, our results indicate that while assuming a $\Delta^{13}C$ value of 1‰ may be appropriate for cetaceans, $\Delta^{15}N$ values may be closer to 1.5‰ rather than the commonly assumed 3‰. Our data also suggest that understanding seasonal variability in prey composition is another significant consideration when applying discrimination factors or turnover times to field studies focused on feeding habits. Isotope retention times of only a few weeks suggest that, in addition, these isotope data could play an important role in interpreting recent fine-scale habitat utilization and residency patterns.

KEY WORDS: Feeding habits, Isotope turnover time, Cetaceans

INTRODUCTION

In recent years, stable isotope analysis has been effectively used to gain a better understanding of the feeding ecology of many marine mammal species (e.g. Ramsay and Hobson, 1991; Ames et al., 1996; Walker et al., 1999; Kurle and Worthy, 2002; Yamamuro et al., 2004; Lee et al., 2005; Newsome et al., 2006; Reich and Worthy, 2006; Alves-Stanley and Worthy, 2009; Witteveen et al., 2011). Stable isotope analysis utilizes naturally occurring isotopes, such as those of nitrogen and carbon, which are passed from prey to consumer and are reflected in the consumer's tissues. The stable isotope ratio in the tissues of a consumer reflects its diet over some period of time, not just the most recent prey eaten. Sampling for this technique is minimally invasive, allowing for repeated sampling, and can be safely carried out on free-ranging animals using a biopsy dart.

Although stable isotope analysis is a powerful and important tool to gain insight into the ecology of animals, there are additional measurements that are imperative to the proper interpretation of such data. Many assumptions that are crucial to applying these approaches in the field have not been assessed using controlled feeding studies. While these captive studies may not precisely replicate what *in situ* animals are consuming, they do give invaluable insights into how they function. In order to accurately interpret and apply stable isotope analysis to understanding the feeding ecology of wild cetaceans, the rates at which cetaceans incorporate stable isotopes into tissues must first be determined (Tieszen et al., 1983; Hobson et al., 1996; Seminoff et al., 2006), perhaps even at a species-specific level (e.g. DeMots et al., 2010; Wyatt et al., 2010).

The stable isotope ratio of a consumer will differ slightly from that of its prey. This shift is referred to as diet–tissue discrimination (or fractionation) and occurs because even though isotopes of a given element undergo the same biochemical processes in the body, they react differently as a result of differences in their atomic weights. The different affinities for the heavier and lighter isotopes result in the consumer tissue being more enriched in ^{13}C, compared with the diet, by an average of ~1‰ (DeNiro and Epstein, 1978), and the diet–tissue discrimination for ^{15}N averages 2–5‰ (Rau et al., 1983; Minagawa and Wada, 1984; Hobson et al., 1994). It is this latter shift that is generally used to assess step-wise trophic level changes and allows the potential interpretation of feeding history (DeNiro and Epstein, 1981; Peterson and Fry, 1987). Discrimination values can vary dramatically depending on the tissue being sampled and the species in question. In order to more accurately evaluate stable isotope data, tissue-specific diet–tissue discrimination values need to be determined for cetacean skin, as that is the tissue most easily obtained. There are currently only two published studies on cetacean diet–tissue discrimination values for skin (Caut et al., 2011; Borrell et al., 2012) but neither study was undertaken under controlled conditions or for an extended period of time.

To interpret temporal changes in the isotopic values of consumer tissues as diet composition changes, the turnover rates of those tissues must be known (Bosley et al., 2002). If turnover rate and diet–tissue discrimination are known, one can ultimately infer changes in habitat, diet and/or migratory patterns (Mitani et al., 2006; Marcoux et al., 2007; Witteveen et al., 2011). Turnover rates of stable isotopes will vary depending on the species involved and type of tissue being sampled. Plasma or liver tissue, for example, will have fast turnover rates and provide information on recently consumed diets, whereas bone tissue will have a slower turnover time and provide information over a longer dietary history (Tieszen et al., 1983; Ogden et al., 2004). There are currently no data on turnover times for cetaceans and in order to appropriately use stable isotope data to answer ecological questions, these rates need to be established.

Studies undertaken under controlled conditions are crucial to establish discrimination values and turnover rates because the assessment of these values requires the opportunity to experimentally switch an animal from one isotopically distinct diet

[1]Physiological Ecology and Bioenergetics Lab, Department of Biology, University of Central Florida, Orlando, FL 32816-2368, USA. [2]SeaWorld Parks and Entertainment, Orlando, FL 32821, USA. [3]Farglory Ocean Park, Hualien, Taiwan, ROC.

*Author for correspondence (Graham.Worthy@ucf.edu)

Received 15 July 2013; Accepted 9 September 2013

The Journal of Experimental Biology

to another isotopically distinct diet under controlled conditions. Despite the large number of field studies that have applied stable isotope analysis to marine mammals (e.g. Ramsay and Hobson, 1991; Ames et al., 1996; Walker et al., 1999; Kurle and Worthy, 2002; Yamamuro et al., 2004; Lee et al., 2005; Newsome et al., 2006; Reich and Worthy, 2006; Alves-Stanley and Worthy, 2009; Witteveen et al., 2011), there are no turnover rates available for skin (the most commonly available tissue) for any cetacean species. The only marine mammal species for which there are any data on turnover time is the West Indian manatee (*Trichechus manatus*) (Alves-Stanley and Worthy, 2009). Because turnover rates and diet–tissue discrimination values often differ between tissues and species, it is crucial to broaden marine mammal isotope turnover research in order to accurately interpret stable isotope data. Having these critical values will also allow researchers to go back and more accurately interpret historical data.

Data for carbon and nitrogen turnover rates are scarce in the literature and most studies have been performed on birds and small mammals (e.g. Teiszen et al., 1983; Voigt et al., 2003; Ogden et al., 2004). Some studies have reported turnover rates for larger mammals, including alpacas, bears, horses, seals and cattle; however, none of these studies measured turnover rates of carbon and nitrogen specifically in skin (e.g. Hilderbrand et al., 1996; Zhao and Schell, 2004; Sponheimer et al., 2006). The quality and quantity of protein in a consumer's diet can affect the turnover rate of proteins, thereby altering carbon and nitrogen turnover rates (Lobley, 2003; Zhao et al., 2006). Isotopic turnover rates can also vary among tissue types, species, body sizes and nutritional states (Martínez del Rio et al., 2009; Newsome et al., 2010), making comparisons among studies difficult.

In a field study where large biopsy samples (3–5 cm in diameter and 1 cm deep) were surgically removed from bottlenose dolphins, there was almost complete healing after 42 days with only a slight discoloration left on the surface of the skin (Weller et al., 1997). These rates of wound healing lend insight into potential turnover rates for dolphin integument and, in the absence of empirical data for these species, we hypothesized that turnover rates in cetaceans would be considerably faster than might be suggested from currently available literature values for other species.

Knowledge of turnover rates and diet–tissue discrimination values is crucial in interpreting isotope-based feeding ecology studies involving free-ranging cetaceans in order to assess feeding habits and evaluate critical habitat needs. Stable isotope data not only enable important insight into dietary habits but also potentially allow identification of habitat preferences and give insight into habitat usage. By establishing these much-needed values for delphinids, stable isotope data will be better used to evaluate important aspects of the ecology of these animals, and scientists, ultimately, will be better equipped to make important conservation decisions for these species. The goal of the present study was to ascertain stable isotope discrimination factors and turnover rates of skin collected from *ex situ* bottlenose dolphins, *Tursiops truncatus* (Montagu 1821), held on controlled diets.

RESULTS

Diet

Sea World of Florida (SWF), USA

A total of 52 fish from three species were analyzed from SWF (Table 1). Isotope data ($\delta^{15}N$ and $\delta^{13}C$) were normally distributed for all species (Shapiro–Wilk test, S–W; $P \geq 0.186$) except for capelin $\delta^{13}C$ (S–W=0.85, $P=0.04$). Fish species were found to be significantly different from each other (Tukey's HSD, $P<0.001$) for both $\delta^{15}N$ ($F_{2,79}=162.91$, $P<0.001$) and $\delta^{13}C$ ($F_{4,156}=701.51$, $P<0.001$) (Table 2). Herring (11.87±0.09‰), capelin (11.74±0.06‰) and night smelt (13.45±0.04‰), at SWF, had significantly different $\delta^{15}N$ values (Tukey's HSD, $P \leq 0.01$). Night smelt (−15.67±0.08‰) was significantly different from both herring (−19.86±0.09‰) and capelin (−19.87±0.06‰) in terms of $\delta^{13}C$ values (Tukey's HSD, $P<0.001$), while capelin and herring were not significantly different from each other (Tukey's HSD, $P=0.297$). Lipid content was relatively low, ranging from 2.3±0.2% (night smelt) to 7.3±0.2% for herring, with C:N ratios of 2.93±0.02 and 2.95±0.04, respectively (Table 1).

SWF-1 dolphins received 60% (by mass) capelin and 40% herring, resulting in a combined $\delta^{15}N$ value of 11.79‰, $\delta^{13}C$ value of −19.87‰ and fat content of 6% (Tables 1, 2). Proportions of each species of fish fed to individual SWF-2 dolphins varied according to age and reproductive status, and therefore diets for these individuals ranged between values of 12.04‰ and 12.46‰ for $\delta^{15}N$ and between values of −18.19‰ and −19.27‰ for $\delta^{13}C$, with a fat content of ~5% (Tables 1, 2).

Far Glory Ocean Park (FGOP), Taiwan

A total of 56 fish from four species ($N=14$ for each species) were analyzed from FGOP (Table 1). Isotope data ($\delta^{15}N$ and $\delta^{13}C$) were normally distributed for all species (S–W; $P \geq 0.09$) except for $\delta^{15}N$ values in Pacific saury (S–W=0.85, $P=0.02$). Fish species were found to be significantly different in terms of both $\delta^{15}N$ ($F_{3,52}=477.91$, $P<0.001$) and $\delta^{13}C$ ($F_{3,52}=202.48$, $P<0.001$) values. Pacific saury (7.55±0.11‰) and capelin (12.83±0.06‰) were significantly different (Tukey's HSD, $P<0.05$) from other species for $\delta^{15}N$ value and Atlantic mackerel (11.22±0.14‰) and horse mackerel (11.38±0.09‰) were not significantly different from each other (Tukey's HSD, $P>0.05$) (Table 2). Pacific saury (−21.39±0.09‰) and capelin (−19.73±0.05‰) were significantly different from other species for $\delta^{13}C$ values (Tukey's HSD, $P<0.05$),

Table 1. Mass, total length and nutritional value of fish utilized in diets of *ex situ* bottlenose dolphins

Species	Mass (g)	Total length (mm)	Lipid (%)	C:N ratio
SWF				
Herring (*N*=15)	172.8±10.5	257.7±9.6	7.3±0.2%	2.95±0.04
Capelin (*N*=17)	28.9±0.6	161.9±1.3	5.2±0.3%	2.92±0.03
Night smelt (*N*=20)	9.7±0.3	98.6±1.0	2.3±0.2%	2.93±0.02
FGOP				
Pacific saury (*N*=14)	66.5±1.9	274.6±2.2	17.0±0.2%	3.15±0.01
Atlantic mackerel (*N*=14)	121.1±4.9	239.9±3.9	3.4±0.1%	3.10±0.03
Capelin (*N*=14)	21.9±0.5	151.6±1.3	1.5±0.2%	3.09±0.02
Horse mackerel (*N*=14)	115.4±4.9	234.6±4.3	6.7±0.2%	2.99±0.01

SWF, SeaWorld of Florida; FGOP, Far Glory Ocean Park.
Data are means ± s.e.m.

The Journal of Experimental Biology (2014) doi:10.1242/jeb.093963

Table 2. Isotopic composition of prey items offered to *ex situ* dolphins and overall isotopic composition of diets used in the present study.

Dietary components	$\delta^{15}N$	$\delta^{13}C$
SWF		
Herring (*N*=15)	11.87±0.09‰	−19.86±0.09‰
Capelin (*N*=17)	11.74±0.08‰	−19.87±0.05‰
Night smelt (*N*=20)	13.45±0.04‰	−15.67±0.08‰
SWF-1 diet offered to dolphins (60% capelin, 40% herring)	11.79‰	−19.87‰
SWF-2 diet offered to dolphins (variable amounts: capelin, herring and night smelt)	12.04‰ to 12.46‰	−18.19‰ to −19.27‰
FGOP		
Pacific saury (*N*=14)	7.55±0.11‰	−21.39±0.09‰
Atlantic mackerel (*N*=14)	11.22±0.14‰	−16.87±0.28‰
Capelin (*N*=14)	12.83±0.06‰	−19.73±0.05‰
Horse mackerel (*N*=14)	11.38±0.09‰	−17.02±0.06‰
FGOP diet A offered to dolphins (80% Pacific saury, 20% Atlantic mackerel)	8.30‰	−20.48‰
FGOP diet B offered to dolphins (80% capelin, 20% horse mackerel)	12.54‰	−19.19‰

Data are means ± s.e.m.

while Atlantic mackerel (−16.87±0.28‰) and horse mackerel (−17.02±0.06‰) were not significantly different from each other (Tukey's HSD, $P>0.05$) (Table 2). Lipid content ranged from 1.5±0.2% (capelin) to 17.0±0.2% for Pacific saury (Table 1) with C:N ratios of 3.09±0.02 and 3.15±0.01, respectively (Table 1).

At FGOP, diet A (80% Pacific saury, 20% Atlantic mackerel) had an overall nitrogen value of 8.30‰, carbon value of −20.48‰ and lipid content of 13.9% (Tables 1, 2). Diet B (80% capelin, 20% horse mackerel) had an overall nitrogen value of 12.54‰, carbon value of −19.19‰ and lipid content of 2.5% (Tables 1, 2).

Dolphin skin isotopic values and diet discrimination

Dolphin skin differed significantly in both $\delta^{15}N$ ($F_{4,44}=14.90$, $P<0.0001$) and $\delta^{13}C$ ($F_{4,44}=3.89$, $P<0.009$) values between different groups (Table 3). There were no significant differences between SWF dolphins regardless of treatment in terms of either $\delta^{15}N$ or $\delta^{13}C$ values (Tukey's HSD, $P>0.05$) (Table 3, Fig. 1). FGOP dolphins consuming the pre-experiment diet were not significantly different in $\delta^{15}N$ values from those consuming either diet A or diet B (Tukey's HSD, $P>0.05$) (Table 3, Fig. 1), but diets A and B differed significantly in $\delta^{15}N$ value (Tukey's HSD, $P<0.05$). FGOP dolphins consuming the pre-experiment diet had $\delta^{13}C$ values that were significantly higher than those of dolphins consuming diet A (Tukey's HSD, $P<0.05$), but were not significantly different from those of dolphins consuming diet B ($P=0.66$) (Tukey's HSD, $P>0.05$) (Table 3, Fig. 1).

Diet–tissue discrimination values were significantly different for both $\Delta^{15}N$ ($F_{3,38}=19.98$, $P<0.0001$) and $\Delta^{13}C$ ($F_{3,38}=20.333$, $P<0.001$) values between groups. SWF-1 and SWF-2 adults did not differ in $\Delta^{15}N$ (Tukey's, $P>0.05$), but SWF-2 juveniles had significantly higher $\Delta^{15}N$ values (Tukey's, $P<0.05$) (Table 3, Fig. 2). FGOP dolphins consuming diet B had significantly lower $\Delta^{15}N$ values than animals consuming diet A (Tukey's, $P<0.05$) but were not significantly different from SWF-1 or SWF-2 adults (Tukey's, $P>0.05$). SWF-2 juveniles were not significantly different from FGOP diet A dolphins (Tukey's, $P>0.05$) (Table 3, Fig. 2).

SWF-2 juvenile and adult SWF-2 dolphins did not differ significantly from each other in $\Delta^{13}C$ values (Tukey's, $P>0.05$) but collectively had significantly lower $\Delta^{13}C$ values than all other dolphin groups (Tukey's, $P<0.05$). SWF-1 adults were not significantly different from FGOP diet B dolphins (Tukey's, $P>0.05$) (Table 3, Fig. 2) and there were no significant differences between FGOP dolphins consuming diet A and diet B (Tukey's, $P>0.05$) ($P=0.127$) (Table 3, Fig. 2).

Diet–tissue discrimination values for nitrogen ($\Delta^{15}N$) were not significantly different between SWF-1 adults, SWF-2 adults and FGOP dolphins consuming diet B (Tukey's, $P>0.05$) (Table 3). SWF-2 juveniles and FGOP dolphins consuming diet A were also not significantly different (Tukey's, $P>0.05$) but were significantly different from other groups (Table 3). Diet–tissue discrimination values for carbon ($\Delta^{13}C$) were not significantly different for SWF-2 adults and SWF-2 juveniles (Tukey's, $P>0.05$), but those dolphins

Table 3. Isotope values and diet–tissue discrimination values for SWF and FGOP dolphins

Group	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Isotope values		
SWF-1 – adults	13.66±0.15 (*N*=8)^{b,c}	−18.26±0.19 (*N*=8)^{a,b,c}
SWF-2 – adults	14.21±0.10 (*N*=14)^{c}	−17.91±0.07 (*N*=14)^{a,b,c}
SWF-2 – juveniles	14.58±0.15 (*N*=5)^{c}	−18.53±0.20 (*N*=5)^{a}
FGOP – pre-experiment	12.61±0.31 (*N*=12)^{a,b}	−17.71±0.21 (*N*=12)^{c}
FGOP – diet A	11.81±0.55 (*N*=6)^{a}	−18.41±0.18 (*N*=6)^{a}
FGOP – diet B	13.73±0.44 (*N*=6)^{b,c}	−17.81±0.19 (*N*=6)^{b,c}
Diet–tissue discrimination values		
SWF-1 – adults	2.09±0.07 (*N*=8)^{a}	1.28±0.16 (*N*=8)^{b}
SWF-2 – adults	1.90±0.08 (*N*=14)^{a}	0.66±0.07 (*N*=14)^{a}
SWF-2 – juveniles	2.61±0.14 (*N*=5)^{b}	0.53±0.19 (*N*=5)^{a}
FGOP – diet A	2.96±0.12 (*N*=6)^{b}	2.04±0.14 (*N*=6)^{c}
FGOP – diet B	1.68±0.11 (*N*=6)^{a}	1.60±0.09 (*N*=6)^{b,c}

FGOP dolphins were sampled after being on either diet A or diet B for 7–8 weeks. Superscript letters denote the lack of a significant difference.
Data are means ± s.e.m.

The Journal of Experimental Biology (2014) doi:10.1242/jeb.093963

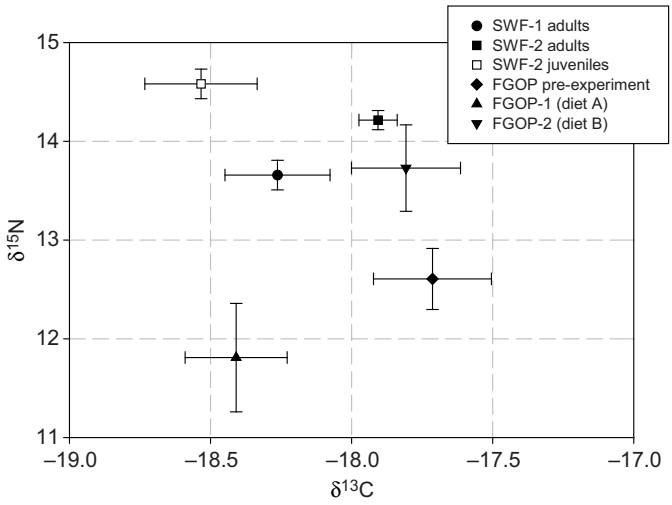

Fig. 1. Carbon and nitrogen isotope values (means ± s.e.m.) of epidermal tissue of bottlenose dolphins at Sea World of Florida (SWF) and Far Glory Ocean Park (FGOP). SWF-1: adult males (*N*=8); SWF-2: adult males (*N*=9), adult females (*N*=5) and juveniles (*N*=4 females, *N*=1 male). All SWF-1 and SWF-2 dolphins were fed a consistent mixture of herring and capelin for 2 months prior to sample collection. SWF-2 animals also received additional capelin and night smelt in variable amounts. FGOP-1 (*N*=4) and FGOP-2 (*N*=2) received two diets over the course of the study. All FGOP animals were on a common diet consisting of variable proportions of Pacific saury, Atlantic mackerel, capelin and horse mackerel prior to the experimental diets. Experimental diet A was 80% (by mass) Pacific saury and 20% Atlantic mackerel, while diet B was 80% capelin and 20% horse mackerel. FGOP-1 dolphins were fed diet A for 8 weeks before switching to diet B for 9 weeks. FGOP-2 dolphins were fed diet B for 8 weeks then transitioned to diet A for 9 weeks. Assessment of turnover time and diet–tissue discrimination occurred after the diet switch.

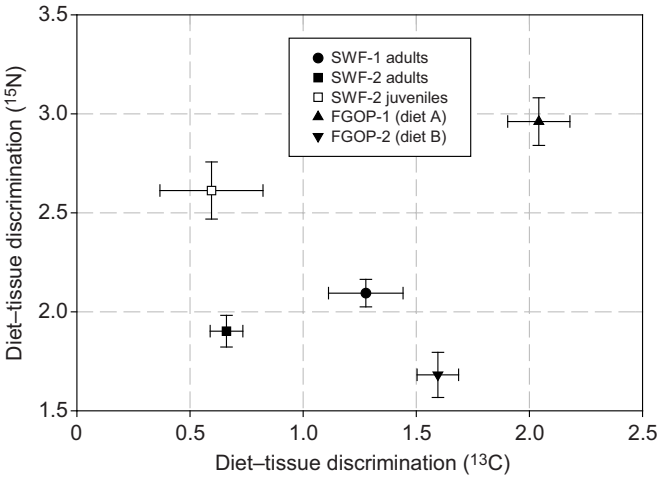

Fig. 2. Diet–tissue discrimination values (means ± s.e.m.) were significantly different for both $\Delta^{15}N$ values and $\Delta^{13}C$ values. SWF-1 and SWF-2 adults did not differ in $\Delta^{15}N$ value, but SWF-2 juveniles had significantly greater $\Delta^{15}N$ values. FGOP diet B dolphins had significantly lower $\Delta^{15}N$ values than diet A animals, but were not significantly different from SWF-1 or SWF-2 adults. $\Delta^{15}N$ values for SWF-2 juveniles were not significantly different from those of FGOP diet A dolphins. SWF-2 juvenile and SWF-2 adult dolphins did not differ significantly from each other in $\Delta^{13}C$ values but were significantly lower than all other groups. SWF-1 adults were not different from FGOP diet B dolphins in $\Delta^{13}C$ values, nor were there were significant differences in $\Delta^{13}C$ between FGOP dolphins consuming diet A and diet B. See Fig. 1 for details of dolphins and diets.

were significantly different from SWF-1 adults (Tukey's, $P<0.05$). SWF-1 adults were not significantly different from FGOP dolphins consuming diet B (Tukey's, $P>0.05$), which in turn were not significantly different from FGOP dolphins consuming diet A (Tukey's, $P<0.05$).

Isotopic half-life and retention time

Half-lives were calculated for dolphins at FGOP and ranged from 14 to 23 days for $\delta^{15}N$ (Table 4, Fig. 3A). Mean half-life for diet B was 18.2±3.6 days (*N*=4), which was not significantly different from diet A with a mean half-life of 15.3±1.7 days (*N*=2) (*t*=–1.33, d.f.=3, *P*=0.256). The combined half-life for these two groups was 17.2±1.3 days for $\delta^{15}N$ (*N*=6). Mean retention times ranged from 22.1±2.4 days (diet A) to 26.2±5.2 days (diet B) for $\delta^{15}N$ with an overall mean of 24.9±1.9 days.

Half-lives calculated for dolphins at FGOP ranged from 11 to 23 days for $\delta^{13}C$ (Table 4, Fig. 3). Diet B had a mean half-life of 13.9±4.8 days (*N*=4), which was significantly shorter than 22.1±0.5 days (*N*=2) (*t*=3.39, d.f.=3, *P*<0.04) for diet A. Mean retention times ranged from 20.0±6.9 days (diet B) to 31.8±0.7 days (diet A) for $\delta^{13}C$ (Table 4).

DISCUSSION

This is the first time that diet–tissue discrimination values and turnover times have been assessed for the skin of any cetacean using a controlled feeding experiment. Discrimination factors used in isotope studies are typically assumed to range from 2 to 5‰ for nitrogen and 1 to 2‰ for carbon (DeNiro and Epstein, 1981; Das et al., 2000; Kelly, 2000; Herman et al., 2005); however, these data are generally derived from studies of unrelated species. Diet–tissue discrimination values for nitrogen, calculated in the present study, fell in the low end of this range, with evidence of significant differences based on the quality of the diet (lipid content) and age of the dolphin. Actively growing, but independently feeding, juvenile dolphins had significantly higher $\Delta^{15}N$ values (2.46±0.17‰) than adults consuming the same diet (1.92±0.14‰) but did not differ significantly in $\Delta^{13}C$ values (0.53±0.17‰ versus 0.84±0.18‰). FGOP dolphins consuming a low fat diet (diet B) had significantly lower $\Delta^{15}N$ values (1.68±0.11‰) than FGOP dolphins consuming a high fat (diet A) diet (2.96±0.12‰), but the two diets resulted in similar $\Delta^{13}C$ values (1.60±0.09‰ versus 2.04±0.14‰). This result might be expected given the relatively small difference in $\delta^{13}C$ values between diets A and B (1.3‰).

At present, there are few studies that have measured discrimination factors for marine mammal skin. Alves-Stanley and Worthy (Alves-Stanley and Worthy, 2009) calculated discrimination values in the skin of *ex situ* manatees to be 2.8‰ for carbon, lower than the 4.1‰ carbon discrimination reported by Ames and colleagues (Ames et al., 1996). Hobson et al. (Hobson et al., 1996) found skin of *ex situ* harbor seals to be enriched by 2.3‰ for nitrogen and 2.8‰ for carbon. More recently, Caut and colleagues (Caut et al., 2011) were able to calculate discrimination factors using skin from a single killer whale and found $\Delta^{15}N$ to be 3.2‰ and $\Delta^{13}C$ to be 2.4‰; and Borrell et al. (Borrell et al., 2012) estimated nitrogen and carbon discrimination factors for the skin of *in situ* fin whales to be 2.8‰ and 1.3‰, respectively, similar to measured values in the present study.

It has been previously noted that diet–tissue discrimination factors differ between different diets and between tissues (e.g. Roth and Hobson, 2000; Pearson et al., 2003; Mirón et al., 2006). Diet–tissue discrimination factors are thought to be influenced by a variety of variables such as the metabolic rate of the animal and/or the tissue

The Journal of Experimental Biology (2014) doi:10.1242/jeb.093963

Table 4. Exponential decay equations, half-lives and retention times for $\delta^{15}N$ and $\delta^{13}C$ turnover in epidermal tissue of *ex situ* dolphins at FGOP

Dolphin	Diet	Equation	R^2	Half-life (days)	Retention time (days)	Initial isotope value (‰)
$\delta^{15}N$						
Dolphin 13	B	$y=14.64+(-3.63)e^{-0.043t}$	0.93	16.1	23.3	11.16
Dolphin 3	B	$y=14.29+(-3.08)e^{-0.048t}$	0.70	14.4	20.8	10.87
Dolphin 5	B	$y=14.13+(-2.93)e^{-0.035t}$	0.98	19.8	28.6	11.31
Dolphin 7	B	$y=14.68+(-3.03)e^{-0.031t}$	0.99	22.4	32.3	11.46
Mean				18.2±3.6	26.2±5.2	11.2±0.2
Dolphin 6	A	$y=11.36+(3.13)e^{-0.042t}$	0.94	16.5	23.8	14.24
Dolphin 9	A	$y=11.06+(3.89)e^{-0.049t}$	0.99	14.1	20.4	14.08
Mean				15.3±1.7	22.1±2.4	14.2±0.1
$\delta^{13}C$						
Dolphin 13	B	$y=-17.63+(-1.06)e^{-0.063t}$	0.83	11.0	15.9	−18.61
Dolphin 3	B	$y=-17.70+(-1.14)e^{-0.059t}$	0.87	11.7	16.9	−18.84
Dolphin 5	B	$y=-17.86+(-0.76)e^{-0.059t}$	0.77	11.7	16.9	−18.52
Dolphin 7	B	$y=-17.52+(-1.13)e^{-0.033t}$	0.95	21.0	30.3	−18.68
Mean				13.9±4.8	20.0±6.9	−18.7±0.1
Dolphin 6	A	$y=-18.81+(1.70)e^{-0.031t}$	0.90	22.4	32.3	−17.38
Dolphin 9	A	$y=-18.59+(1.11)e^{-0.032t}$	0.87	21.7	31.3	−17.48
Mean				22.1±0.5	31.8±0.7	−17.4±0.2

Exponential decay curves [$y(t)=y_a+ae^{-bt}$] were fitted with y_a set to the expected value based on diet and the calculated diet-specific value for $\Delta^{15}N$ or $\Delta^{13}C$ as appropriate.

being studied, the age of the animal, the nutritional quality of the diet, the tissue being sampled and the taxon being investigated (Hobson and Clark, 1992; Martínez del Rio et al., 2009; Newsome et al., 2010; Borrell et al., 2012). Tissues of the integumentary system generally tend to have higher carbon discrimination values compared with those of other tissues in mammals as they are mostly composed of keratin (Borrell et al., 2012). Tieszan and colleagues (Tieszen et al., 1983) and Hobson and colleagues (Hobson et al., 1996) found hair to have the highest carbon discrimination values compared with other types of tissues. Differing food composition can also result in differential rates of assimilation and storage of nutrients. Howland and colleagues (Howland et al., 2003) reported that pigs fed diets with differing amino acid composition exhibited diet–tissue fractionation factors for bone collagen ranging from 0.5‰ to 6.1‰.

Some researchers have suggested that $\Delta^{15}N$ should increase as protein content (%N) increases in the diet (quantity hypothesis) (e.g. Pearson et al., 2003; Martínez del Rio et al., 2009) while others have suggested that $\Delta^{15}N$ should decrease as dietary protein quality increases (quality hypothesis) (e.g. Roth and Hobson, 2000; Robbins et al., 2010). Florin and colleagues (Florin et al., 2011) found that neither of these hypotheses alone can fully explain the variability in $\Delta^{15}N$. They found that it was likely a combination of protein quality and quantity based on the assessment that low protein quality and high protein content have the potential to increase $\Delta^{15}N$ by increasing protein turnover rates. They (Florin et al., 2011) noted that the diet of piscivores would not vary as extensively in protein quality as would diets consumed by omnivores, suggesting that quantity would play a greater role in determining $\Delta^{15}N$.

Dolphins in the present study were consuming protein of similar biological value (fish) and therefore interpretation of our data relates to the absolute quantity of protein consumed. Dolphins consuming isocaloric diets that vary in fat content will consume different absolute masses of protein. Dolphins consuming the higher fat content diet (FGOP diet A: 13.9%) (i.e. lower protein intake) had significantly greater diet–tissue discrimination factors for both nitrogen and carbon than dolphins consuming diets with lower fat content (2–6%) (i.e. higher protein). Our results are inconsistent with either the protein quantity hypothesis (e.g. Pearson et al., 2003; Martínez del Rio et al., 2009) or the protein quality hypothesis (e.g. Roth and Hobson, 2000; Robbins et al., 2010), but discrimination values are consistent with the variability exhibited by carnivores reported by Florin and colleagues (Florin et al., 2011).

Turnover rates and retention times of stable isotopes quantify the period of time it takes for the isotopic composition of consumer tissues to reflect a new diet. It has been estimated that it takes ~2–3 half-lives for the complete integration of a new isotopic value into consumer tissues (Hobson and Clark, 1992), consistent with retention times measured in the present study (Table 4). The ability to use stable isotopes to quantify dietary choices over these relatively short temporal periods facilitates greater resolution of potential prey switching, seasonal changes in foraging behavior, and/or small spatial scale differences in feeding habits.

The only previous study in the literature that has investigated turnover rates in marine mammal skin was done with manatees (Alves-Stanley and Worthy, 2009). This study investigated half-lives of carbon and nitrogen in the skin of both coastal (marine environment) and riverine (freshwater environment) manatees. Coastal manatees had a mean carbon half-life of 53 days and nitrogen half-life of 27 days, while riverine manatees had a half-life for carbon of 59 days and nitrogen of 58 days. Turnover rates may be slower in manatees than in bottlenose dolphins, due to the lower metabolic rates of manatees (Irvine, 1983). Martínez del Rio et al. (Martínez del Rio et al., 2009) cautioned that incorporation rates cannot be compared among animals of differing body sizes without risking integrating large error into the interpretation of results, and this may be a concern in extrapolating our data to larger cetaceans.

Stable isotope analysis has become increasingly popular in ecological studies to trace movement, analyze habitat usage, compare trophic levels of organisms and recreate food webs. Gannes and colleagues (Gannes et al., 1997) predicted an ‘explosion’ in the usage of stable isotopes and argued that controlled studies are needed to establish species- and tissue-specific baseline values that can be applied in field studies. Despite the wide-spread application of discrimination values, the literature is still lacking information on these values for most cetacean tissue types and turnover rates are

The Journal of Experimental Biology (2014) doi:10.1242/jeb.093963

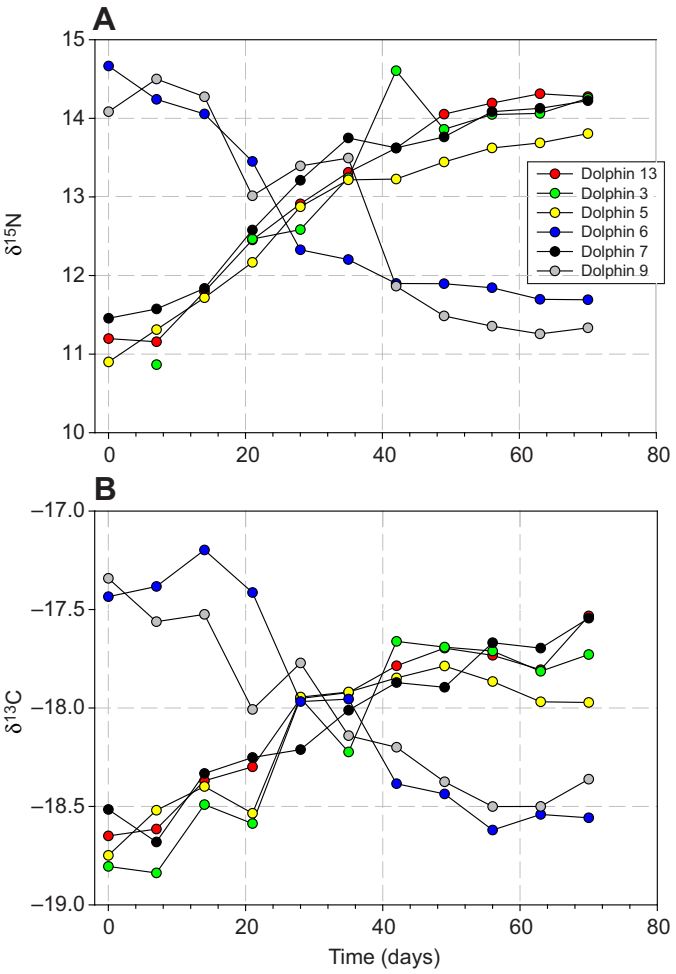

Fig. 3. Nitrogen (δ^{15}N, A) and carbon (δ^{13}C, B) isotope values in bottlenose dolphin skin (FGOP) as a function of days after diet switch. Dolphins 3, 5, 7 and 13 were in FGOP-1 and dolphins 6 and 9 were in FGOP-2.

virtually non-existent. Over the past few years, there have been repeated calls for direct measurements of the basic assumptions and parameters involved in stable isotope ecology (e.g. Gannes et al., 1997; Martínez del Rio et al., 2009; Wolf et al., 2009; Wyatt et al., 2010). Tissue-specific discrimination values, especially for skin, allow ecologists to establish trophic levels for organisms and turnover rate data are necessary for interpreting temporal scales. Both of these values are necessary when trying to recreate dietary history and discrimination factors are crucial for use in mixing models (e.g. Phillips and Gregg, 2001; Phillips and Gregg, 2003; Parnell et al., 2010). Indeed, many of these mixing models have been shown to be very sensitive to variation in discrimination factors (i.e. Bond and Diamond, 2011). To date, cetacean researchers have been relying on published values from organisms ranging from pinnipeds to gerbils and birds.

Collectively, our results suggest that prey composition could be a significant factor to consider when applying discrimination factors to field studies. Frequently, in the absence of species-specific data, average discrimination factors (Δ^{15}N ~3.4‰ and Δ^{13}C ~1‰) are applied in interpreting field studies. Our results suggest that while diet–tissue discrimination factors for Δ^{13}C may be ~1‰ in odontocetes, Δ^{15}N may be closer to 2.0‰ and may show significant variability as dietary quality changes on a seasonal basis. Therefore, relying on the frequently cited average discrimination factors from the literature might lead to misinterpretation of delphinid isotope data.

Application of stable isotopes as a tool for interpreting temporal changes in diet as a result of seasonal changes in prey composition, changes in prey availability or changes in habitat utilization by the consumer requires an understanding of turnover rate and retention time. Retention times ranging from 20 to 32 days suggest that the isotopic composition of dolphin skin is representative of short-term recent feeding history, thereby giving investigators the opportunity to explore both recent feeding history, through various mixing models (e.g. Parnell et al., 2010), and/or changes in residency patterns of the dolphins. Barros et al. (Barros et al., 2010) and Gibbs et al. (Gibbs et al., 2011) have distinguished populations of coastal/bay resident dolphins from offshore populations using stable isotopes. Recently, Wilson and colleagues (Wilson et al., 2012) examined dolphins living in three Florida Bay systems and distinguished distinct residency patterns consistent with sighting and tracking data (Balmer et al., 2008). Assessment of isotopic values over a wider geographic scale could ultimately resolve questions relating to population structure and, ultimately, allow for a better understanding of the roles of dolphins as apex predators.

MATERIALS AND METHODS

Diet

Dietary samples of fish fed to dolphins were collected at both Sea World of Florida (SWF), USA, and Far Glory Ocean Park (FGOP), Taiwan, to assess dietary isotopic composition and serve as a comparison with isotopic composition of skin for calculation of diet–tissue discrimination, turnover rate and retention time. Whole frozen individual herring (*Clupea harengus*, *N*=15), capelin (*Mallotus villosus*, *N*=17) and night smelt (*Spirinchus starksi*, *N*=20) were obtained from SWF and processed at the University of Central Florida (UCF). Mass and standard lengths were obtained and then individual fish were homogenized in a blender. Ground fish were then freeze dried individually and stored in plastic Whirl-Pak bags until further processing.

Whole frozen Pacific saury (*Cololabis saira*, *N*=14), Atlantic mackerel (*Scomber scombrus*, *N*=14), capelin (*Mallotus villosus*, *N*=14) and horse mackerel (*Trachurus japonicus*, *N*=14) were obtained and individually processed from FGOP. Preliminary processing of FGOP fish samples occurred at National Sun Yat-Sen University, Kaohsiung, Taiwan. Standard length and body mass were obtained for intact frozen fish, which were then individually ground into a homogeneous sample using blenders. Homogenized samples were then freeze dried and stored in Whirl-Pak bags for shipment to National Taiwan University, Taipei, Taiwan, for further processing and isotopic analyses (see below).

Proximate composition analyses of fish was undertaken to assess potential impacts of dietary quality. The water content of each individually homogenized fish was determined gravimetrically by drying in a lyophilizer (LabConco, Kansas City, MO, USA) for 96 h. Gross lipid content was determined gravimetrically by extraction with petroleum ether in a Soxhlet extractor for 24 h.

Skin collection

To determine stable isotope values of skin from dolphins held on a known diet, a total of 27 individuals were sampled at SWF. SWF samples were only used in determining diet–tissue discrimination factors. Skin samples were collected by veterinarians during routine health and wellness exams. The first group (SWF-1) were all adult males (*N*=8), while the second group (SWF-2) consisted of adult males (*N*=9), adult females (*N*=3), pregnant females (*N*=2) and juveniles (defined as nutritionally independent animals that had not reached asymptotic length) (*N*=4 females, *N*=1 male). All dolphins (SWF-1 and SWF-2) were fed a mixture of herring and capelin at consistent proportions for a period of 2 months prior to sample collection. SWF-2 animals, however, also received a portion of their diet (capelin and

night smelt) during controlled feeding sessions with the general public. Amounts consumed were estimated by animal care staff, who managed the public interactions.

Small samples of superficial epidermis (~20 mg) were collected from each dolphin at SWF on a single occasion using 7 mm diameter disposable dermal curettes (Miltex, Plainsboro, NJ, USA). The curette was dragged across the epidermal surface, which had been towel dried, with sloughing and intact epidermis being collected in the loop of the curette. The sample was transferred to plastic microcentrifuge vials (Novatech, model D1010; Boston, MA, USA) and kept at −20°C until further processing.

Adult female bottlenose dolphins at FGOP were divided into two groups (FGOP-1, N=4; FGOP-2, N=2) each of which underwent two diet shifts. The initial period (duration 8 weeks) served to establish an isotopically known baseline diet and end-point skin samples were used to calculate diet–tissue discrimination; the second diet (duration 9 weeks) was utilized to calculate both isotope turnover rates and an additional value for diet–tissue discrimination (see Table 2 for bulk isotope values for individual diets). Small samples of skin (ca. 20 mg) were collected weekly using the same approach as above and kept at −20°C until further processing.

Prior to the start of the current study, FGOP dolphins were fed a mixture of four species of fish and two species of squid in varying amounts. Based on the availability of prey items and stable isotope values for each item, two isotopically distinct diets, each consisting of two species of fish, were derived from food items that dolphins were already consuming. To develop these two experimental diets, samples of whole prey items were taken and analyzed to determine isotope values. Diet A consisted of 80% (by mass) Pacific saury and 20% Atlantic mackerel, while diet B consisted of 80% capelin and 20% horse mackerel. FGOP-1 dolphins were fed diet A for 8 weeks to establish a baseline isotope value and then switched to diet B for 9 weeks. Assessment of turnover time and diet–tissue discrimination factors started the day they commenced consuming diet B. FGOP-2 dolphins were initially offered diet B for 8 weeks to establish a baseline and then transitioned to diet A for 9 weeks. Assessment of turnover time and diet–tissue discrimination factors started on the first day of consuming diet A. Skin samples were collected weekly from each animal for 2 weeks prior to the start of the study, throughout the duration of transitioning animals onto the first isotopically known diet, and then for the duration of the shift to the second diet. There were five occasions when samples were not collected from individual dolphins due to the dolphins being uncooperative. Dolphin skin samples were sent to National Taiwan University for sample preparation and isotopic analysis (see below).

Stable isotope analysis

Dolphin skin samples were placed in a drying oven at 60°C for 24 h. Skin samples and freeze-dried ground fish (see above) were then wrapped in glass microfilter paper (Whatman, GF/A), placed in cellulose thimbles (Advantec, grade 84), and lipid extracted for 24 h using petroleum ether in a Soxhlet extractor. Samples were then placed in a drying oven at 60°C for 24 h to remove any remaining solvent. Samples were re-ground into a fine powder using a Wig-L-Bug amalgamator (Dentsply, model MSD; York, PA, USA). Aliquots of skin and fish (0.9–1.5 mg) sampled in Taiwan were sealed into 6 mm×4 mm tin capsules (Elemental Microanalysis, model D1006. Okehampton, UK) and analyzed for carbon and nitrogen stable isotopes using a stable isotope ratio mass spectrometer at National Taiwan University (Thermo Scientific Delta V Advantage isotope-ratio mass spectrometry, IRMS; Waltham, MA, USA). Aliquots of fish and skin samples collected in the USA (0.9–1.5 mg) were sealed into 5 mm×9 mm tin capsules and analyzed by IRMS at the University of Georgia (Finnigan MAT Delta Plus XL; Bremen, Germany). Available sample mass was too small for detection in the case of six FGOP skin samples. Data are expressed in delta notation (δ; ‰):

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

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 Vienna PeeDee Belemnite (V-PDB) and atmospheric nitrogen gas. To assess quality control in sample analysis of stable isotope ratios, a known standard sample (fish muscle at National Taiwan University and bovine tissue at the University of Georgia) was run periodically after unknown samples. Analytical errors for the standard samples (N=38) were ±0.06‰ (mean ± s.d.) for $\delta^{13}C$ and $\delta^{15}N$ at National Taiwan University and ±0.01‰ for $\delta^{13}C$ and $\delta^{15}N$ when running the University of Georgia standard samples (N=10).

Diet–tissue discrimination values for each isotope were calculated by assessing the difference in delta values between FGOP dolphin skin (y) (on days 0 and 63) and the fish diet (w) using the $\Delta_{\text{tissue–diet}}$ notation (Caut et al., 2011):

$$\Delta_{\text{tissue–diet}} = \delta_y - \delta_w . \quad (2)$$

Turnover rates of carbon and nitrogen in FGOP dolphin skin were calculated using the exponential model used by Ogden et al. (Ogden et al., 2004):

$$Y(t) = y_a + ae^{-bt} , \quad (3)$$

in which $Y(t)$ is the $\delta^{13}C$ or $\delta^{15}N$ value of dolphin skin at time t, y_a is the value approached asymptotically, a is the absolute change in value after the diet switch, b is the turnover rate of carbon or nitrogen in dolphin skin, and t is time (days) since the diet switch. Exponential decay curves were fitted with y_a set to the expected skin isotopic value based on diet and the individually calculated diet-specific value for both $\Delta^{15}N$ and $\Delta^{13}C$. Turnover rate was expressed in terms of half-life, the time (b) it takes for the isotopic composition of the skin to reach halfway between the initial and final values:

$$\frac{-\ln(0.5)}{b} . \quad (4)$$

Retention time (T; the time required for an isotope in a consumer tissue to be replaced by isotopes from a new source) was calculated as the inverse of b.

Statistical analyses

Stable isotope data were tested for normality using Shapiro–Wilks' test and tested for homogeneity of variance using Levene's test. Differences in stable isotope values for fish species were explored for $\delta^{15}N$ and $\delta^{13}C$ using GLM multivariate analysis. Tukey's HSD *post hoc* tests were performed to determine homogeneous subsets. All statistical analyses were conducted using SPSS 20.0 with a critical value of α=0.05 and plotted using SigmaPlot (Version 10.0, Systat Software). Values reported are means ± s.e.

Acknowledgements

We especially thank all of the Animal Care and Veterinary staff of both SeaWorld of Florida and Far Glory Ocean Park for their hard work in caring for the dolphins and collecting all of the fish and dolphin skin samples. Many thanks go to Dr Yen-Tsun Yu, National Ilan University, who assisted in preparation of fish samples from Far Glory Ocean Park and to Dr Meng-Hsien Chen, and her students at National Sun Yat-Sen University, for their assistance in preparing dietary samples from FGOP for analysis. We thank Dr Lien-Siang Chou of National Taiwan University, and especially her graduate student, Florence Evacitas, for their help with all of the FGOP isotope analysis. This research was conducted under UCF-IACUC protocol number 10-35W.

Competing interests

The authors declare no competing financial interests.

Author contributions

This work represents a component of the doctoral dissertation of N.E.B. All authors were involved in concept development and data collection/analyses as well as preparing and editing the manuscript prior to submission.

Funding

This research was funded through an award from the Ocean Park Hong Kong Conservation Fund [grant number MM01-1011 to G.A.J.W.] and a Research Enhancement Award, Office of the Provost, University of Central Florida (to G.A.J.W.).

References

Alves-Stanley, C. D. and Worthy, G. A. J. (2009). Carbon and nitrogen stable isotope turnover rates and diet–tissue discrimination in Florida manatees (*Trichechus manatus latirostris*). *J. Exp. Biol.* **212**, 2349-2355.

Ames, A. L., VanVleet, E. S. and Sackett, W. M. (1996). The use of stable carbon isotope analysis for determining the dietary habits of the Florida manatee, *Trichechus manatus latirostris*. *Mar. Mamm. Sci.* **12**, 555-563.
Balmer, B., Wells, R. S., Nowacek, S., Nowacek, D., Schwacke, L., McLellan, W. A., Scharf, F. S., Rowles, T. K., Hansen, L. J., Spradlin, T. R. et al. (2008). Seasonal abundance and distribution patterns of common bottlenose dolphins (*Tursiops truncatus*) near St. Joseph Bay, Florida, USA. *J. Cetacean Res. Manage.* **10**, 157-167.
Barros, N. B., Ostrom, P. H., Stricker, C. A. and Wells, R. S. (2010). Stable isotopes differentiate bottlenose dolphins off west-central Florida. *Mar. Mamm. Sci.* **26**, 324-336.
Bond, A. L. and Diamond, A. W. (2011). Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. *Ecol. Appl.* **21**, 1017-1023.
Borrell, A., Abad-Oliva, N., Gómez-Campos, E., Giménez, J. and Aguilar, A. (2012). Discrimination of stable isotopes in fin whale tissues and application to diet assessment in cetaceans. *Rapid Commun. Mass Spectrom.* **26**, 1596-1602.
Bosley, K. L., Witting, D. A., Chambers, R. C. and Wainright, S. C. (2002). Estimating turnover rates of carbon and nitrogen in recently metamorphosed winter flounder *Pseudopleuronectes americanus* with stable isotopes. *Mar. Ecol. Prog. Ser.* **236**, 233-240.
Caut, S., Laran, S., Garcia-Hartmann, E. and Das, K. (2011). Stable isotopes of captive cetaceans (killer whales and bottlenose dolphins). *J. Exp. Biol.* **214**, 538-545.
Das, K., Lepoint, G., Loizeau, V., Debacker, V., Dauby, P. and Bouquegneau, J. M. (2000). Tuna and dolphin associations in the North-east Atlantic: evidence of different ecological niches from stable isotope and heavy metal measurements. *Mar. Poll. Bull.* **40**,102-109.
DeMots, R. L., Novak, J. M., Gaines, K. F., Gregor, A. J., Romanek, C. S. and Soluk, D. A. (2010). Tissue-diet discrimination factors and turnover of stable carbon and nitrogen isotopes in white-footed mice (*Peromyscus leucopus*). *Can. J. Zool.* **88**, 961-967.
DeNiro, M. J. and Epstein, S. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* **42**, 495-506.
DeNiro, M. J. and Epstein, S. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim. Cosmochim. Acta* **45**, 341-351.
Florin, S. T., Felicetti, L. A. and Robbins, C. T. (2011). The biological basis for understanding and predicting dietary-induced variation in nitrogen and sulphur isotope ratio discrimination. *Funct. Ecol.* **25**, 519-526.
Gannes, L. Z., O'Brian, D. and Martínez del Rio, C. (1997). Stable isotopes in animal ecology: assumptions, caveats, and a call for laboratory experiments. *Ecology* **78**, 1271-1276.
Herman, D. P., Burrows, D. G., Wade, P. R., Durban, J. W., Matkin, C. O., LeDuc, R. G., Barrett-Lennard, L. G. and Krahn, M. M. (2005). Feeding ecology of eastern North Pacific killer whales *Orcinus orca* from fatty acid, stable isotope, and organochlorine analyses of blubber biopsies. *Mar. Ecol. Progr. Ser.* **302**, 275-291.
Hilderbrand, G. V., Farley, S. D., Robbins, C. T., Hanley, T. A., Titus, K. and Servheen, C. (1996). Use of stable isotopes to determine diets of living and extinct bears. *Can. J. Zool.* **74**, 2080-2088.
Hobson, K. A. and Clark, R. G. (1992). Assessing avian diets using stable isotopes. I. Turnover of ^{13}C in tissues. *Condor* **94**, 181-188.
Hobson, K. A., Piatt, J. F. and Pitocchelli, J. (1994). Using stable isotopes to determine seabird trophic relationships. *J. Anim. Ecol.* **63**, 786-798.
Hobson, K. A., Schell, D. M., Renouf, D. and Noseworthy, E. (1996). Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. *Can. J. Fish. Aquat. Sci.* **53**, 528-533.
Howland, M. R., Corr, L. T., Young, S. M. M., Jones, V., Jim, S., van der Merwe, N. J., Mitchell A. D. and Evershed, R. P. (2003). Expression of the dietary isotope signal in the compound-specific δ13C values of pig bone lipids and amino acids. *Int. J. Osteoarchaeol.* **13**, 54-65.
Irvine, A. B. (1983). Manatee metabolism and its influence on distribution in Florida. *Biol. Conserv.* **25**, 315-334.
Kelly, J. F. (2000). Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Can. J. Zool.* **78**, 1-27.
Kurle, C. M. and Worthy, G. A. J. (2002). Stable nitrogen and carbon isotope ratios in multiple tissues of the northern fur seal *Callorhinus ursinus*: implications for dietary and migratory reconstructions. *Mar. Ecol. Prog. Ser.* **236**, 289-300.
Lee, S. H., Schell, D. M., McDonald, T. L. and Richardson, W. J. (2005). Regional and seasonal feeding by bowhead whales *Balaena mysticetus* as indicated by stable isotope ratios. *Mar. Ecol. Prog. Ser.* **285**, 271-287.
Lobley, G. E. (2003). Protein turnover – what does it mean for animal production. *Can. J. Anim. Sci.* **83**, 327-340.
Marcoux, M., Whitehead, H. and Rendell, L. (2007). Sperm whale feeding variation by location, year, social group and clan: evidence from stable isotopes. *Mar. Ecol. Prog. Ser.* **333**, 309-314.
Martínez del Rio, C. M., Wolf, N., Carleton, S. A. and Gannes, L. Z. (2009). Isotopic ecology ten years after a call for more laboratory experiments. *Biol. Rev. Camb. Philos. Soc.* **84**, 91-111.
Minagawa, M. and Wada, E. (1984). Stepwise enrichment of $\delta^{15}N$ along food chains: further evidence and the relation between ^{15}N and animal age. *Geochim. Cosmochim. Acta* **48**, 1135-1140.
Mirón L. L., Herrera L. G., Ramírez N. and Hobson, K. A. (2006). Effect of diet quality on carbon and nitrogen turnover and isotopic discrimination in blood of a New World nectarivorous bat. *J. Exp. Biol.* **209**, 541-548.
Mitani, Y., Bando, T., Takai, N. and Sakamoto, W. (2006). Patterns of stable carbon and nitrogen isotopes in the baleen of common minke whale Balaenoptera acutorostrata from the western North Pacific. *Fish. Sci.* **72**, 69-76.
Newsome, S. D., Koch, P. L., Etnier, M. A. and Aurioles-Gambao, D. (2006). Using carbon and nitrogen isotope values to investigate maternal strategies in northeast Pacific otariids. *Mar. Mamm. Sci.* **22**, 556-572.
Newsome, S. D., Clementz, M. T. and Koch, P. L. (2010). Using stable isotope biogeochemistry to study marine mammal ecology. *Mar. Mamm. Sci.* **26**, 509-572.
Ogden, L. J. E., Hobson, K. A. and Lank, D. B. (2004). Blood isotopic ($\delta^{13}C$ and $\delta^{15}N$) turnover and diet–tissue fractionation factors in captive dunlin (*Calidris alpina pacifica*). *Auk* **121**, 170-177.
Parnell, A. C., Inger, R., Bearhop, S. and Jackson, A. L. (2010). Source partitioning using stable isotopes: coping with too much variation. *PLoS ONE* **5**, e9672. (doi:10.1371/journal.pone.0009672).
Pearson, S. F., Levey, D. J., Greenberg, C. H. and Martínez Del Rio, C. (2003). Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. *Oecologia* **135**, 516-523.
Peterson, B. J. and Fry, B. (1987). Stable isotopes in ecosystem studies. *Annu. Rev. Ecol. Syst.* **18**, 293-320.
Phillips, D. L. and Gregg, J. W. (2001). Uncertainty in source partitioning using stable isotopes. *Oecologia* **127**, 171-179.
Phillips, D. L. and Gregg, J. W. (2003). Source partitioning using stable isotopes: coping with too many sources. *Oecologia* **136**, 261-269.
Ramsay, M. A. and Hobson, K. A. (1991). Polar bears make little use of terrestrial food webs: evidence from stable-carbon isotope analysis. *Oecologia* **86**, 598-600.
Rau, G. H., Mearns, A. J., Young, D. R., Olson, R. J., Schafer, H. A. and Kaplan, I. R. (1983). Animal $^{13}C/^{12}C$ correlates with trophic level in pelagic food webs. *Ecology* **64**, 1314-1318.
Reich, K. J. and Worthy, G. A. J. (2006). An isotopic assessment of the feeding habits of free-ranging manatees. *Mar. Ecol. Prog. Ser.* **322**, 303-309.
Robbins, C. T., Felicetti, L. A. and Florin, S. T. (2010). The impact of protein quality on stable nitrogen isotope ratio discrimination and assimilated diet estimation. *Oecologia* **162**, 571-579.
Roth, J. D. and Hobson, K. A. (2000). Stable carbon and nitrogen isotopic fractionation between diet and tissue of captive red fox: implications for dietary reconstruction. *Can. J. Zool.* **78**, 848-852.
Seminoff, J. A., Jones, T. T., Eguchi, T., Jones, D. R. and Dutton, P. H. (2006). Stable isotope discrimination ($\delta^{13}C$ and $\delta^{15}N$) between soft tissues of the green sea turtle Chelonia mydas and its diet. *Mar. Ecol. Prog. Ser.* **308**, 271-278.
Sponheimer, M., Robinson, T. F., Cerling, T. E., Tegland, L., Roeder, B. L., Ayliffe, L., Dearing, M. D. and Ehleringer, J. R. (2006). Turnover of stable carbon isotopes in the muscle, liver, and breath CO_2 of alpacas (*Lama pacos*). *Rapid Commun. Mass Spectrom.* **20**, 1395-1399.
Tieszen, L. L., Boutton, T. W., Tesdahl, K. G. and Slade, N. A. (1983). Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. *Oecologia* **57**, 32-37.
Voigt, C. C., Matt, F., Michener, R. and Kunz, T. H. (2003). Low turnover rates of carbon isotopes in tissues of two nectar-feeding bat species. *J. Exp. Biol.* **206**, 1419-1427.
Walker, J. L., Potter, C. W. and Macko, S. A. (1999). The diets of modern and historic bottlenose dolphin populations reflected through stable isotopes. *Mar. Mamm. Sci.* **15**, 335-350.
Weller, D. W., Cockroft, V. G., Würsig, B., Lynn, S. K. and Fertl, D. (1997). Behavioral responses of bottlenose dolphins to remote biopsy sampling and observations of surgical biopsy wound healing. *Aquatic Mammals* **23**, 49-58.
Wilson, R. M., Kucklick, J. R., Balmer, B. C., Wells, R. S., Chanton, J. P. and Nowacek, D. P. (2012). Spatial distribution of bottlenose dolphins (*Tursiops truncatus*) inferred from stable isotopes and priority organic pollutants. *Sci. Total Environ.* **425**, 223-230.
Witteveen, B. H., Worthy, G. A. J., Wynne, K. M., Hirons, A. C., Andrews, A. G. and Markel, R. W. (2011). Trophic levels of North Pacific humpback whales (*Megaptera novaeangliae*) through analysis of stable isotopes: implications on prey and resource quality. *Aquatic Mammals* **37**, 101-110.
Wolf, N., Carleton, S. A. and Martínez del Rio, C. (2009). Ten years of experimental animal isotopic ecology. *Funct. Ecol.* **23**, 17-26.
Wyatt, A. S. J., Waite, A. M. and Humphries, S. (2010). Variability in isotope discrimination factors in coral reef fishes: implications for diet and food web reconstruction. *PLoS ONE* **5**, e13682.
Yamamuro, M., Aketa, K. and Uchida, S. (2004). Carbon and nitrogen stable isotope ratios of the tissues and gut contents of a dugong from the temperate coast of Japan. *Mammal Study* **29**, 179-183.
Zhao, L. and Schell, D. M. (2004). Stable isotope ratios in harbor seal *Phoca vitulina vibrissae*: effects of growth patterns on ecological records. *Mar. Ecol. Prog. Ser.* **281**, 267-273.
Zhao, L. Y., Schell, D. M. and Castellini, M. A. (2006). Dietary macronutrients influence 13C and 15N signatures of pinnipeds: captive feeding studies with harbor seals (*Phoca vitulina*). *Comp. Biochem. Physiol.* **143A**, 469-478.