Journal of Experimental Marine Biology and Ecology 475 (2016) 54–61

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

From the pool to the sea: Applicable isotope turnover rates and diet to skin discrimination factors for bottlenose dolphins (*Tursiops truncatus*)

Joan Giménez [a,*], Francisco Ramírez [a], Javier Almunia [b], Manuela G. Forero [a], Renaud de Stephanis [a,c]

[a] Department of Conservation Biology, Estación Biológica de Doñana (EBD—CSIC), Américo Vespucio s/n, Isla de la Cartuja, 42092, Sevilla, Spain
[b] Loro Parque Fundation, Avda. Loro Parque, s/n, 38400 Puerto de la Cruz, Tenerife, Spain
[c] Conservation, Information and Research on Cetaceans (CIRCE), Cabeza de Manzaneda 3, Algeciras-Pelayo, 11390 Cádiz, Spain

ARTICLE INFO

Article history:
Received 7 January 2015
Received in revised form 3 July 2015
Accepted 3 November 2015
Available online 6 December 2015

Keywords:
Stable isotopes
Isotope turnover rates
Diet-tissue discrimination factor
Bottlenose dolphins
Tursiops truncatus

ABSTRACT

One of the most common applications in isotopic ecology is the assessment of animal's assimilated diet through mass-balance mixing models. Its applicability relies on the use of accurate diet to tissue discrimination factors and turnover rates, which are known to vary as a function of several factors including taxon or tissue type. To date, few studies have assessed isotopic discrimination factors and turnover rates in cetacean species under controlled conditions. Previous experimental studies focused on blood, a difficult sample to obtain in the wild, or on a more appropriate tissue, the skin, but assessed in short experimental trials without arriving to the isotopic equilibrium. We carried out the longest controlled feeding experiment available (350 days) in bottlenose dolphins (*Tursiops truncatus*) in order to assess discrimination factors and turnover rates in skin. Animals' isotopic composition was first stabilized by maintaining individuals under an isotopically constant diet during 172 days. Afterwards, diet was shifted and maintained during 178 days to calculate isotopic discrimination and turnover rates. Estimates for isotopic discrimination factors were 1.01 ± 0.37‰ (mean ± sd) for $\delta^{13}C$ and 1.57 ± 0.52‰ for $\delta^{15}N$. Half-life turnover rates were estimated to be 24.16 ± 8.19 days for carbon and 47.63 ± 19 days for nitrogen. This is the first time that applicable values are available to assess the diet of free ranging small cetaceans through stable isotope mixing model analysis.

1. Introduction

Marine top predators inhabit one of the most inaccessible and hardest environments to perform ecological research, thus information on their trophic ecology is scarce and lacks in reliability. Predation events are rarely observed and stomach contents are only available for stranded or bycaught animals (e.g. Santos et al., 2001), thus likely providing unrealistic or biased information on feeding preferences. Within this scenario, stable isotope analysis has emerged as a suitable alternative to conventional approaches to reconstruct individuals and populations' diet through mass-balance mixing models (e.g. Fernández et al., 2011; Mèndez-Fernandez et al., 2012; Kiszka et al., 2014).

The reliability of dietary assessments thought isotopic approach relies on the use of accurate diet to tissue discrimination factors and turnover rates (Phillips et al., 2014). Indeed, the use of inappropriate isotopic discrimination factors has been recognized as one of the biggest sources of uncertainty in using mixing models to assess diet (Gannes et al., 1997; Phillips et al., 2014; Wolf et al., 2009). Traditionally, it was assumed that isotopic discrimination factors linking diet and consumer tissues were ca. +1‰ for carbon-13 (DeNiro and Epstein, 1978) and +3‰ for nitrogen-15 (DeNiro and Epstein, 1981). However, recent research showed considerable variation in isotopic discrimination factors as a function of various extrinsic (e.g. diet quality or composition (McCutchan et al., 2003; Robbins et al., 2005) and intrinsic factors (e.g. taxa, (Caut et al., 2009; Vanderklift and Ponsard, 2003); or age (Hobson and Quirk, 2014; Minagawa and Wada, 1984)). Taxon and tissue-specific isotopic discrimination factors obtained under experimental trials or studies of wild populations where their diets are well-known are likely to produce the most accurate dietary estimates (Bond and Diamond, 2011; Caut et al., 2008; Gannes et al., 1997; McCutchan et al., 2003; Pilot et al., 2012; Post, 2002; Spence and Rosenheim, 2005).

On the other hand, an accurate knowledge on isotopic turnover rates is mandatory to determine the time window in which researchers can perceive the course of dietary changes (Dalerum and Angerbjörn, 2005). As for isotopic discrimination, turnover rates may vary according to taxa (Boecklen et al., 2011) or tissue type (MacNeil et al., 2006). Therefore, accurate and reliable estimates of turnover rates for specific tissues and different taxa are needed to depict the time frame integrated in diet studies (Dalerum and Angerbjörn, 2005; Martínez del Rio et al., 2009).

To date, few studies have assessed isotopic diet to tissue discrimination factors and turnover rates in dolphins under controlled conditions

* Corresponding author.
E-mail addresses: gimenez.verdugo@gmail.com, joan.gimenez@csic.es (J. Giménez).

http://dx.doi.org/10.1016/j.jembe.2015.11.001
0022-0981/

(Browning et al., 2014; Caut et al., 2011). Previous experimental studies focused on blood (Caut et al., 2011), a difficult sample to obtain in the wild, or on a more appropriate tissue, the skin (Browning et al., 2014), but assessed in short experimental trials without reaching the isotopic equilibrium. Although valuable information was provided by these authors, their isotopic discrimination factors and turnover rates should be taken with caution when reconstructing animals' diet through mass-balance mixing models (see Bates and Watts, 1988; Berges et al., 1994; and Martínez del Rio et al., 2009). Accordingly, the call for comparative laboratory experiments repeatedly recommended by several authors (e.g. Gannes et al., 1997; Wolf et al., 2009) is still needed for dolphins in order to properly interpret isotopic data of wild populations, as well as to make inferences on their diets through mass-balance mixing models. Bottlenose dolphins (Montagu, 1821) are a good model to study these parameters because they are among the most widespread of the small cetaceans, occurring in nearly all tropical and temperate seas (Leatherwood and Reeves, 1983) and they adapt well to captivity, being the most common specie in dolphinariums.

In this study, a controlled feeding experiment was conducted during 350 days monitoring carbon and nitrogen stable isotope ratios in bottlenose dolphins, *Tursiops truncatus* skin and their diet. This extensive dataset is the longest available so far for cetaceans. Therefore, applicable diet to skin discrimination factors and turnover rates are provided to be used in future diet studies of this species and probably in taxonomically close species such as other small cetaceans in the wild.

2. Methods

2.1. Experimental design

Six bottlenose dolphins, one male (Paco) and five females (Clara, Pacina, Sanibel, Ruffles, and Luna) were maintained under a controlled diet during 350 days in "Loro Parque" facilities (Tenerife, Spain). The experimental trial was twofold: (1) dolphins were first fed with diet A [i.e. 90% sprat (*Sprattus sprattus*) + 10% herring (*Clupea harengus*)] during 172 days to be confident that the skin of all individuals reached the isotopic equilibrium with their diet, thus establishing a known and stable isotopic baseline; (2) dolphin's diet was then shifted to diet B [i.e. 10% sprat + 90% capelin (*Mallotus villosus*)] during 178 days, allowing us to calculate isotopic discrimination factors and turnover rates. These parameters were only assessed for the second phase, when isotopic differences between skin and diet were larger, thus maximizing the fit of incorporation models. Experimental diets were designed to maximize isotopic differences, while ensuring the energy and nutritional requirements of the dolphins. Diet quantity provided to the dolphins was assessed by the veterinarian and keeper team depending on the physical condition and energy requirements of the dolphins, but respecting the proportions of species given. Health and wellness conditions were monitored to guarantee the best conditions for the dolphins. Indeed, two of them (Sanibel and Ruffles) did not complete the experiment due to weight loss or the rejection of fish items in the established proportion. Therefore, both animals were omitted in further analysis.

Skin samples were collected with a scalpel from the dorsal fin of each individual, which is a common zone to biopsy in the wild. Skin was removed every 14 days along the entire experiment, kept frozen at −20 °C in plastic microcentrifuge vials, and sent to the laboratory for stable isotope ratio measurements. Isotopic analyses were performed concurrently with the experiment to ensure that dolphins' diet reached the isotopic equilibrium.

2.2. Stable isotope analysis

Muscle subsamples were obtained from each fish and processed separately from the remaining fish, to test the effect of obtaining turnover rates and isotopic discrimination factor from muscle vs the entire fish (Table 1). Both fish and dolphin skin samples were oven-dried at 60 °C during 48 h and powdered with a mortar and pestle. Two aliquots were extracted from each powdered sample. One aliquot was immediately processed for dual isotopic determinations ($\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$), whereas the other underwent lipid extraction with several rinses of chloroform:methanol (2:1) solution prior to stable isotope ratio measurements ($\delta^{15}N_{del}$ and $\delta^{13}C_{del}$) in order to reduce the isotopic variability due to the differential content of lipids (Logan et al., 2008). Subsamples of powdered materials were weighed to the nearest μg and placed into tin capsules for $\delta^{13}C$ and $\delta^{15}N$ determinations. Isotopic analyses were carried out at the "Laboratorio de Isótopos Estables" of the "Estación Biológica de Doñana" (LIE-EBD, Spain; www.ebd.csic.es/lie/index.html). All samples were combusted at 1020 °C using a continuous flow isotope-ratiomass spectrometry system by means of Flash HT Plus elemental analyzer coupled to a Delta-V Advantage isotope ratio mass spectrometer via a CONFLO IV interface (Thermo Fisher Scientific, Bremen, Germany). The isotopic compositions are reported in the conventional delta (δ) per mil notation (‰), relative to Vienna Pee Dee Belemnite ($\delta^{13}C$) and atmospheric N_2 ($\delta^{15}N$). Replicate assays of standards routinely inserted within the sampling sequence indicated analytical measurement errors of ±0.1‰ and ±0.2‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. The internal standards used were: EBD-23 (cow horn), LIE-BB (whale baleen), and LIE-PA (feathers of razorbill). These laboratory standards were previously calibrated with international standards supplied by the International Atomic Energy Agency (IAEA, Vienna).

2.3. Isotopic discrimination factors and turnover rate

Mean estimates of isotopic discrimination factors, linking consumer tissues and its diet, were expressed in Δ notation as the ‰ difference between the average isotopic composition of dolphin skin on day 350 and that for fish diet. Variability (sd; standard deviation) associated to such isotopic discrimination factors was estimated as the square root of summed variances for skin and fish samples. Isotopic turnover rates are commonly expressed as half-life, which is the time required for an isotope ratio to change halfway from its initial equilibrium value to its new equilibrium value (Boecklen et al., 2011). Here, this parameter was quantified by fitting our data using a non-linear procedure NLS and following the exponential decay curve: $y = a + be^{-ct}$ where y is $\delta^{13}C$ or $\delta^{15}N$, a is the isotopic value approached asymptotically ($\delta X(\infty)$), b is the total change in isotopic values after the diets were switched at t_0 ($\delta X(\infty) - \delta X(t)$), c is the turnover rate, and t is the time in days since the switch. To find the time span required for α% turnover, the following equation was solved: $T = ln(1 - \alpha / 100) / c$; where T is the time in days, α is % turnover, and c is the turnover rate of the tissue

Table 1
Mean $\delta^{13}C$ and $\delta^{15}N$ values (±sd) for the composition of cetacean diets with different treatments (with and without lipids) and only the muscle tissue or the whole fish.

Fish specie	N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
*Capelin (**Mallotus villosus**)*			
Muscle	21	−21.65 ± 0.63	11.11 ± 0.52
Delipidated muscle	21	−20.48 ± 0.34	11.78 ± 0.54
Whole fish	11	−23.34 ± 0.59	10.97 ± 0.48
Delipidated whole fish	14	−20.42 ± 0.58	11.32 ± 0.44
*Sprat (**Sprattus sprattus**)*			
Muscle	39	−19.35 ± 0.55	12.99 ± 0.43
Delipidated muscle	37	−17.99 ± 0.49	13.63 ± 0.47
Whole fish	18	−20.48 ± 0.41	12.57 ± 0.43
Delipidated whole fish	28	−17.70 ± 0.39	13.02 ± 0.43
*Herring (**Clupea harengus**)*			
Muscle	13	−19.17 ± 0.75	11.59 ± 0.45
Delipidated muscle	14	−18.40 ± 0.70	11.97 ± 0.49
Whole fish	9	−20.20 ± 0.92	11.70 ± 0.50
Delipidated whole fish	11	−17.99 ± 0.51	11.94 ± 0.62