Zoological Studies **60**:32 (2021)
doi:10.6620/ZS.2021.60-32

Zoological Studies

Open Access

Tissue-specific Isotopic Incorporation Turnover Rates and Trophic Discrimination Factors in the Freshwater Shrimp *Macrobrachium borellii* (Crustacea: Decapoda: Palaemonidae)

María Florencia Viozzi[1,*], Carlos Martínez del Rio[2], and Verónica Williner[1,3]

[1]*Laboratorio de Macrocrustáceos, Instituto Nacional de Limnología, Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina. *Correspondence: E-mail: fviozzi@inali.unl.edu.ar (Viozzi)*
[2]*Department of Zoology and Physiology, University of Wyoming, Laramie, Wyoming 82071, USA. E-mail: CmDelRio@uwyo.edu (Martínez del Rio)*
[3]*Universidad Nacional del Litoral, Santa Fe, Argentina. E-mail: vwilliner@inali.unl.edu.ar (Williner)*

Received 8 April 2020 / Accepted 15 March 2021 / Published 2 July 2021
Communicated by Benny K.K. Chan

The interpretation of isotopic data in ecology requires knowledge about two factors: turnover rate and the trophic discrimination factor, which have not been well described in freshwater shrimps. We performed a 142-day diet shift experiment on 174 individuals of the omnivorous shrimp *Macrobrachium borellii*, measured their growth, and temporally serially sampled muscle and hepatopancreas tissue to quantify carbon and nitrogen incorporation rates and isotope discrimination factors. Shrimps were fed with artificial diets ($\delta^{13}C$ = -26.1‰, $\delta^{15}N$= 2.1‰) for 45 days in attempt to standardize the shrimps' initial $\delta^{13}C$ and $\delta^{15}N$ values for subsequent experiments. Shrimps were then fed with another artificial diet ($\delta^{13}C$ = -16.1‰, $\delta^{15}N$ = 15.8‰) and the change in $\delta^{13}C$ and $\delta^{15}N$ was observed for a period of 97 days. The trophic discrimination factor (Δ) for $\delta^{13}C$ was significantly higher in hepatopancreas (0.7 ± 0.36‰) than in muscle (-0.1 ± 0.83‰); however, the opposite was the case for $\delta^{15}N$ (1.7 ± 0.43‰ and 3.6 ± 0.42‰, respectively). In the hepatopancreas the mean residence time (τ) of ^{13}C was 26.3 ± 4.3 days compared to a residence time of 16.6 ± 5.51 days for $\delta^{15}N$, whereas the τ in muscle was 75.8 ± 25 days for $\delta^{13}C$ and 40 ± 25 days for $\delta^{15}N$. The rate of incorporation of carbon into muscle was higher than that predicted by allometric equations relating isotopic incorporation rate to body mass that was developed previously for invertebrates. Our results support ranges of traditional trophic discrimination factor values observed in muscles samples of different taxa ($\Delta^{15}N$ around 3–3.5‰ and $\Delta^{13}C$ around 0–1‰), but our work provides evidence that these traditionally used values may vary in other tissues, as we found that in the hepatopancreas $\Delta^{15}N$ is around 1.7‰.

Key words: Stable isotopes, Carbon, Nitrogen, Muscle, Hepatopancreas.

BACKGROUND

The study of the relative importance of the different food sources in freshwater food webs stands as a key issue to understanding ecosystem function (Marchese et al. 2014). An important tool to assess the flow from different resources to consumers is $\delta^{13}C$ and $\delta^{15}N$ stable isotope analyses (SIA). These analyses have become increasing available and affordable and a central tool to investigate the structure and dynamics of ecological communities (Peterson and Fry 1987; Post 2002; Benstead et al. 2006). The interpretation of isotopic patterns in ecological systems hinges on two pieces of information: The small difference often

Viozzi MF, Martínez del Rio C, Williner V. 2021. Tissue-specific isotopic incorporation turnover rates and trophic discrimination factors in the freshwater shrimp *Macrobrachium borellii* (Crustacea: Decapoda: Palaemonidae). Zool Stud **60**:32. doi:10.6620/ZS.2021.60-32.

observed between the isotopic value of tissues and those of diet (DeNiro and Epstein 1978), and the temporal response of change in the consumer's tissues' isotopic values (Tieszen et al. 1983; Karasov and Martinez del Rio 2007).

The difference in isotopic values between a consumer's tissues and its diet is called the trophic discrimination factor and symbolized by Δ ($\Delta = \delta_{tissue} - \delta_{diet}$) (Peterson and Fry 1987; Post 2002; Mccutchan et al. 2003). It is an important component as it represents a correction factor of the mixing models used to estimate the proportions of different diet components (Phillips and Gregg 2003; Caut et al. 2010). Estimating the value of Δ is central to assessing assimilated diets of wild animals when using stable isotopes (Robbins et al. 2010). Despite the large variability in nitrogen and carbon discrimination factors, most isotope model studies have used a single discrimination factor for carbon and nitrogen 0–1‰ for $\delta^{13}C$ and 3.4‰ for $\delta^{15}N$. These values are, often obtained from published reviews (Fry and Sherr 1984; Minagawa and Wada 1984; Post 2002; Vanderklift and Ponsard 2003; Caut et al. 2009; Sabat et al. 2013). But these values represent averages of large data sets that combine laboratory and field measurements and that have relatively large amounts of variation. Because using inappropriate values for discrimination factors can lead to large errors or meaningless results (Caut et al. 2009), empirically determined discrimination factors are most reliable for diet reconstruction in mixing models (Boecklen et al. 2011).

Since different tissues incorporate assimilated dietary elements at different rates, they integrate this information over different temporal scales (Bearhop et al. 2002). Isotopic ecologists should be interested in the time course of the incorporation of the isotopic signature into an animal's tissues for two reasons: first, this information determines the time window through which they can perceive the course of changes in the isotopic composition of an animal's diet (Newsome et al. 2007). Second, by sampling different types of tissues in a single individual, SIA permits can be used to explore how an animal uses resources over a variety of temporal scales (Martinez del Rio 2008). The time window that a tissue represents is determined with diet shift experiments designed to measure the rates of incorporation, or turnover, and the residence times of different elements in a tissue (Martínez del Rio et al. 2008; Martínez del Rio and Carleton 2012).

Conducting one more isotopic incorporation experiment on a species, and on a variety of tissues is not only important for understanding the isotopic data for that particular species in the field, it also adds to a body of data that will make performing those experiments unnecessary in the future (Martínez del Rio and Carleton 2012). Numerous studies have investigated the trophic roles and interactions of macroinvertebrates using stable isotope analysis (March et al. 2002; Atkinson et al. 2010; Schoeller et al. 2010; Marchese et al. 2014; Saigo et al. 2016; Mao et al. 2016; Lipták et al. 2019). Nevertheless, to our knowledge, there is only a single published estimated value of $\Delta^{15}N$ and $\Delta^{13}C$ in a freshwater crustacean: a study on *Cherax destructor* (Carolan et al. 2012) that reported an unusually low value for $\Delta^{15}N$ (1.5 ± 1.0‰) in muscle. This observation raises the question of whether the typically assumed ± 3.4‰ is appropriate for freshwater crustaceans. For that reason, we aimed to determine the turnover of carbon and nitrogen in the hepatopancreas and muscle of *Macrobrachium borellii* as well as the trophic discrimination factors ($\Delta^{15}N$ and $\Delta^{15}C$) and to assess how these values compared with values for other organisms (reviewed by Vanderfklift and Ponsard 2003; Vander Zanden et al. 2015).

Our study is the first to directly evaluate and compare muscle and hepatopancreas' tissue-specific isotopic incorporation rates and trophic discrimination factors in an omnivorous macroinvertebrate of the Paraná River's floodplain. Within the group of omnivorous macrocrustaceans that inhabit the floodplain of the Paraná River, *Macrobrachium borellii* stands out as the most suitable species to carry out this type of study, since is a common and widespread shrimp in the southern cone of South America (Brazil, Argentina, Paraguay and Uruguay). As an omnivore, *M. borellii* has a large impact on energy flow in the food web, having a higher interaction index in the trophic webs than other macrocrustacean species (Carvalho et al. 2016), and making it a key species in the food webs of freshwater environments (Collins et al. 2007 2012). Moreover, *M. borellii* has an adequate size and life cycle (Vogt 2012) to carry out this type of study that requires at least one year of extension and a minimum amount of sample to perform stable isotope analysis in the tissues of interest (Martínez del Rio and Carleton 2012).

The natural diet of *M. borellii* is characterized mainly by animal items and but also by algae (Collins and Paggi 1998; Collins 2005; Carvalho et al. 2016). However, the variety and availability of potential prey changes with the unstable environmental conditions of floodplain rivers. Saigo et al. (2015) constructed a food web model for benthic invertebrates in different lakes belonging to the Paraná River floodplain and highlighted that *M. borellii* may experience changes in its trophic relationships caused by the periodic fluctuations in the availability of resources given by the dynamics of pulses in floodplain rivers. Consequently, it is relevant to know the isotopic incorporation rates

of $\delta^{13}C$ and $\delta^{15}N$ in different tissues, since these cover different time windows which can be inserted in the different phases of the hydrosedimentological regime of the Paraná River, and therefore be related to the changes that occur in the availability of trophic resources. In this sense, the hepatopancreas of crustaceans would reflect recent additions to the metabolic circuit and muscle tissue would provide information on a wider time window (Martínez del Rio et al. 2009). Therefore, by determining both the turnover rates and trophic discrimination factor of *M. borellii*, we will be able to analyze more precisely its trophic role in aquatic food webs and elucidate how this can vary over time in subsequent studies.

MATERIALS AND METHODS

Origin and maintenance of shrimps

Adult *M. borellii* individuals (with a carapace length greater than 15 mm) were collected in the Salado River (Santa Fe, Argentina 31°40'28.4"S; 60°45'16.7"W) during May 2017, and transported to National Institute of Limnology (INALI, Santa Fe, Argentina). A total of 174 shrimps were distributed in nine 35-liter aquariums (18–20 shrimp per aquarium) holding dechlorinated water at a constant temperature (25.4 ± 0.7°C) and a 12/12 (light/darkness) photoperiod. Dissolved oxygen (6.5 ± 0.47 mg/l), oxygen saturation percentage (79.1 ± 6.0 %) pH (7.6 ± 0.2), conductivity (145.7 ± 18.7 ppm), ammonium (0.14 ± 0.19 ppm), nitrite (1.26 ± 0.80) and nitrates (0.19 ± 0.30 ppm) were recorded weekly with electronic sensors (Hanna HI 98130/9146). To record ammonium levels, the Nessler method was used, for nitrites the NitriVer® 3 1 method, HACH 8507 method with USEPA compliance as an effluent report and for nitrates the Nitra-Ver® 5 method, cadmium reduction according to method Ac 8171. Shrimp were fed daily *ad libitum* (to avoid starvation), and after feeding 30% of the water of the aquaria was renewed to avoid contamination. This design was maintained throughout the experiment.

Experimental setup

Selection of diets used in the trial

Initially, artificial diets with proven survival rates were chosen because we sought to reduce the variability associated with the type of food supplied (Choi et al. 2016; Collins and Petriella 1999). For this, two diets (Table 1) with known $\delta^{13}C$ and $\delta^{15}N$ isotopic differences (two-sample Welch test: $\delta^{15}N$: t = -386.32, $\delta^{13}C$: t = -363.88, $p < 0.0001$) were selected (see Table 1 for details about the composition and isotopes values of experimental diets) to maximize the separation of the values obtained before and after the diet change and to obtain reliable parameters (Martínez del Rio and Carleton 2012). We also sought to ensure that the diets had a high nutritional quality to rule out unwanted metabolic responses with respect to a low-quality diet (Carvalho et al. 2020). Both diets had a similar protein, lipid, and carbohydrate content (diet 1: protein 45.7%, lipid 24.4%, and carbohydrate 29.57%, diet 2: protein 49.4%, lipid 20%, and carbohydrate 28.6%) and essential nutrients that cannot be synthesized by shrimp (Carvalho et al. 2020). This was achieved by incorporating *Chlorella vulgaris* in diet 1 (Choi et al. 2016) and fishmeal in diet 2 (Table 1) (Carvalho et al. 2020). Finally, to elaborate the diets, both diets were considered to represent feeding pathways recorded in previous studies (Marchese et al. 2014; Saigo et al. 2015; Carvalho et al. 2016), so diet 1 mainly comprised algae (a particularly important source for this species at a certain time of year) and diet 2 represented sources of animal origin (items of animal origin are recorded in the diet of *M. borellii* throughout the year with variations according to the environmental offer).

For 45 days, from the time of capture until the start of the diet-switch experiment, shrimps were fed on diet 1 with low $\delta^{13}C$ and $\delta^{15}N$ values (Table 1). This

Table 1. Composition and isotope values of experimental diets (‰)

	Composition	Isotope value
Diet 1	wheat flour 22%, concentrated soybean meal 33%, *Chlorella vulgaris* 12%, canola oil 16%, soybean meal 6%, cholesterol 1%, grenetin 4%, mixture of vitamins and minerals[a] 2%, bicalcium phosphate 4%.	$\delta^{13}C$ = -26.1‰ $\delta^{15}N$ = 2.1‰
Diet 2	fish flour 60%, starch 27%, fish oil 2%, cholesterol 1%, grenetin 4%, commercial fish diet containing mixture vitamins and minerals[a] 2%, bicalcium phosphate 4%	$\delta^{13}C$ = -16.1‰ $\delta^{15}N$ = 15.8‰

[a]Manufactured by Nutralia S.R.L. (Santa Fe, Santa Fe, Argentina). Maximum values of active principles in g/1,000 g: vitamin B1 ((0.550); vitamin B2 (1.925); vitamin B6 (1,238); vitamin B12 (4.125); niacin; pantothenic acid (5.978); vitamin C (27.500); biotin (5.500); vitamin A (3.385); vitamin D (0.550); vitamin E (44.000); vitamin K (11.000); iron (50.417); zinc (64,706; copper (15.714); manganese (0.917); selenium (18.750); phosphorous (0.314); and maltodextrin (excipient).

timescale was used because 45 days was assumed to be approximately four isotopic half-lives according to the body mass of individual shrimps (average: 0.6 g), considering specific growth rate during this period, and according to values in literature (Hobson and Clark 1992; Karasov and Martínez del Rio 2007; Thomas and Crowther 2015; deVries et al. 2015). Consequently, the shrimps should have been close to isotopic equilibrium at the end of this period. Then individuals were switched to a new a diet 2—hereinafter called Day 0 of the experiment—with significant statistically higher $\delta^{13}C$ and $\delta^{15}N$ values (Table 1).

To measure growth over the course of the experiment, the mass of a subset of 64 individuals (three of the initial nine aquariums) was recorded on Day 0 and at the end of the experiment (day 97). Each individual was measured at the beginning (cephalothorax length mean: 18.20 ± 0.7) and at the end of the trial (cephalothorax length mean: 18.26 ± 0.9) with an electronic digital caliper (0–150 mm; Schwyz), and weighted three times at 15-second intervals to the nearest μg. At the end of the experiment, the parameters weight gain (WG), specific growth rate (SGR), and survival (S) were calculated were calculated using the following formulae:

$$WG(g): final\ weight - initial\ weigth$$

$$SGR(\%day^{-1}) = \frac{ln\ final\ weight - ln\ initial\ weigth}{Number\ of\ days} \times 100$$

$$S(\%) = \frac{final\ number\ of\ shrimps}{initial\ number\ of\ shrimps} \times 100$$

Significant changes in growth variables were evaluated using a Wilcoxon two-sample test.

From the remaining 110 individuals (six of the nine initial aquariums), we took muscle and hepatopancreas samples to later perform the stable isotope analysis. For this reason, on Day 0 of the experiment nine individuals were randomly selected to be euthanized on ice and their hepatopancreas dissected. After being switched to the new diet (diet 2), 3 to 6 individuals were randomly sampled on days 1, 2, 4, 7, when isotopic change was greatest. Then samples were taken on days 13, 19, 25, 31, 40, 64, and the latest samples were collected after 97 days of the diet switch (Fig. 1).

All the samples were lipids extracted following Ingram et al. (2007) recommendations in order to adjust $\delta^{13}C$ values and minimize shifts in $\delta^{15}N$. To do this, chloroform-methanol extractions based on the original protocols of Bligh and Dyer (1959) were performed. Then the samples were dried at 50°C to constant mass, ground to a fine powder with a mortar and a pestle, placed in sealed tin capsules (580–1920 μg), and stored in a desiccator.

Stable isotope analysis

Samples' $\delta^{13}C$ and $\delta^{15}N$ isotopic compositions were analyzed with Carlo Erba 1110 Elemental

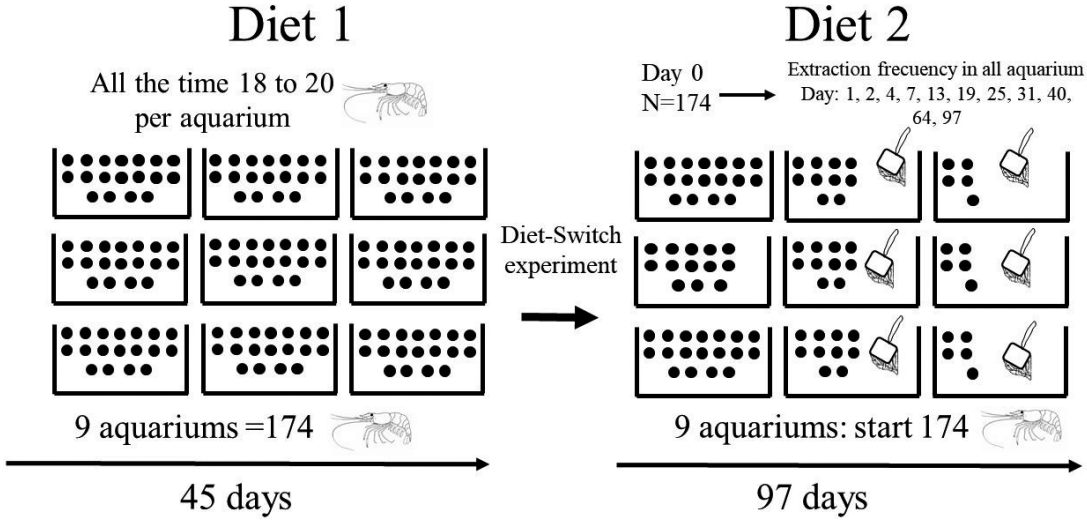

Fig. 1. Scheme of the trial design to determine the turnover rates and the trophic discrimination factor of the freshwater shrimp *Macrobrachium borellii*. The black circles represent the shrimp. The composition of the diets is detailed in table 1. During the trial period, the following variables were controlled for: temperature, photoperiod, dissolved oxygen, oxygen saturation percentage, pH, conductivity, ammonium, nitrite, and nitrates. The extraction of the specimens was carried out completely at random. On the extraction days, samples of hepatopancreas and muscle were obtained.

Analyzer coupled to a Thermo Delta V IRMS at the Wyoming University's Stable Isotope Facility (EEUU). Stable isotope ratios were expressed using standard δ notation in parts per mil (‰) as:

$$\delta X - (\frac{Rsample}{Rstandard} - 1) \times 1000$$

where R_{sample} and $R_{standard}$ are the molar ratios of the heavy/light isotope of the sample and the reference, respectively. Samples were referenced against the international standard, the VPDB for $\delta^{13}C$ and atmospheric N (AIR) for $\delta^{15}N$. We use Glutamic 1 (Standard reference material 36-UWSIF-Glutamic 1, n =24, $\delta^{13}C$ = -28.3 ± 0.3‰ and $\delta^{15}N$ = -4.6 ± 0.09‰), Glutamic 2 (Standard reference material 39-UWSIF-Glutamic 2, n = 20, $\delta^{13}C$ = 24.4 ± 0.14‰ and $\delta^{15}N$ = 27.9 ± 0.06‰), and liver (Standard reference material UWSIF01 n = 28, $\delta^{13}C$ = -17.8 ± 0.03‰ and $\delta^{15}N$ = 6.8 ± 0.06‰) as internal references.

Statistical analysis

Statistical analyses were done with R Core Ream (2019) (Develop-men Core Team, The R Foundation for Statistical Computing, Vienna, Austria). We used coexponential one- and two-compartment models to describe the time course of isotopic incorporation and AICc to assess whether one or two compartment models were better supported by data and used r^2 as a qualitative estimate of the fit of the fitted non-linear models of isotopic incorporation (Martínez del Rio and Anderson-Sprecher 2008). Because in all cases, one-compartment models received better support (Δ AIC_c > 3.0), we only presented data from these models. Briefly, these models are of the form:

$$\delta X(t) = a + b(exp(-\lambda t))$$

In this equation X is the stable isotope (^{13}C or ^{15}N), $\delta X(t)$ is the isotopic value of the tissue at time (t), a is the asymptotic isotopic value of the tissue after a diet switch, and b is the difference between the asymptotic isotopic value of the tissue and the isotopic value of the tissue prior to a diet switch. The value of λ represents the instantaneous rate of isotopic incorporation (with units equal to time -1), and its reciprocal (1/ λ**)** equals the mean residence time τ (in units = $days^{-1}$). The latter parameter provides an intuitive measure of residence time in days.

The diet-to-tissue discrimination factor also can be calculated from the incorporation rate model. Discrimination factors were calculated for both isotopes as:

$$\Delta^h = \delta^h X_\infty \, shrimp - \delta^h prey$$

where Δ^h is the trophic discrimination factor for h stable isotope, $\delta^h X_\infty \, shrimp$ is the estimated value of the steady-state isotopic composition of the shrimp tissue and $\delta^h prey$ is the mean isotopic value of new diet (diet 2).

RESULTS

The weight gain (WG) at the end of the trial was 0.195 ± 0.45 g (initial weight: 0.692 ± 0.23; final weight: 0.886 ± 0.39), specific growth rate (SGR) was 0,003%, and survival (S) was 52%. There were no significant differences found between the final and initial masses (Wilcox two-sampled test Z = 1.7497, p = 0.08057) or in the final and initial lengths of the cephalothorax (Wilcox two-sampled test Z = 0.30458, p = 0.7633).

The composition of $\delta^{13}C$ and $\delta^{15}N$ rapidly increased over time immediately following the change in diet (Fig. 2). The isotopic composition of *M. borellii* changed during the experiment (97 days) and approached diet 2, as expected. The values of $\Delta^{13}C$ for muscle tissue range from -0.93 to 0.73‰ and $\Delta^{15}N$ from 3.18 to 4.02‰, while for the hepatopancreas the $\Delta^{13}C$ values range from 0.34 to 1.06‰ and for $\Delta^{15}N$ from 1.27 to 2.13‰.

In the two tissues the isotopic discrimination factor (Δ) was greater for $\delta^{15}N$ than for $\delta^{13}C$. This parameter for $\delta^{13}C$ was significantly higher in hepatopancreas than muscle. However, in the case of $\delta^{15}N$ the values were in the opposite direction: the isotopic discrimination factor (Δ) was greater in the muscle than in hepatopancreas (Table 2). Because shrimp did not grow during our experiments, λ can be interpreted as an estimate of steady elemental turnover (Martínez del Rio and Carleton 2012). In this case, the incorporation of both elements conforms to a single compartment model. The isotopic incorporation rate of hemolymph was faster than that of muscle, both for $\delta^{15}N$ and for $\delta^{13}C$. In the hepatopancreas turnover rate λ for $\delta^{13}C$ was 0.038 ± 0.006 -days. This parameter takes more time for $\delta^{15}N$ in the hepatopancreas and presents a value of 0.063 ± 0.020 -days. In the muscle the registered values of the turnover rate for both $\delta^{15}N$ and $\delta^{13}C$ are lower than in the hepatopancreas. The respective values were 0.0132 ± 0.004 -days for $\delta^{13}C$ and 0.023 ± 0.01315N -days for $\delta^{15}N$ (Table 2). In consequence, in the hepatopancreas the mean residence time (τ) of $\delta^{13}C$ was 26.3 ± 4.3 days compared to a residence time of 16.6 ± 5.51 days for $\delta^{15}N$, whereas the τ in muscle was 75.8 ± 25 days for

$\delta^{13}C$ and 40 ± 25 days for $\delta^{15}N$ (Fig. 2).

DISCUSSION

The incorporation of the carbon and nitrogen isotopic values of diet into the tissues of *Macrobrachium borellii* was well described by a single compartment model. These models allowed estimating the fractional incorporation rate of carbon and nitrogen stable isotopes into the shrimps' muscle and hepatopancreas, and the isotopic discrimination factor (Δ) between these tissues and diet. Here we interpret these values by considering the absence of growth during the experiment, and by comparing the values with those found in other species of shrimp and with the values expected from allometric models.

During the experiment, the specimens of *M. borellii* did not grow significantly. In the revision of Vogt (2012) it is explained that although this species continues to molt throughout its life, the time intervals between molts increases with age and the growth that accompanies each molt declines (Vogt 2012). Because our experiments included adult individuals of the largest sizes observed under natural conditions, the negligible growth rate and survival observed is expected (Collins and Petriella 1999). Regarding the survival rate obtained, this may be due to the species' own senescence period, which has a life expectancy of 2 years (Vogt 2012), which was remarkably close to being reached at the end of the experiment, due to the experiment extension and the size of shrimp used in

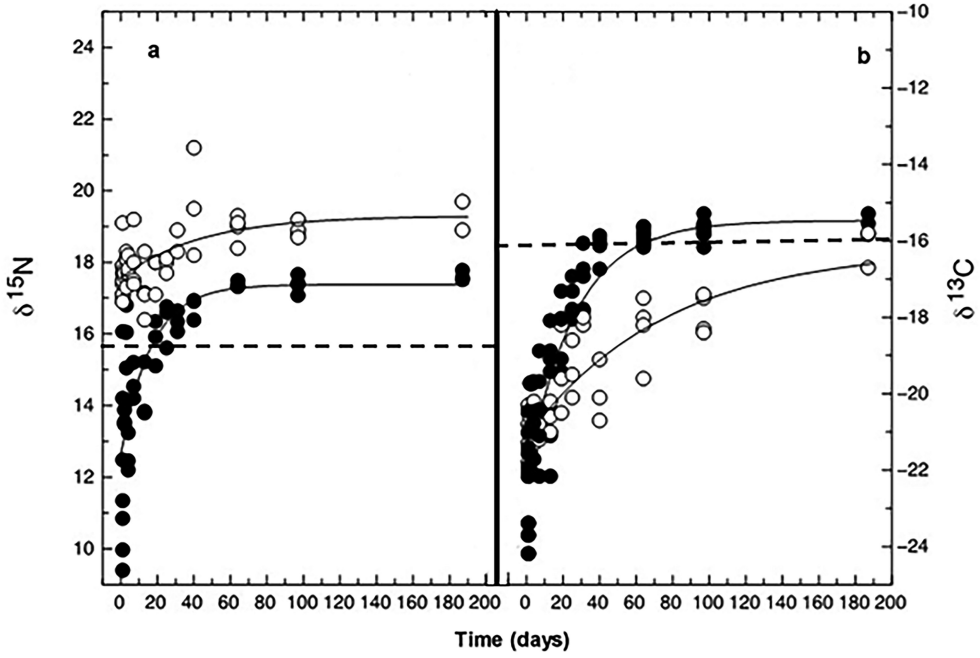

Fig. 2. Temporal changes in the isotopic value of the tissues of the freshwater shrimp *Macrobrachium borellii* after a diet shift. One-compartment models described the changes in changes in $\delta^{13}C$ (a) and $\delta^{15}N$ (b) adequately well. For each graph, the curve is the best fit. Circles indicate individual shrimp sampled at each time (1, 2, 4, 7, 13, 19, 25, 31, 40, 64, 97 days). The black circles represent the hepatopancreas and the white circles the muscle. Dashed black line represents the $\delta^{13}C$ and $\delta^{15}N$ value of diet 2. The isotope values for diet 1 were $\delta^{13}C$ = -26.1‰ and 1 $\delta^{15}N$ = 2.1‰.

Table 2. Model parameters ± standard error from one-compartment isotopic incorporation rate models predicted for the freshwater shrimp *Macrobrachium borellii*

Tissue	Isotope	*n*	Model	r^2 for model fit	Mean residence time τ (d)	Elemental turnover rate λ (d^{-1})	Equilibrium Value δ_∞ (‰)	Discrimi-nation factor Δ (‰)
H	^{13}C	47	$-15.47 - (6.7)\, e^{-0.038t}$	0.88***	26.3 ± 4.3	0.038 ± 0.006	-15.47 ± 0.36	0.7 ± 0.36
H	^{15}N	47	$17.38 - (4.73)\, e^{-0.063t}$	0.70***	16.6 ± 5.51	0.063 ± 0.020	17.38 ± 0.43	1.7 ± 0.43
M	^{13}C	48	$-16.19 - (4.95)\, e^{-0.0132t}$	0.78***	75.8 ± 25	0.0132 ± 0.004	-16.19 ± 0.83	-0.1 ± 0.83
M	^{15}N	48	$19.3 - (1.74)\, e^{-0.023t}$	0.59***	40.0 ± 25	0.023 ± 0.013	19.3 ± 0.42	3.6 ± 0.42

M = muscle tissue, H = hepatopancreas tissue, *n* = sample size. * = $P < 0.001$.

it. However, the shrimp were not growing, they were feeding normally and as demonstrated by the positive values of incorporation rate, they were assimilating the food offered.

In a great variety of animal species, it has been acknowledged that the average values for $\Delta^{13}C$ and $\Delta^{15}N$ range from -0.6‰ to + 2.7‰ and 3 to 5‰, respectively (DeNiro and Epstein 1978). In our study the values for $\Delta^{13}C$ for muscle tissue -0.93 to 0.73‰ agreed with Post (2002) suggesting a mean of 0.39 ± 1.3‰ and with those obtained by Latli et al. (2017) in two species of freshwater fish larvae (-0.3 and -0.1‰). Nevertheless, they contrast with those values suggested in other works about a greater fractionation in freshwater fish muscle samples ($\Delta^{13}C \sim 2$‰) and in crustaceans' ($\Delta^{13}C$ from 2 to 4‰) (Parker et al. 1989; Yokoyama et al. 2005; deVries et al. 2015). The same is true for nitrogen, although the values obtained in the present study (3.18 to 4.02‰) agree with those commonly cited $\Delta^{15}N$ in the literature and are like the fractionation found in muscle of marine crustaceans (3.6 to 4.0‰) (Yokoyama et al. 2005). They differ from the values obtained in other crustaceans; for instance, 2.2‰ in *Litopenaeus vannamei* (Downs et al. 2014) and 0–1‰ for mantis shrimp *Neogonodactylus bredini* (deVries et al. 2015). In the last two cases, the quality of the diet provided and whether the animals grew during the trial may have had impact. Robbins et al. (2010) reported a highly significant interspecific negative correlation between $\Delta^{15}N$ and the diet's protein value. Due to growth, Martínez del Rio et al. (2009) predicted that $\Delta^{15}N$ should be lower in growing than in non-growing animals. Moreover, deVries et al. (2015) affirmed that in rapidly growing crustaceans low $\Delta^{15}N$ values can result from consuming diets with a high protein content which have C: N ratios less than 6. In our case, the food supplied for shrimps contained high quality protein C: N = 5.9 but the animals did not grow during the trial, and that could be the reason why we did not obtain low $\Delta^{15}N$ values. In the trials conducted by deVries et al. (2015), the diet they used had a higher protein value than our diet (C: N ratio of 3. 9), yet (and similarly to our case) the animals did not show a significant growth rate. In the essay conducted by Downs et al. (2014) with the Pacific white shrimp *Litopenaeus vannamei* the fact that shrimp have grown during the test can at least partly explain the obtained $\Delta^{15}N$ value. Lefrebvre and Dubois (2016) showed evidence that trophic discrimination factor (Δ) values are linked to growth of individuals and that it is highly relevant to estimate growths in experiments and in field studies to estimate trophic discrimination factor values. However, more studies are necessary to clarify the relevance of both factors, growth, and protein content on the diet, on Δ values in crustaceans.

The factors that shape trophic discrimination values in crustaceans are still unknown. Possible reasons for the interspecific variation observed in Δ values are differences in the quality of the diet fed in experiments (Robbins et al. 2010) and differences in growth (Martínez del Rio et al. 2009). A recent experiment on the Mysid *Neomysis integer* demonstrated a strong negative relationship between $\Delta^{15}N$ and growth rate (Gorokhova 2018). Our results revealed a small (≈ 0.8‰) and statistically non-significant differences between the $\Delta^{13}C$ of muscle and hepatopancreas, but a larger and statistically significant difference between the $\Delta^{15}N$ values of muscle and hepatopancreas (mean difference ≈ 2‰, $t = 4.4$, $p < 0.01$). Differences in discrimination factors among tissues can be due to a variety of causes, including differences in amino acid composition (amino acids and other compounds can vary widely in $\delta^{13}C$ and $\delta^{15}N$ values, according to Macko et al. (1986) and Perga and Grey (2010)) and differences in in-situ deamination (Hobson et al. 1997; Logan et al. 2006; Schmidt et al. 2007; Tieszen et al. 1983; Wolf et al. 2009; Yokoyama et al. 2005). Independently of the causes of these differences, and because Δ values can have strong effects on the application of isotopic values for ecological inferences (for example in the estimation of trophic position (Post 2002) and in mixing models (Jackson et al. 2014)) our results suggest that ecological inferences likely demand using tissue-specific Δ values.

The rate at which tissues of an animal incorporate the isotopic value of resources is determined by both the addition of new material (growth) and by the replacement of material exported from the tissue as a result of catabolism turnover (Fry et al. 1982). Because experimental shrimp did not grow during the experiment their rates of isotopic incorporation, and therefore the average retention time of elements in the tissue, was only determined by catabolic turnover (Martínez del Rio and Carleton 2012). Rapidly growing crustaceans have been reported to have faster incorporation rates than those reported here for *M. borellii.* In this sense, Bójorquez-Mascareño and Soto-Jiménez (2016) reported nitrogen half-life time to be between two and five in muscle of postlarvae of *Litopenaeus vannamei* marine shrimps. Glon et al. (2016) reported $\delta^{15}N$ and δ^{13} half-lives in muscle of two freshwater crayfish: *Orconectes rusticus* and *O. virilis*. In the first of them, $\delta^{15}N$ and δ^{13} half-lives were 30.38 and 36.71 days, respectively, while mean $\delta^{15}N$ and δ^{13} half-lives of *O. virilis* were 27.96 and 33.20 days, respectively. Carolan et al. (2012) estimated a $\delta^{15}N$ half-live 19 days in muscle of the freshwater crayfish *Cherax destructor*. However, Busst and Britton (2017) estimated a muscle $\delta^{15}N$ half-time of 84 days for a slow growing freshwater fish *Barbus barbus* and deVries et al. (2015) estimated a

muscle $\delta^{15}N$ half- live time of 50 days and a δ^{13} half-live of 62 days in adults of mantis shrimps, *Neogonodactylus bredini*, which also did not grow significantly during the trial. Considering the aforementioned, we hypothesize that faster values in early-stage decapods are due to the contribution of growth rate to isotopic turnover.

The residence time of carbon was longer than that of nitrogen, and the residence time for both carbon and nitrogen and nitrogen was higher in hepatopancreas than in muscle (Table 2). The former results suggest higher turnover of nitrogen due to amination and transamination coupled to conservation of the carbon skeletons of some indispensable amino acids (Mente et al. 2002). The higher turnover of both $\delta^{13}C$ and $\delta^{15}N$, and the concomitant shorter average isotopic retention times in hepatopancreas than in muscle is consistent with the observation that isotopic retention times appear to be higher in splanchnic than in structural tissues (Martinez del Rio et al. 2009). Whether this is the case in crustaceans is unknown, although higher protein turnover in hepatopancraes than in muscle has been reported in *Penaeus esculentus* (Hewitt, 1992). This difference between tissues offers the opportunity to use different tissues to assess dietary changes at different time scales.

deVries et al. (2015) and Vander Zanden et al. (2015) compiled large data sets to construct allometric equations that predict the isotopic incorporation from body mass. Our experimental estimates for *M. borellii* allow us to assess those predictions. To maintain consistency with Vander Zanden et al. (2015), we transformed the estimated average retention times to half-lives ($t_{1/2}$ = Ln (2)τ) and used only values for muscle. We compared these values with those predicted by the equation derived by Vander Zanden et al. (2015) for invertebrate muscle. We used 0.60 grams as the average body mass of *M. borellii.* The equation estimates a half-life of roughly 23 days. The average half-lives (± SE) estimated experimentally for $\delta^{13}C$ and $\delta^{15}N$ in the muscle of *M. borellii* are 75.8 ± 25 and 40.0 ± 25 days, respectively. These values are higher than those estimated by allometric equations, although the values are well within the 95% confidence intervals of the predicted values. Allometric equations are often accurate but imprecise (Martínez del Rio 2008; Vander Zanden et al. 2015), especially when body mass is the sole predictor of a given trait. Their imprecision is perhaps expected given the relatively rough categories included as covariates in allometric analyses, and the observation that these equations do not incorporate some of the primary determinants of isotopic incorporation such as growth (Vander Zanden et al. 2015). The usefulness of allometric predictions might be limited when precise values of isotopic incorporation are needed, although predictions remain useful to guide experimental designs of isotopic incorporation experiments and when relatively coarse estimates suffice.

CONCLUSIONS

Despite the widespread use of stable isotopes to reconstruct trophic webs in freshwater environments, there are very few works tending to estimate the specific trophic position and the average residence time of carbon and nitrogen isotopes in different tissues. Our results confirm traditional trophic discrimination factor values observed in muscles samples of different taxa, namely a $\Delta^{15}N$ around 3–3.5‰ and $\Delta^{15}C$ around 0–1‰ (Post 2002), but our work also provides evidence that these traditionally used values may vary in other tissues, as we found that $\Delta^{15}N$ in the hepatopancreas is around 1.7‰.

Finally, we found a higher turnover rate of both $\delta^{13}C$ and $\delta^{15}N$ in the hepatopancreas than in muscle, which is consistent with the observation that isotopic retention times appear to be higher in splanchnic than in structural tissues (Martínez del Rio et al. 2009).

Acknowledgments: This work was funded by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) (PIP No. N°790, PICT 2018 0174). We thank Dr. Franco Teixeira de Mello (Universidad de la República de Uruguay-CURE-Maldonado) for helping us ship the samples. The authors thank the Argentinian national government for supporting relevant scientific and technological development from 2011 to 2015. We are also grateful to Dr. Amy C. Krist and the anonymous reviewers for providing constructive comments on the manuscript.

Authors' contributions: MFV conceived and designed the study, performed the sampling, diet preparation, trial maintenance, processed the organism, analyzed the data, prepared figures and/or tables, authored and reviewed drafts of the paper, approved the final draft. CMdR analyzed the data, prepared figures and/or tables, authored and reviewed drafts of the paper, approved the final draft. VW conceived and designed the study, performed the sampling, processed the organism, authored and reviewed drafts of the paper, approved the final draft.

Competing interests: None of the authors presents a conflict of interest regarding the realization or publication of this work.

Availability of data and materials: The data generated in this study is included in supplementary material.

Consent for publication: All the authors have agreed to this submitted version of the manuscript.

Ethics approval consent to participate: Not applicable

REFERENCES

Atkinson CL, Opsahl SP, Covich AP, Golladay SW, Conner ML. 2010. Stable isotopic signatures, tissue stoichiometry, and nutrient cycling (C and N) of native and invasive freshwater bivalves. J N Am Benthol Soc **29(2):**496–505. doi:10.1899/09-083.1.

Bearhop S, Waldron S, Votier SC, Furness RW. 2002. Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. Physiol Biochem Zool **75(5):**451–58. doi:10.1086/342800.

Benstead JP, March JG, Fry B, Ewel KC, Pringle CM. 2006. Testing isosource: Stable isotope analysis of a tropical fishery with diverse organic matter sources. Ecology **87:**326–333. doi:10.1890/05-0721.

Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. Can J Biochem Physiol **37:**911–917.

Boecklen WJ, Yarnes CT, Cook BA, James AC. 2011. On the use of stable isotopes in trophic ecology. Annu Rev Ecol Evol Syst **42:**411–440. doi:10.1146/annurev-ecolsys-102209-144726.

Bójorquez-Mascareño EI, Soto-Jiménez MF. 2016. Isotopic turnover rate and trophic fractionation of nitrogen in shrimp *Litopenaeus vannamei* (Boone) by experimental mesocosms: implications for the estimation of the relative contribution of diets. Aquac Res **47(10):**3070–3087. doi:10.1111/are.12757.

Busst GM, Britton JR. 2017. Tissue-specific turnover rates of the nitrogen stable isotope as functions of time and growth in a cyprinid fish. Hydrobiologia **805:**49–60. doi:10.1007/s10750-017-3276-2.

Carolan JV, Mazumder D, Dimovski C, Diocares R, Twining J. 2012. Biokinetics and discrimination factors for $\delta^{13}C$ and $\delta^{15}N$ in the omnivorous freshwater crustacean, *Cherax destructor*. Mar Freshwater Res **63:**1–9. doi:10.1071/MF11240.

Carvalho D, Williner V, Giri F, Vaccari C, Collins PA. 2016. Quantitative food webs and invertebrate assemblages of a large river: a spatiotemporal approach in floodplain shallow lakes. Mar Freshwater Res **68(2):**293–307. doi:10.1071/MF15095.

Carvalho DA, Reyes P, Williner V, Mora MC, Viozzi MF, De Bonis CJ, Collins PA. 2020. Growth, survival, body composition and amino acid profile of *Macrobrachium borellii* against the limitation of feeds with different C: N ratios with comments about application in integrated multi-trophic aquaculture. Aquac Res **51:**3947–3958. doi:10.1111/are.14696.

Caut S, Angulo E, Courchamp F. 2009. Variation in discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$): the effect of diet isotopic values and applications for diet reconstruction. J Appl Ecol **46(2):**443–53. doi:10.1111/j.1365-2664.2009.01620.x.

Caut S, Angulo E, Courchamp F, Figuerola J. 2010. Trophic experiments to estimate isotope discrimination factors. J Appl Ecol **47(4):**948–954. doi:10.1111/j.1365-2664.2010.01832.x.

Choi JY, Kim SK, La GH, Chang KH, Kim DK, Jeong KY, Park MS, Joo GJ, Kim HW, Jeong KS. 2016. Effects of algal food quality on sexual reproduction of *Daphnia magna*. Ecol Evol **6(9):**2817–2832. doi:10.1002/ece3.2058.

Collins PA. 2005. A coexistence mechanism for two freshwater prawns in the Paraná River floodplains, Argentina. J Crustac Biol **25(2):**219–225. doi:10.1651/C-2528.

Collins PA, Carnevali R, Carvalho D, Williner V. 2012. Dynamics of decapod crustaceans in a trophic web of continental aquatic environments in Southern South America. Chapter 5. *In*: Daniels JA (ed) Advances in Environmental Research, Vol. 21. Nova Publishers, New York, pp. 160–185.

Collins PA, Paggi JC. 1998. Feeding ecology of *Macrobrachium borellii* (Nobili) (Decapoda: Palaemonidae) in the flood valley of the River Paraná, Argentina. Hydrobiologia **362:**21–30. doi:10.1023/A:1003166116086.

Collins PA, Petriella A. 1999. Growth pattern of isolated prawns of *Macrobrachium borellii* (Crustacea, Decapoda , Palaemonidae). Invertebr Reprod Dev **36:**87–91. doi:10.1080/07924259.1999.9652682.

Collins PA, Williner V, Giri F. 2007. Littoral communities. Macrocrustaceans. *In*: Iriondo MH, Paggi JC, Parma JM (eds) The Middle Paraná River: Limnology of a Subtropical Wetland. Springer-Verlag. Heidelberg, Germany, pp. 227–301.

DeNiro MJ, Epstein S. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta **42:**495–506. doi:10.1016/0016-7037(78)90199-0.

deVries MS, Martínez Del Rio C, Tunstall TS, Dawson TE. 2015. Isotopic Incorporation rates and discrimination factors in mantis shrimp crustaceans. PLoS ONE **10(4):**1–16. doi:10.1371/journal.pone.0122334.

Downs EE, Popp BN, Holl CM. 2014. Nitrogen isotope fractionation and amino acid turnover rates in the pacific white shrimp *Litopenaeus vannamei*. Mar Ecol Prog Ser **516:**239–250. doi:10.3354/meps11030.

Fry B, Arnold C, Pierce F. 1982. Rapid $^{13}C/^{12}C$ turnover during growth of brown shrimp (*Penaeus aztecus*). Oecologia **54:**200–204. doi:10.1007/BF00378393.

Fry B, Sherr EB. 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Contrib Mar Sci **27:**13–47. doi:10.1007/978-1-4612-3498-2_12.

Glon MG, Larson ER, Pangle KL. 2016. Comparison of ^{13}C and ^{15}N discrimination factors and turnover rates between congeneric crayfish *Orconectes rusticus* and *O. virilis* (Decapoda, Cambaridae). Hydrobiologia **768:**51–61. doi:10.1007/s10750-015-2527-3.

Gorokhova E. 2018. Individual growth as a non-dietary determinant of the isotopic niche metrics. Methods Ecol Evol **9:**269–277. doi:10.1111/2041-210X.12887.

Hewitt DR. 1992. Response of protein turnover in the brown tiger prawn *Penaeus esculentus* to variation in dietary protein content. Comp Biochem Phys A **103(1):**183–187. doi:10.1016/0300-9629(92)90261-N.

Hobson KA, Atwell L, Wassenaar LI. 1999. Influence of drinking water and diet on the stable hydrogen isotope ratios of animal tissues. Proc Natl Acad Sci U.S.A. **96:**8003–8006. doi:10.1073/pnas.96.14.8003.

Hobson KA, Clark RG. 1992. Assessing avian diets using stable isotopes I: turnover of ^{13}C in tissues. Condor **94:**181–188.

Ingram T, Matthews B, Harrod C, Stephens T, Grey J, Markel R, Mazumder A. 2007. Lipid extraction has little effect on the $\delta^{15}N$ of aquatic consumers. Limnol Oceanogr Methods **5:**338–343. doi:10.4319/lom.2007.5.338.

Jackson AL, Moore JW, Parnell AC, Inger R, Bearhop S, Semmens BX, Phillips DL, Ward EJ. 2014. Best practices for use of stable isotope mixing models in food-web studies. Can J Zool **92(10):**823–835. doi:10.1139/cjz-2014-0127.

Karasov WH, Martínez del Rio C. 2007. Physiological ecology: How animals process energy, nutrients, and toxins. Chapter Eigth. Book for Princeton University Press, Princeton, New Jersey, USA, pp. 433–472.

Latli A, Sturaro N, Desjardin N, Michel LN, Otjacques W, Lepoint G, Kestemont P. 2017. Isotopic half-life and enrichment factor in two species of European freshwater fish larvae: an experimental approach. Rapid Commun Mass Spectrom **31(8):**685–692. doi:10.1002/rcm.7838.

Lefebvre S, Dubois S. 2016. The stony road to understand isotopic enrichment and turnover rates: insight into the metabolic part. Vie Et Milieu-life And Environment **66 (3-4):**305–314.

Lipták B, Veselý L, Ercoli F, Bláha M, Buřič M, Ruokonen TJ, Kouba A. 2019. Trophic role of marbled crayfish in a lentic freshwater ecosystem. Aquat Invasions **14(2):**299–309. doi:10.3391/ai.2019.14.2.09.

Logan JM, Haas H, Deegan L, Gaines E. 2006. Turnover rates of nitrogen stable isotopes in the salt marsh mummichog, *Fundulus Heteroclitus*, following a laboratory diet switch. Oecologia **147(3):**391–395. doi:10.1007/s00442-005-0277-z.

Macko SA, Fogel ML, Engel MH, Hare PE. 1986. Kinetic fractionation of stable nitrogen isotopes during amino acid transamination. Geochim Cosmochim Acta **50:**2143–2146. doi:10.1016/0016-7037(86)90068-2.

Mao Z, Xiaohong G, Zeng Q. 2016. Food sources and trophic relationships of three decapod crustaceans: insights from gut contents and stable isotope analyses. Aquac Res **47:**2888–2898. doi:10.1111/are.12739.

March JG, Pringle CM, Townsend MJ, Wilson AI. 2002. Effects of freshwater shrimp assemblages on benthic communities along an altitudinal gradient of a tropical island stream. Freshw Biol **47(3):**377–390. doi:10.1046/j.1365-2427.2002.00808.x.

Marchese MR, Saigo M, Zilli FL, Capello S, Devercelli M, Montalto L, Paporello G, Wantzen KM. 2014. Food webs of the Paraná River floodplain: assessing basal sources using stable carbon and nitrogen isotopes. Limnologica **46:**22–30. doi:10.1016/j.limno.2013.11.004.

Martínez del Rio C. 2008. Metabolic theory or metabolic models? Trends Ecol Evol **23(5):**256–260. doi:10.1016/j.tree.2008.01.010.

Martínez del Rio C, Anderson-Sprecher R. 2008. Beyond the reaction progress variable: the meaning and significance of isotopic incorporation data. Oecologia **156:**765–772. doi:10.1007/s00442-008-1040-z.

Martínez del Rio C, Carleton SA. 2012. How fast and how faithful: the dynamics of isotopic incorporation into animal tissues. J Mammal **93(2):**353–359. doi:10.1644/11-MAMM-S-165.1.

Martínez del Rio C, Wolf N, Carleton SA, Gannes LZ. 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biol Rev Camb Philos Soc **84(1):**91–111. doi:10.1111/j.1469-185X.2008.00064.x.

Mccutchan JH, Lewis WM, Kendall C, Mcgrath CC. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos **102:**378–390. doi:10.1034/j.1600-0706.2003.12098.x.

Mente E, Coutteau P, Houlihan D, Davidson I, Sorgeloos P. 2002. Protein turnover, amino acid profile and amino acid flux in juvenile shrimp *Litopenaeus vannamei*: effects of dietary protein source. J Exp Biol **205:**3107–3122. doi:10.1242/jeb.205.20.3107.

Minagawa M, Wada E. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between δ^{15}N and animal age. Geochim Cosmochim Acta **48:**1135–1140. doi:10.1016/0016-7037(84)90204-7.

Newsome SD, Martínez del Río C, Bearhop S, Phillips DL. 2007. A niche for stable isotope ecology. Front Ecol Environ **5(8):**429–436. doi:10.1890/060150.01.

Parker PL, Anderson RK, Lawrence A. 1989. A δ^{13}C and δ^{15}N tracer study of nutrition in aquaculture: *Penaeus vannamei* in a pond growout system. Stable isotopes in ecological research. Springer, New York, pp. 288–303.

Perga ME, Grey J. 2010. Laboratory measures of isotope discrimination factors: comments on Caut, Angulo & Courchamp (2008, 2009). J Appl Ecol **47(4):**942–947. doi:10.1111/j.1365-2664.2009.01730.x.

Peterson B, Fry BJ. 1987. Stable isotopes in ecosystem. Annu Rev Ecol Evol Syst **18(1):**293–320. doi:10.1146/annurev.es.18.110187.001453.

Phillips DL, Gregg JW. 2003. Source partitioning using stable isotopes: coping with too many sources. Oecologia **136:**261–269. doi:10.1371/journal.pone.0009672.

Post DM. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology **83(3):**703–718. doi:10.1111/fwb.12727.

R Core Team. 2019. R: a Language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available at http://www.R-project.org/. Accessed 5 Feb. 2018.

Robbins CT, Felicetti LA, Florin ST. 2010. The impact of protein quality on stable nitrogen isotope ratio discrimination and assimilated diet estimation. Oecologia **162(3):**571–579. doi:10.1007/s00442-009-1485-8.

Sabat P, Ramirez-Otarola N, Bozinovic F, Martínez del Rio C. 2013. The isotopic composition and insect content of diet predict tissue isotopic values in a south american passerine assemblage. J Comp Physiol B, Biochem Syst Environ Physiol **183(3):**419–430. doi:10.1007/s00360-012-0711-6.

Saigo M, Marchese MR, Wantzen KM. 2016. Sources contribution for benthic invertebrates: an inter-lake comparison in a flood plain system. Hydrobiologia **770(1):**27–36. doi:10.1007/s10750-015-2565-x.

Saigo M, Zilli FL, Marchese MR, Demonte D. 2015. Trophic level, food chain length and omnivory in the Paraná River: A food web model approach in a floodplain river system. Ecol Res **30:**843–852. doi:10.1007/s11284-015-1283-1.

Schmidt K, McClelland J, Mente E, Montoya J, Atkinson A, Voss M. 2007. Trophic-level interpretation based on δ^{15}N values: implications of tissue-specific fractionation and amino acid composition. Mar Ecol Prog Ser **266:**43–58. doi:10.3354/meps266043.

Schoeller DA, Minagawa M, Slater R, Kaplan IR. 2010. Stable isotopes of carbon, nitrogen and hydrogen in the contemporary north American human food web. Ecol Food Nutr **18:**159–170. doi:10.1080/03670244.1986.9990922.

Thomas SM, Crowther TW. 2015. Predicting rates of isotopic turnover across the animal kingdom: a synthesis of existing data. J Anirn Ecol **84:**861–870. doi:10.1111/1365-2656.12326.

Tieszen LL, Boutton TW, Tesdahl KG, Slade NA. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for δ^{13}C analysis of diet. Oecologia **57:**32–37. doi:10.1007/BF00379558.

Vander Zanden MJ, Clayton MK, Moody EK, Solomon CT, Weidel BC. 2015. Stable isotope turnover and half-life in animal tissues: A literature synthesis. PLoS ONE **10:**1–16. doi:10.1371/journal.pone.0116182.

Vanderklift MA, Ponsard S. 2003. Sources of variation in consumer-diet δ^{15}N enrichment: a meta-analysis. Oecologia **136(2):**169–182. doi:10.1007/s00442-003-1270-z.

Vogt G. 2012. Ageing and longevity in the Decapoda (Crustacea): A review. Zool Anz **251(1):**1–25. doi:10.1016/j.jcz.2011.05.003.

Wolf N, Carleton S, Martínez del Rio C. 2009. Ten years of experimental animal isotopic ecology. Funct Ecol **23:**17–26. doi:10.1111/j.1365-2435.2009.01529.x.

Yokoyama H, Tamaki A, Harada K, Shimoda K, Koyama K, Ishihi Y. 2005. Variability of diet-tissue isotopic fractionation in estuarine macrobenthos. Mar Ecol Prog Ser **296:**115–128. doi:10.3354/meps296115.

Supplementary materials

Original data in the present study. (download)