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Estimating tissue-specific discrimination factors and turnover rates of stable isotopes of nitrogen and carbon in the smallnose fanskate *Sympterygia bonapartii* (Rajidae)

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This study aimed to estimate trophic discrimination factors (TDFs) and metabolic turnover rates of nitrogen and carbon stable isotopes in blood and muscle of the smallnose fanskate *Sympterygia bonapartii* by feeding six adult individuals, maintained in captivity, with a constant diet for 365 days. TDFs were estimated as the difference between δ^{13}C or δ^{15}N values of the food and the tissues of *S. bonapartii* after they had reached equilibrium with their diet. The duration of the experiment was enough to reach the equilibrium condition in blood for both elements (estimated time to reach 95% of turnover: C $t95\%_{blood} = 150$ days, N $t95\%_{blood} = 290$ days), whilst turnover rates could not be estimated for muscle because of variation among samples. Estimates of Δ^{13}C and Δ^{15}N values in blood and muscle using all individuals were $\Delta^{13}C_{blood} = 1{\cdot}7‰$, $\Delta^{13}C_{muscle} = 1{\cdot}3‰$, $\Delta^{15}N_{blood} = 2{\cdot}5‰$ and $\Delta^{15}N_{muscle} = 1{\cdot}5‰$, but there was evidence of differences of *c.*0·4‰ in the Δ^{13}C values between sexes. The present values for TDFs and turnover rates constitute the first evidence for dietary switching in batoids based on long-term controlled feeding experiments. Overall, the results showed that *S. bonapartii* has relatively low turnover rates and isotopic measurements would not track seasonal movements adequately. The estimated Δ^{13}C values in *S. bonapartii* blood and muscle were similar to previous estimations for elasmobranchs and to generally accepted values in bony fishes ($\Delta^{13}C = 1{\cdot}5‰$). For Δ^{15}N, the results were similar to published reports for blood but smaller than reports for muscle and notably smaller than the typical values used to estimate trophic position (Δ^{15}N *c.* 3·4‰). Thus, trophic position estimations for elasmobranchs based on typical Δ^{15}N values could lead to underestimates of actual trophic positions. Finally, the evidence of differences in TDFs between sexes reveals a need for more targeted research.

Key words: δ^{13}C; δ^{15}N; elasmobranch; fractionation; mixing models; trophic position.

INTRODUCTION

Elasmobranchs are widely affected by human activities such as overfishing (Stevens *et al.*, 2000; Simpfendorfer *et al.*, 2002; Ferreti *et al.*, 2010) and habitat degradation (Lotze *et al.*, 2006), resulting in many populations being depleted and some species considered threatened with extinction (Baum *et al.*, 2003; Dulvy *et al.*, 2014). Because

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of the current conservation status of elasmobranchs and their ability, as top predators, to influence ecosystem functioning and trophic structure (Acevedo-Gutiérrez, 2002; Baum & Worm, 2009), there are important global efforts to improve their management and create effective conservation activities. There is a lack of data, however, on diet, life history and behaviour that compromises these goals (Shiffman *et al.*, 2012). Simpfendorfer *et al.* (2011) analysed critical research needs for conservation of elasmobranchs and listed, among them, gathering information on ontogenetic trophodynamics and dietary habits. Stable isotope analysis (SIA) is an appropriate method to provide accurate estimations of the trophic position (Albo-Puigserver *et al.*, 2015) and about dietary contributions of the main prey items (Navarro et al., 2014) using small sample sizes (Galván *et al.*, 2010). It is a cost-effective tool (Shiffman *et al.*, 2012) that was successfully used to study rare species (Barría *et al.*, 2015). In addition, robust results can be obtained with small muscle biopsies and blood extraction, without sacrificing fishes, which is desirable for most species (Huntingford *et al.*, 2006), but particularly for endangered groups. The usefulness of SIA, however, is constrained by specific knowledge about isotopic trophic discrimination factors (TDFs) and turnover times in fishes (Gannes *et al.*, 1997), particularly in elasmobranchs (Shiffman *et al.*, 2012), principally due to the limited number of controlled dietary experiments (Martínez del Rio *et al.*, 2009).

The analysis of carbon and nitrogen stable isotope ratios has increased exponentially since 2000 in trophic ecology (Boecklen *et al.*, 2011), because $\delta^{13}C$ or $\delta^{15}N$ values are powerful ecological tracers to elucidate features of food webs (Vander Zanden *et al.*, 1999; Pinnegar & Polunin, 2000), to estimate consumer trophic position (Post, 2002), to asses diet composition (Phillips & Gregg, 2003; Parnell *et al.*, 2010) and to track animal movements (Rubestein & Hobson, 2004). This indirect approach to studying animal diet is based on the assumption that the stable isotope ratios of consumer tissues are similar to those of dominant food sources after accounting for TDF (DeNiro & Epstein, 1976). TDF values, however, can vary among taxa, tissues of the same species and even within the same species and type of tissue because of diet quality or isotopic composition (Vander Zanden & Rasmussen, 2001; Vanderklift & Ponsard, 2003; Caut *et al.*, 2009; Hussey *et al.*, 2014). In addition, if the diet of an individual changes over time, the isotopic composition of specific tissues would integrate different periods because of differences in their turnover rates (Pinnegar & Polunin, 1999). The differences in the isotopic composition among tissues are a powerful tool to infer movement patterns and home range shifts by comparing tissues that integrate the stable isotope composition of the food items at different rates (Rodgers & Wing, 2008; Heady & Moore, 2014). Thus, estimating the tissue-specific TDFs and turnover rates is of great importance to food web studies.

Estimations of $\Delta^{13}C$ and $\Delta^{15}N$ values are scarce for elasmobranchs and the results are highly variable (Table I). For example, estimations of $\Delta^{15}N$ in muscle, the most common tissue in SIA, range between $-1{\cdot}8$ and $3{\cdot}7$‰ and for $\Delta^{13}C$ range between $0{\cdot}4$ and $3{\cdot}2$‰ (Table I). Those values of TDFs vary between currently accepted estimations for aquatic organisms and bony fishes, with $\Delta^{15}N = 3–3{\cdot}4$‰ (Vander Zanden & Rasmussen, 2001; Sweeting *et al.*, 2007*a*) and $\Delta^{13}C = 1–1{\cdot}5$‰ (Vander Zanden & Rasmussen, 2001; Sweeting *et al.*, 2007*b*), but can be profoundly different. Such magnitude of variation could lead to dramatic changes in diet assessment (Caut *et al.*, 2008; Galván *et al.*, 2012) or estimates of trophic position (Post, 2002). Although some multisource mass-balance mixing models that are used to estimate dietary proportions

can formally incorporate uncertainty in the discrimination values (Moore & Semmens, 2008; Parnell *et al.*, 2010), researchers need accurate estimations to make informed choices about what TDF values to input into the models. Thus, the goal of this study was to obtain estimations of Δ^{15}N and Δ^{13}C values and turnover rates of δ^{15}N and δ^{13}C values in two tissues, muscle and blood, of the smallnose fanskate *Sympterygia bonapartii* Müller & Henle 1841, conducting a long-term experiment. This is the first attempt to estimate TDFs for batoids under controlled conditions.

MATERIALS AND METHODS

CONTROL FEEDING EXPERIMENT

The experiment was conducted in the Bioparque Temaiken aquarium (www.temaiken.org.ar). The aquarium provides a warm-temperate climate; water temperature varied between 16 and 19° C, a 12L:12D photoperiod was fixed all year around and salinity was *c*. 36. For the present experiment, six adult *S. bonapartii* were used, three males and three females, that were maintained at the internal facilities of the aquarium isolated in a circular 85001 3 m diameter tank. All individuals were born and grown in captivity in the aquarium (Jañez & Sueiro, 2007) and had never been part of the exhibition tanks. At the beginning of the experiment the individuals were between 4 and 5 years old with total lengths (L_T) ranging from 70 to 58 cm (Table II). Up to the beginning of the experiment they were fed with *Engraulis anchoita* Hubbs & Marini 1935, *Scomber japonicus* Houttuyn 1782, *Pogonias cromis* (L. 1766), *Parona signata* (Jenyns 1841), *Mullus argentinae* Hubbs & Marini 1933, *Percophis brasiliensis* Quoy & Gaimard 1835 and *Illex argentinus*, offering more than one item in each feeding in variable quantities. From 17 November 2011 to 16 November 2012, the six *S. bonapartii* were fed only with fillets of *P. brasiliensis* taken from a single lot. The aquarium staff fed the individuals by offering 25 g of food every day to each animal and, after checking that all the individuals had eaten, they removed the remaining food from the tanks. *Percophis brasiliensis* was selected because it has low lipid content, presumably a higher isotopic composition given its piscivorous diet and because the aquarium staff, to preserve animal welfare, did not agree to a major diet switch to a completely new food source that would have ensured a large shift in dietary isotopic composition. On the other hand, the limited dietary change resembles a level of change that would be expected to occur in the wild.

SAMPLING AND STABLE ISOTOPE ANALYSIS

Samples of blood and muscle were taken from each individual at the beginning of the experiment (day 1, 17 November 2011). Then, blood was sampled after 15, 60, 150 and 360 days from the beginning and muscle was sampled after 60, 150 and 360 days. During sampling, each individual was taken from the tank and placed in a plastic tray, weighed and measured. Then, 1 ml of blood was drawn from the caudal vein and a biopsy was taken from the dorsal region, separating dermis and epidermis from the muscle. All samples were placed in individual vials and frozen at −20° C. Twelve pieces of *c*. 1 cm^3 of *P. brasiliensis* fillet were randomly sampled from the frozen lot throughout the experiment and kept individually separated at −20° C. Whole blood and muscle samples were dried at 60° C for 2 days or more until a constant mass, then ground to a powder. Neither urea nor lipids were extracted from samples given the evidence that δ^{15}N values of muscle and blood are not affected by tissue urea content (Logan & Lutcavage, 2010) and given the low lipid content (*i.e.* C:N < 3·5; Post *et al.*, 2007) of elasmobranch tissues. In order to estimate analytical precision, six samples of each tissue were analysed in duplicate. Stable isotope analysis was performed by a mass spectrometer in the stable isotope facility of the University of California Davis. The stable isotope ratios were expressed as δ values as ‰: $\delta x = 10^3 \left(R_{\text{sample}} - R_{\text{standard}}\right) R_{\text{standard}}^{-1}$, where x is ^{13}C or ^{15}N and R is the corresponding ratio ^{13}C:^{12}C or ^{15}N:^{14}N. The standards used were Vienna Peedee belemnite for δ^{13}C and atmospheric

TABLE I. List of published estimates of trophic discrimination factors (TDF) and isotopic turnover rates in elasmobranchs kept under controlled or semi-controlled conditions indicating published reference, type of experiment and duration (number of days), main objective of each study, marine (M) or freshwater (F) environment, common and scientific names of the species used in each experiment [with number of individuals (*n*) in parentheses], life stage of the specimens used [length range (*L*, cm) in parentheses], tissues sampled (RBCl = red blood cells), TDFs for nitrogen (Δ^{15}N‰) and carbon (Δ^{13}C‰) and time to reach 95% of isotopic turnover (*t*95%, days)

Reference	Experiment duration (days)	Objective	Environment	Species (*n*) life stage (*L*, cm)	Tissue	Δ^{15}N (‰)	Δ^{13}C (‰)	*t*95% N (days)	*t*95% C (days)
MacNeil *et al.*, 2006	Controlled feeding dietary switch (63)	Turnover rate	F	Stingray *Potamotrygon motoro* (18) Juveniles	Liver	–	–	166	–
					Blood	–	–	265	–
					Muscle	–	–	422	–
					Cartilage	–	–	576	–
Logan & Lutcavage, 2010	Controlled feeding dietary switch (55)	TDF Turnover rate Influence of urea	M	Sandbar shark *Carcharhinus plumbeus* (5) Juveniles (77–86.5)	Liver	–	–	>200	>300
					Blood	–	–	>300	>500
					Muscle	–	–	–	–
Hussey *et al.*, 2010	Semicontrolled multispecific preys in captive	TDF	M	Sand tiger shark *Carcharias taurus* (3) Adults (198–261)	Liver	1·5	0·835	–	–
					Muscle	2·19	1·01	–	–
					Cartilage	1·12	3·515	–	–
				Lemon shark *Negaprion brevirostris* (1) Sub-adult (199)	Liver	1·5	−1	–	–
					Muscle	2·6	0·55	–	–
					Cartilage	2·11	4·22	–	–

TABLE I. Continued

Reference	Experiment duration (days)	Objective	Environment	Species (*n*) life stage (*L*, cm)	Tissue	$\Delta^{15}N$ (‰)	$\Delta^{13}C$ (‰)	*t*95% N (days)	*t*95% C (days)
Kim *et al.*, 2012	Controlled feeding dietary switch (1000)	TDF	M	Leopard shark *Triakis semifasciata* (3) Juveniles	RBCl	2·4	2·3	–	–
					Plasma	2·2	2·8	–	–
					Muscle	3·7	1·7	–	–
Malpica-Cruz *et al.*, 2012	Controlled feeding dietary switch (192)	TDF	M	Leopard shark *Triakis semifasciata* (16) Juveniles (31 ± 1)	Liver	1·4	2·4	–	–
					Blood	1·8	3·3	–	–
					Muscle	2·3	3·2	>300	>200
					Fin	1·9	4·2	–	–
					Cartilage	1·1	4·1	–	–
Caut *et al.*, 2013	Controlled feeding dietary switch (240)	TDF Turnover rate	M	Nursehound dogfish *Scyliorhinus stellaris* (26) Juveniles (50 ± 1)	Plasma	3–0·4	2·8–3·2	170–467	252–263
					RBCl	3·2–0·7	1·2–2	258–582	405–461
					Muscle	−1·8–3·5	0·5–4·3		
					Fin	−1·9–0·5	1·1–5·1		
This study	Controlled feeding dietary switch (365)	TDF Turnover rate	M	Smallnose fanskate *Sympterygia bonapartii* (6) Adults (57·5–68)	Blood	2·5	1·7	290	150
					Muscle	1·5	1·3	250	250–400

TABLE II. Sex (M, male; F, female), total length (L_T) at the beginning of the experiment and mass at the beginning (T_1) and the end of the experiment (T_{360}) for six *Sympterygia bonapartii* used in the controlled feeding experiment. L_T is only given for the beginning of the experiment as there was no discernible increase in length between days 1 and 360

Individual identification number	Sex	L_T (cm)	Mass (g)	
			T_1	T_{360}
1	M	57·5	1570	1594
2	M	63·0	1950	1922
3	M	60·0	1946	1946
4	F	65·0	2672	2636
5	F	68·0	2920	2880
6	F	65·5	2548	2556

N_2 for $\delta^{15}N$. The laboratory internal standards used were G11 (nylon), G13 (bovine liver), G17 (USGS-41 glutamic acid) and G9 (glutamic acid).

DATA ANALYSES AND ESTIMATES OF DIET-TISSUE DISCRIMINATION FACTORS

Diet-tissue discrimination factors were calculated as the difference between food and consumer isotopes ratios: $\Delta x_{\text{diet-tissue}} = \delta x_{\text{eq.tissue}} - \delta x_{\text{diet}}$. This equation assumed that consumer tissues reach isotopic equilibrium with the diet. The isotopic composition at equilibrium condition was estimated modelling the turnover rate of $\delta^{15}N$ and $\delta^{13}C$ values as a time-dependent model that follows an exponential decay curve (Boecklen *et al.*, 2011): $\delta x_{\text{t.tissue}} = \delta x_{\text{eq.tissue}} + b e^{-(m)t}$, where $\delta x_{\text{t.tissue}}$ is the isotopic composition of the tissue at the time t (days since the beginning of the experiment), $\delta x_{\text{eq.tissue}}$ is the isotopic composition at equilibrium with the controlled diet as indicated by asymptotic tissue isotope values, b is the difference between $\delta x_{\text{eq.tissue}}$ and the isotopic composition at $t = 0$ and m is the coefficient of isotopic turnover plus the growth rate. Given that all individuals were 5 year-old adults, it was assumed that the growth rate was negligible and m was an estimation of isotopic turnover; this assumption was tested comparing initial and final masses by t-test (Crawley, 2007).

Prior to estimating the TDF values, the differences between the initial and final mean isotopic compositions ($\delta x_{t=0}$ and $\delta x_{t=360}$) were tested. Given that *S. bonapartii* shows feeding differences between sexes in the wild (Estalles, 2012), possible differences between sexes for mean isotopic compositions were also tested at the beginning of the experiment, which could reflect sex feeding preferences, and at the end of the experiment, which could show differences in TDF between sexes. Differences between $\delta^{13}C$ and $\delta^{15}N$ mean values were tested by a t-test, for two paired samples (comparison between $\delta x_{t=0}$ and $\delta x_{t=360}$) or unpaired samples (comparison between sexes), using the Welch's correction for unequal variances (Crawley, 2007). The half-life of the isotopic composition of each element ($t50\%$, *i.e.* time to 50% of new diet equilibrium) and time to reach 95% of turnover ($t95\%$) were calculated from the equation: $T = \ln[(1-\alpha)100^{-1}]m^{-1}$, where T is the time in days, α is per cent turnover and m is the turnover rate. Non-linear models were fitted using the nls function of the statistical software R (www.r-project.org).

RESULTS

The isotopic composition of the experimental food (fillets of *P. brasiliensis*) was $\delta^{13}C_{\text{food}} = -17{\cdot}10 \pm 0{\cdot}22‰$ and $\delta^{15}N_{\text{food}} = 17{\cdot}11 \pm 0{\cdot}21‰$ and the C:N elemental

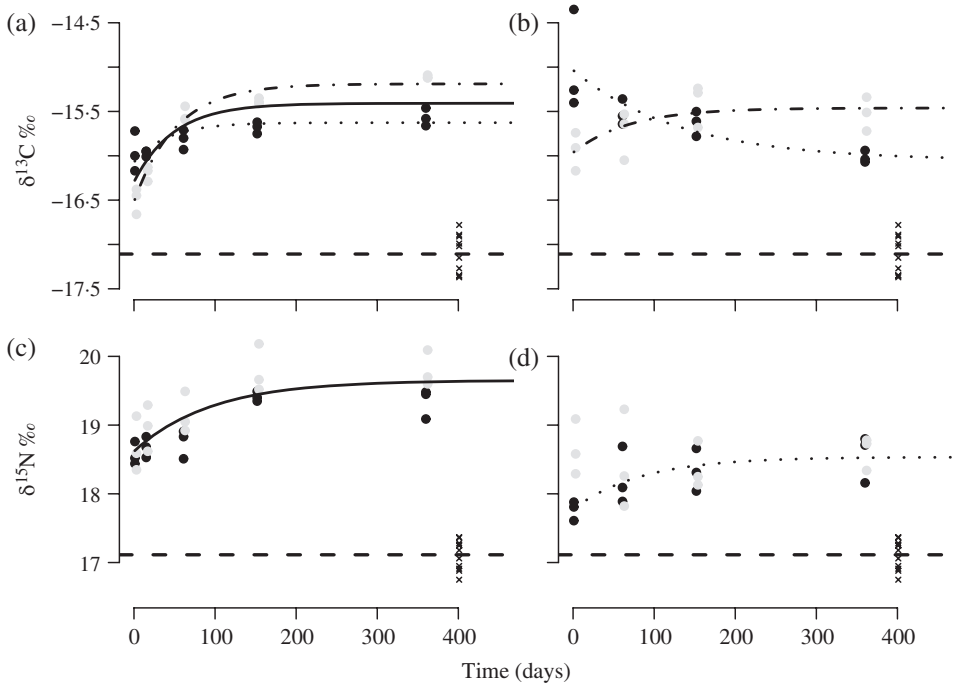

FIG. 1. Stable isotope ratios of (a, b) carbon (δ^{13}C) and (c, d) nitrogen (δ^{15}N) in (a, c) blood and (b, d) muscle of *Sympterygia bonapartii*. -×-, food values (*Percophis brasiliensis*); ●, values for females; ●, values for males; ·····, model fitted to female data; - - -, model fitted to male data; ——, model fitted to data from both sexes combined.

ratio was $C:N_{food} = 3{\cdot}53 \pm 0{\cdot}18$ (mean ± S.D.). During the experiment, the mass of each individual *S. bonapartii* did not change significantly (*t*-test, $t_5 = 1{\cdot}8$, $P > 0{\cdot}05$; Table II). The mean ± S.D. elemental compositions for muscle and blood were $C:N_{muscle} = 2{\cdot}57 \pm 0{\cdot}10‰$ and $C:N_{blood} = 2{\cdot}32 \pm 0{\cdot}15‰$, respectively.

The mean blood isotopic composition changed during the experiment [$t_5 = 4{\cdot}3$, $P < 0{\cdot}01$ for δ^{13}C and $t_5 = 5{\cdot}03$, $P < 0{\cdot}01$ for δ^{15}N values; Fig. 1(a), (c)]. Mean δ^{13}C values were different between sexes at the beginning ($t_{3{\cdot}4} = 4{\cdot}3$, 0, $P < 0{\cdot}05$) and at the end ($t_2 = 6{\cdot}3$, $P < 0{\cdot}05$) of the experiment, with lower values for females than males at the beginning, but higher at the end [Fig. 1(a)]. Females and males did not show differences between either initial or final δ^{15}N values ($t_{2{\cdot}6} = 0{\cdot}5$, $P > 0{\cdot}05$ and $t_{3{\cdot}8} = 2{\cdot}3$, $P > 0{\cdot}05$, respectively). Thus, turnover models were fitted considering all individuals for both elements and for each sex separately for C. All the fitted curves had estimated parameters *m* (turnover rate) and *b* ($\delta x_{ini.tissue} - \delta x_{eq.tissue}$) that were significantly different from zero (Table III). The estimated *t*50% of each element in blood were 35 days (females = 30 days and males = 35 days) for C and 65 days for N. The estimated *t*95% values were 150 days (females = 155 days and males = 125 days) and 290 days for δ^{13}C or δ^{15}N values, respectively [Fig. 1(a), (c)]. Given the observed turnover rates, it was assumed that the isotopic composition of both elements reached equilibrium with the diet by the end of the experiment. The diet-blood discrimination factors, estimated with all individuals, were $\Delta^{13}C_{blood} = 1{\cdot}7 \pm 0{\cdot}1‰$ ($\Delta^{13}C_{blood\text{-}females} = 1{\cdot}5 \pm 0{\cdot}1‰$ and

TABLE III. Non-linear models fitted to describe the isotope turnover process in *Sympterygia bonapartii*: $\delta x_{\text{eq.tissue}}$ represents the isotopic composition at isotopic equilibrium to the diet; b is the difference between $\delta x_{\text{eq.tissue}}$ and the isotopic composition at $t = 0$ and m is the coefficient of isotopic turnover. Confidence intervals (C.I.) for the parameters are given where it was possible estimate them. Differences between m and 0: $^{*}P < 0{\cdot}05$), $^{**}P < 0{\cdot}01$), $^{***}P < 0{\cdot}001$

	$\delta x_{\text{eq.tissue}}$	C.I. $\delta x_{\text{eq.tissue}}$	b	C.I. b	m	C.I. m
$\Delta^{13}C_{\text{blood}}$	−15·41	−15·55 to −15·22	0·887***	0·687 to 1·086	0·204**	0·009 to 0·426
$\Delta^{13}C_{\text{blood-males}}$	−15·19	−15·31 to −15·05	1·330***	1·167 to 1·494	0·019***	0·012 to 0·029
$\Delta^{13}C_{\text{blood-females}}$	−15·62	−15·71 to −15·51	0·444***	0·319 to 0·569	0·024*	0·008 to 0·058
$\Delta^{15}N_{\text{blood}}$	19·65	19·40 to 20·15	1·035***	0·709 to 1·460	0·010*	0·003 to 0·023
$\Delta^{13}C_{\text{muscle-males}}$	−15·46	–	0·050***	–	0·014	–
$\Delta^{13}C_{\text{muscle-females}}$	−16·05		−1·019**	–	0·007	–
$\Delta^{15}N_{\text{muscle-females}}$	18·53	–	0·760*	–	0·012	–

$\Delta^{13}C_{\text{blood-males}} = 1{\cdot}9 \pm 0{\cdot}1‰$) and $\Delta^{15}N_{\text{blood}} = 2{\cdot}5 \pm 0{\cdot}2‰$ (mean ± S.E.). The difference between the $\Delta^{13}C$ values of males and females was 0·4‰; that difference was significant when the overlap between the 95% C.I. of $\delta x_{\text{eq.tissue}}$ values was compared (Table III).

There were no differences between the initial and the final isotopic composition of the muscle samples when all individuals were considered together [$t_5 = 0{\cdot}8$, $P > 0{\cdot}05$ for $\delta^{13}C$ and $t_5 = 1{\cdot}05$, $P > 0{\cdot}05$ for $\delta^{15}N$ values; Fig. 1(b), (d)]. Although mean $\delta^{13}C$ values did not differ between sexes at the beginning of the experiment ($t_{2{\cdot}5} = 2{\cdot}6$, $P > 0{\cdot}05$), however, they differed at the end ($t_{2{\cdot}5} = 4{\cdot}2$, $0{\cdot}05 > P > 0{\cdot}01$). Similar to blood samples, females had lower values than males at the beginning but higher at the end [Fig. 1(a), (b)]. Females and males showed differences between initial $\delta^{15}N$ values ($t_{2{\cdot}5} = 3{\cdot}5$, $P < 0{\cdot}05$) but not final values ($t_{3{\cdot}5} = 0{\cdot}24$, $P > 0{\cdot}05$). The $\delta^{15}N$ of males did not differ from beginning to end of the experiment ($t_2 = 0{\cdot}13$, $P > 0{\cdot}05$). Thus, turnover models were fitted separately for each sex for $\delta^{13}C$ values and only for females for $\delta^{15}N$ values. Although the differences between $\delta x_{\text{ini.tissue}} - \delta x_{\text{eq.tissue}}$ values were significant (Table III, parameter b), given that data were more variable than for blood samples and because of the low sample size, m values included zero as a possible result and hence C.I. could not be estimated (Table III). The lack of significance was interpreted as an effect of the low sample size in the number of individuals of each sex ($n = 3$) and not necessarily as a lack of an actual trend, thus the estimations of the $\delta x_{\text{eq.tissue}}$ values were reported and discussed. Carbon diet–muscle discrimination factors were $\Delta^{13}C_{\text{muscle-females}} = 1{\cdot}1 \pm 0{\cdot}3‰$ and $\Delta^{13}C_{\text{muscle-males}} = 1{\cdot}6 \pm 0{\cdot}2‰$ and combined $\Delta^{13}C_{\text{muscle}} = 1{\cdot}3‰$ (mean ± S.E.). The difference between sexes at equilibrium values was 0·5‰. In the case of N, it was possible to estimate the equilibrium value only for females and the estimated TDF was $\Delta^{15}N_{\text{muscle-females}} = 1{\cdot}5 \pm 0{\cdot}2‰$ (mean ± S.E.).

DISCUSSION

As far as is known, the results of this study constitute the first estimations of $\Delta^{13}C$ or $\Delta^{15}N$ values and isotopic turnover rates of $\delta^{15}N$ and $\delta^{13}C$ values based on a long-term

experiment for marine batoids and is the sixth for marine elasmobranchs (Hussey *et al.*, 2010; Logan & Lutcavage, 2010; Kim *et al.*, 2012; Malpica-Cruz *et al.*, 2012; Caut *et al.*, 2013). It is noteworthy that the six *S. bonapartii* had reached adulthood by the time the experiment started and they were no longer growing during the experiment. Hence, the isotopic turnover can be attributed solely to the effect of metabolic turnover.

TURNOVER RATES

The isotopic turnover rates presented here were similar to previous findings in elasmobranchs (Table I). The estimated times to reach 95% isotopic turnover were shorter than the experiment duration; thus, although the food used during the experiment did not constitute a real trophic shift, results were sound estimates of isotopic replacement in blood samples.

Given the turnover rates observed here for blood samples and previous published values for blood, muscle or liver (Table I), the isotopic composition of these tissues in combination may not be adequate to track movements, unless the isotopic difference between locations is relatively large and the movement scale longer than 6 months (Caut *et al.*, 2013). On the other hand, both muscle and blood seem appropriate as long-term integrators of the isotopic composition of the diet, particularly in combination with more metabolically active tissues. In this regard, batoids produce large quantities of mucus, a promising tissue to integrate diet in shorter dietary periods and that has been used successfully in bony fishes (Church *et al.*, 2009).

DISCRIMINATION FACTORS

The estimated values of TDFs for C isotopes in the blood and muscle of *S. bonapartii* ($\Delta^{13}C = 1{\cdot}7–1{\cdot}3‰$) were similar to generally accepted values for bony fishes ($\Delta^{13}C = 1{\cdot}5‰$; Sweeting *et al.*, 2007*b*). Similarly, the estimations presented here also concur with previous $\Delta^{13}C$ values reported for elasmobranchs in controlled and semi-controlled feeding studies for muscle (Table I). They were less than most previous estimations for blood (Table I), however, regardless of whether authors used whole blood (as in this study), plasma or red blood cells. Regarding $\Delta^{15}N$ estimations, the results presented here were similar to previous estimations for blood but less than previous estimations for muscle (Table I). Food isotopic composition is an important variable that affects the discrimination process (Caut *et al.*, 2009, 2013). Hussey *et al.* (2014) estimated a general regression model to scale $\Delta^{15}N$ by food $\delta^{15}N$ values and Caut *et al.* (2013) estimated specific regression models for elasmobranchs to correct both $\Delta^{13}C$ and $\Delta^{15}N$ values. The $\Delta^{15}N$ value reported here (Table I) was similar to the value estimated from the equations reported in Hussey *et al.* (2014), $\Delta^{15}N = 1{\cdot}3‰$, but higher than estimated from the equations reported in Caut *et al.* (2013), $\Delta^{13}C = 0{\cdot}8‰$ and $\Delta^{15}N = 0{\cdot}4‰$. Overall, despite existing differences among $\Delta^{15}N$ estimations, most studies have shown that isotopic discrimination in elasmobranchs could be smaller than the values that are generally used ($\Delta^{15}N = 3–3{\cdot}4‰$). Thus, these results demonstrate that published trophic position estimations for elasmobranchs, based on $\delta^{15}N$ values and assuming $\Delta^{15}N$ *c.* 3·4‰, could be underestimating actual trophic positions, as previously shown by Hussey *et al.* (2014). Carrying out a comparison between published trophic position estimations derived from $\delta^{15}N$ values (TP_{15N}) and estimations derived from stomach contents is difficult. This is because there are other

assumptions involved in estimating trophic position, such as an adequate selection of an isotopic baseline (Post, 2002), that influence results and in which most studies differ. There are a few examples, however, of TP_{15N} estimations using muscle samples and assuming $\Delta^{15}N = 3{\cdot}4$, *e.g.* from 11 TP_{15N} for Indian elasmobranchs, seven were less than diet derived estimations, one matched and three were greater (Borrell *et al.*, 2010) and from four TP_{15N} estimations for shark species, three values were less and one more than diet-derived TP (Estrada *et al.*, 2003). This evidence, although partial for the reason previously mentioned, seems to corroborate a tendency to underestimate the trophic position of elasmobranchs (Hussey *et al.* 2014).

The differences mentioned between the generally assumed $\Delta^{15}N$ values and the specific TDF estimated for elasmobranchs are a warning to avoid the use of average values in multisource mass-balance mixing models to estimate diet composition (Galván *et al.*, 2012). In accordance with this point, Olin *et al.* (2013) used an indirect approach, deriving TDF values from stomach contents and the isotopic composition of prey and predators, to find that $\Delta^{15}N$ values for species within the subclass Elasmobranchii vary greatly. This reinforces the importance of using species-specific TDFs. Despite the increasing development of controlled-diet experiments, however, this study provides one of the few estimations for elasmobranchs and there are many species where questions about their trophic ecology hinder development of management plans (Simpfendorfer *et al.*, 2011). A possible solution, which contributes to understanding the trophic ecology of elasmobranchs, is the use of TDF presented here and in former studies (Table I) to evaluate whether the trophic enrichment factors create a suitable mixing geometry (Smith *et al.*, 2013).

Another important matter is the difference between $\Delta^{13}C$ values of males and females. The differences in TDFs between sexes, in combination with the possible initial differences in diet selection, caused the exponential decay curves of males and females to cross. After reaching the equilibrium state with diet, both tissues showed a difference of *c*. 0·4‰ between sexes, which represents one-fourth of the estimated TDF. This appears to be the first report of sex differences in TDFs in a fish species. Moreover, few experimental designs have tested for effects of sex on TDFs other than on mammals (Lecomte *et al.*, 2011; Kurle *et al.*, 2014). Interestingly, both studies found significant effects.

Overall, the results presented here can be used in conjunction with estimations of $\Delta^{13}C$ and $\Delta^{15}N$ to estimate sound TDFs, scaled by food isotopic composition. The present estimations will help to pursue detailed questions about elasmobranch trophic ecology using SIA, to calculate TP_{15N} using specific TDFs for apex predators and to estimate dietary proportions evaluating the suitability of different mixing geometries.

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