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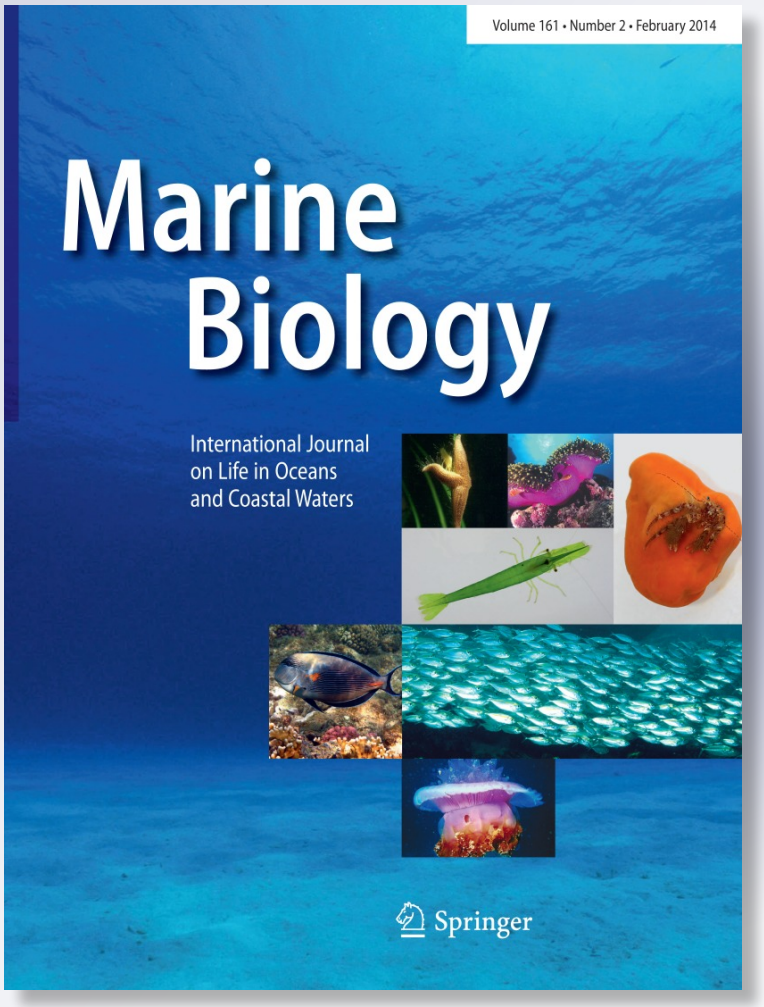

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METHOD

Determination of $\delta^{13}C$ and $\delta^{15}N$ and trophic fractionation in jellyfish: implications for food web ecology

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Abstract Application of stable isotope analysis (SIA) in jellyfish allows definition of trophic patterns not detectable using gut content analysis alone, but analytical protocols require standardization to avoid bias in interpreting isotopic data. We determined $\delta^{13}C$ and $\delta^{15}N$ in *Aurelia* sp. from the northern Gulf of Mexico (30°00′N, 89°00′W–30°24′N, 88°00′W) to define differences in stable isotope composition between body parts and whole body, the effect of lipid extraction on $\delta^{13}C$ in tissues, and fractionation values from medusa to prey. The isotopic composition of bell and whole *Aurelia* sp. was not different. The increase in $\delta^{13}C$ values after lipid removal suggested a correction is needed. To aid future analyses, we derived a correction equation from empirical data for jellyfish samples. Laboratory feeding experiments indicated medusae increased +4 ‰ in $\delta^{13}C$ and +0.1 ‰ in $\delta^{15}N$ compared to their diet. These results suggest protocols commonly applied for other species may be inaccurate to define *Aurelia* sp. trophic ecology. Because *Aurelia* spp. are commonly found in marine ecosystems, accurately defining their trophic role by use of SIA has implications for understanding marine food webs worldwide.

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Introduction

Despite their potential importance within marine food webs, the trophic position of jellyfish is not well defined (Pauly et al. 2009). In part, understanding jellyfish trophic ecology is difficult due to their relatively fast digestion rates, heterogeneous prey fields, and diel feeding patterns (Pitt et al. 2009a; Purcell 2009). Jellyfish digestion rates average 2 ± 1 h (Purcell 2009), with significant species-specific and individual differences (Båmstedt and Martinussen 2000; Purcell 2009). There is also variation due to the type of prey ingested, with hard-bodied zooplankton having relatively longer gut residence times compared to soft-bodied prey (Purcell et al. 1991; Martinussen and Båmstedt 1999). Vertical movements of jellyfish and their zooplankton prey often result in different feeding patterns between day and night (Pitt et al. 2008), further complicating dietary analyses. Because jellyfish are relatively abundant and ubiquitous to coastal ecosystems worldwide, difficulty in accurately defining their trophic position and prey base limits our understanding of marine food webs in general (Pauly et al. 2009).

Most studies to date have relied on gut content analysis to define jellyfish trophic ecology (Purcell 2009). These studies focused primarily on trophic linkages between jellyfish and readily visualized mesozooplankton, but may have underestimated the role of microzooplankton and dissolved organic matter that are more difficult to detect (Purcell 2009). Gut contents also have limited application to the quantification of energy transfer via jellyfish in food

webs because they define ingested rather than assimilated prey (Pitt et al. 2009a). Hence, gut content analysis must be combined with other approaches to better define jellyfish trophic ecology (Pitt et al. 2009a).

Stable isotope analysis (SIA) provides a suitable method to define assimilated foods and trophic linkages not detectable using gut contents alone (Pitt et al. 2009a). Stable isotopes yield a relatively longer-term integrated reconstruction of assimilated prey (Peterson and Fry 1987) that has allowed better understanding of temporal and spatial variation in jellyfish diet, including identification of diel feeding patterns (Malej et al. 1993; Brodeur et al. 2002; Pitt et al. 2008; Frost et al. 2012). However, few studies have applied SIA to assess jellyfish diet, and the results have been inconsistent, likely due to analysis of different tissue types and diverse sample processing and handling. This variation among findings from previous studies suggests protocols to determine and interpret stable isotopes of jellyfish need to be standardized (Pitt et al. 2009a; Fleming et al. 2011).

Based on previous application of SIA to jellyfish trophic ecology (Malej et al. 1993; Brodeur et al. 2002; Pitt et al. 2008; Frost et al. 2012), three major sources of bias challenge the determination and interpretation of $\delta^{13}C$ and $\delta^{15}N$ values in such species. Firstly, there may be differences in the stable isotope composition of body parts compared to the whole body. The one study that attempted to determine $\delta^{13}C$ and $\delta^{15}N$ in the scyphomedusa *Phacellophora camtschatica* found $\delta^{13}C$ and $\delta^{15}N$ varied among oral arms, gonads, and whole (Towanda and Thuesen 2006). Detailed tissue-specific study is needed to define the significance of these differences to interpreting trophic linkages for jellies. Secondly, the lipid content of jellies (22 ± 12 % of total organic matter, Pitt et al. 2009b) may result in decreased $\delta^{13}C$ values that require correction to accurately define diet (Post et al. 2007; Pitt et al. 2009a; Frost et al. 2012). Thirdly, the magnitude of fractionation from consumer to diet has not been clearly defined for jellyfish. To determine potential prey of jellyfish, $\delta^{13}C$ and $\delta^{15}N$ of predators and prey have been interpreted using standard expected trophic shifts +1 ‰ for C and 3–4 ‰ for N (Pitt et al. 2008; Frost et al. 2012). Because field data indicate jellyfish have large fractionation values for C and small for N (D'Ambra 2012; Frost et al. 2012), determining fractionation values in the laboratory is necessary to accurately define jellyfish trophic linkages.

In this study, we collected data to inform unbiased application of SIA to better define the trophic ecology of jellyfish, using *Aurelia* sp. (Scyphozoa, Semaeostomeae) as a globally relevant model genus (Purcell 2009). Previously reported variation in dietary composition for *Aurelia* spp., which are commonly found worldwide, has been attributed to a combination of location-specific feeding patterns and failure of gut content analysis to accurately detect consumed prey (Purcell 2009). To define trophic linkages and account for the three major sources of bias (tissue-specific variation, lipid composition, species-specific fractionation), we determined (1) potential differences in stable isotope composition between body parts by comparing $\delta^{13}C$ and $\delta^{15}N$ of bell, oral arms, gonads, and whole *Aurelia* sp., (2) the effect of lipid removal on $\delta^{13}C$ by comparing the $\delta^{13}C$ of bulk (before lipid removal) and lipid-extracted (after lipid removal) samples, and (3) fractionation values from medusae to their diet in a controlled laboratory setting using a diet of known and constant stable isotope composition.

Materials and methods

Sample collection and processing for SIA

Aurelia spp. were collected in the northern Gulf of Mexico coastal waters (30°00′N, 89°00′W–30°24′N, 88°00′W) from August to October 2010 using a 1-cm mesh dip net ($N = 31$). Medusae were kept in buckets with filtered seawater from the sampling site to allow gut evacuation and then were transported to the laboratory in acid-washed plastic jars on ice. Bell diameter of individual medusae was measured as inter-rhopalia distance (±0.1 cm). To make direct comparison of whole-body samples to individual body parts, we divided freshly caught medusae in half. One half was processed undissected as a whole-body sample, the other half was dissected into bell, oral arms, and gonads. Samples were rinsed with ultrapure water, dried to constant mass at 60 °C, and homogenized using mortar and pestle. To determine the percentage contribution of each body part to the mass of the whole body, we weighed each dried sample on an analytical scale (±0.1 mg).

Lipid removal

The effect of lipid removal on $\delta^{13}C$ values was quantified by extracting lipids from 31 dried samples of body parts and whole *Aurelia* spp. selected on the basis of their C:N values to ensure a representative subsample of lipid contents as expected from the relationship between C:N and lipid content of tissues (Post et al. 2007; Logan et al. 2008). Homogenized tissue (5 mg) was immersed in 2:1 chloroform–methanol in a 15-ml vial for 30 min. Samples were centrifuged at 5,000 cycles min^{-1} for 10 min, and the supernatant was removed. New solution was added to ensure the sample was fully immersed in the solution and the procedure repeated three times. Samples were then centrifuged twice with ultrapure water to remove the solvent. Samples were dried at 60 °C to constant mass and re-homogenized using mortar and pestle.

Determination of fractionation values

A total of 24 medusae were collected at the same site using a dip net and transported in buckets with filtered seawater from the collection site to a wet laboratory facility at the Dauphin Island Sea Lab to determine fractionation values from *Aurelia* spp. to their prey. Three medusae were immediately processed for SIA of whole body and body parts as described above. The remaining 21 medusae were placed in kreisel tanks (Raskoff et al. 2003) containing 200 L of 0.2 μm filtered seawater maintained at 28.0 ± 0.5 °C temperature and 30.0 ± 0.7 psu salinity, conditions similar to those at the sampling site. To ensure jellies acclimated to their controlled diet in the laboratory and to monitor the rate of stable isotope ratio acquisition, we removed and analyzed three *Aurelia* spp. for SIA every 3 days after capture for a total of 21 days. In the kreisels, medusae were fed ad libitum with Cyclopeeze®, frozen *Cyclops* spp. copepods. We determined $\delta^{13}C$ and $\delta^{15}N$ in the diet at the beginning, middle, and end of the experiment. Diet samples were dried at 60 °C to constant mass and homogenized using mortar and pestle. $\delta^{15}N$ of the copepod diet averaged 12.5 ± 0.1 ‰ ($N = 3$). Because C:N values of diet averaged 5.59 ± 0.03, samples were lipid-extracted as described above, based on expected relationship between C:N and lipid contents described by Post et al. (2007). After lipid removal, C:N values of diet lowered to 3.6 ± 0.2 and the $\delta^{13}C$ increased from −25.3 ± 0.1 to −22.6 ± 0.1 ‰.

Stable isotope analyses and C and N contents

$\delta^{13}C$, $\delta^{15}N$ and C and N contents of samples were determined from 4.0 ± 0.3 mg of bulk and 8.0 ± 0.5 mg of lipid-extracted dried biomass of *Aurelia* spp. and 1.0 ± 0.2 mg of bulk and lipid-extracted dried diet to provide sufficient organic sample for SIA and account for the relatively high salt content of jellyfish (Frost et al. 2012) and low carbon content of samples (Table 1). Samples were sent to the Stable Isotope Facility for Environmental Research (SIRFER) at the University of Utah (USA) for analysis by isotope ratio mass spectroscopy. C and N stable isotope ratios were determined using a Finnigan Delta Plus (Bremen, Germany) isotope ratio mass spectrometer coupled with a Carlo Erba elemental analyzer (model 1110; Milan, Italy) through a Finnigan CONFLO III open split interface (Bremen, Germany). During analyses, samples were interspersed with replicates of at least two different laboratory standards. These laboratory standards, which were selected to be compositionally similar to the samples being analyzed, were previously calibrated against standard reference materials from the National Institute of Standards and Technology and International Atomic Energy Agency. Final δ values were expressed as ‰ relative to international standards, PeeDee Belemnite for C, and Air for N. The long-term standard deviations were 0.1 ‰ for $\delta^{13}C$ and 0.2 ‰ for $\delta^{15}N$. C and N contents in samples was determined during SIAs.

Table 1 Biochemical and stable isotope composition (mean ± SD) of bell, oral arms, gonads, and whole wild-caught *Aurelia* spp. ($N = 31$) collected in the northern Gulf of Mexico from August to October 2010 (plain font) and laboratory-reared medusae ($N = 6$) after reaching steady state with the laboratory diet (italics)

Body part	Dry mass (%)	$\delta^{13}C$ (‰)	$\delta^{15}N$(‰)	C:N
Bell	84.1 ± 5.3	−18.3 ± 0.4	11.5 ± 0.5	3.5 ± 0.2
	–	*−18.5 ± 0.3*	*12.5 ± 0.3*	*3.5 ± 0.1*
Oral arms	10.6 ± 4.3	−18.8 ± 0.9	12.0 ± 0.7	3.5 ± 0.3
	–	*−19.9 ± 0.4*	*13.2 ± 0.3*	*3.8 ± 0.1*
Gonads	5.3 ± 2.7	−19.6 ± 0.7	11.6 ± 0.9	3.7 ± 0.3
	–	*−20.4 ± 0.8*	*12.9 ± 0.4*	*3.9 ± 0.2*
Whole body	–	−18.7 ± 0.7	11.8 ± 0.7	3.6 ± 0.2
	–	*−19.0 ± 0.5*	*12.8 ± 0.3*	*3.7 ± 0.1*

Statistical analyses

A one-way analysis of variance (ANOVA) was performed for each stable isotope ratio to determine differences in $\delta^{13}C$ and $\delta^{15}N$ among individual body parts and whole body in wild-caught and laboratory-fed medusae (after reaching steady state with their diet in laboratory at day 16). Normal distribution and homogeneity of variance of the parameters were verified prior to ANOVA using Bartlett's and Levene's tests, followed by Tukey's post hoc comparisons in the case of significant differences.

Bulk (before lipid removal) and lipid-extracted (after lipid removal) $\delta^{13}C$, $\delta^{15}N$, and C:N of *Aurelia* spp. were compared by paired *t* test to determine the effect of lipid removal on $\delta^{13}C$ and $\delta^{15}N$. To derive the mathematical correction for lipid-extracted samples, we regressed $\Delta\delta^{13}C$ (the difference between lipid-extracted and bulk $\delta^{13}C$) against the bulk C:N of all samples from which lipids were extracted according to Post et al. (2007) and used the equation for the best fit line through the data points. This equation is presented as a potential tool to increase the accuracy of $\delta^{13}C$ determinations for *Aurelia* spp. in the same range of C:N values in this study. We applied a generalized lipid correction equation (Post et al. 2007) to the *Aurelia* sp. dataset and compared the difference between our equation and the generalized correcting equation (Post et al. 2007) using a paired *t* test.

Fractionation values were determined for C and N as the difference between the corrected $\delta^{13}C$ and bulk $\delta^{15}N$ values of *Aurelia* sp. tissues after reaching steady state with the

diet and the mean lipid-free $\delta^{13}C$ and bulk $\delta^{15}N$ of the diet. To determine the stable isotope ratios of medusa tissues after reaching steady state with the diet, we fitted lipid-extracted $\delta^{13}C$ and bulk $\delta^{15}N$ values as a function of experimental days with the sigmoid function:

$$y(t) = y_0 + a/\left(1 + e^{-(t-t_{50})/b}\right) \quad (1)$$

where *y(t)* is the stable isotope ratio in *Aurelia* sp. tissues at time *t*, y_0 is the stable isotope ratio in *Aurelia* sp. tissues after reaching steady state with the diet, *a* is the absolute difference between initial and final (after reaching steady state) stable isotope ratios in *Aurelia* sp. tissues, *b* is the isotopic turnover rate, and t_{50} is the isotopic half-life.

Tests were considered significant at $P < 0.05$. Errors were propagated throughout calculations according to Taylor (1996).

Results

Tissue-specific differences in stable isotope composition

One-way ANOVA comparing stable isotope ratios in different tissues of wild-caught *Aurelia* sp. showed at least one tissue had significantly different $\delta^{13}C$ ($F_{(3,80)} = 12.27$, $P < 0.001$) and C:N ($F_{(3,80)} = 3.42$, $P = 0.02$) compared to the other tissues. Tukey's post hoc comparisons indicated gonads had significantly lower $\delta^{13}C$ and higher C:N compared to bell, oral arms, and whole-body tissues (Table 1). In contrast, $\delta^{15}N$ values did not differ among tissue types ($F_{(3,80)} = 2.09$, $P = 0.11$). The 31 *Aurelia* spp. analyzed to determine differences in the stable isotope composition of tissues averaged 28.2 ± 3.6 cm in bell diameter. Bell made the highest contribution to the dry mass of whole *Aurelia* spp. (~84 %), while the contribution of oral arms and gonads was ~16 % (Table 1).

Similarly, one-way ANOVA highlighted differences among tissue of laboratory-fed medusae in $\delta^{13}C$ ($F_{(3,32)} = 25.15$, $P < 0.001$) and C:N ($F_{(3,32)} = 14.39$, $P < 0.001$). Tukey's post hoc comparisons indicated oral arms and gonads had significantly lower $\delta^{13}C$ and higher C:N compared to bell and whole-body tissues. In contrast to wild-caught *Aurelia* spp., $\delta^{15}N$ was significantly different in tissues of laboratory-fed medusae at steady state with their diet ($F_{(3,32)} = 7.63$, $P = 0.001$). Oral arms resulted to have higher $\delta^{15}N$ compared to bell, gonads, and whole *Aurelia* spp. The bell diameter of laboratory-fed medusa used for the inter-tissue comparison averaged 29.7 ± 1.6 cm. In conclusion, inter-tissue comparison of both wild-caught and laboratory-fed medusae after reaching steady state with the laboratory diet indicated no difference in $\delta^{13}C$ and $\delta^{15}N$ of bell and whole *Aurelia* spp.

Lipid removal and correction

Lipid extraction resulted in a significant decrease in C:N from 3.7 ± 0.3 to 3.5 ± 0.1 ($t_{30} = -4.26$, $P < 0.001$) among the 31 *Aurelia* spp. samples. $\delta^{15}N$ values increased from 10.9 ± 1.0 ‰ in bulk samples to 11.8 ± 0.8 ‰ in lipid-free samples ($t_{30} = -4.14$, $P < 0.001$). Bulk $\delta^{13}C$ values averaged −19.0 ± 1.0 ‰, which was significantly lower compared to $\delta^{13}C$ values in lipid-extracted samples (−18.3 ± 0.6 ‰; $t_{30} = -6.58$, $P < 0.001$). The regression equation comparing $\Delta\delta^{13}C$ to bulk C:N ($r^2 = 0.69$, $F_{(1,21)} = 47.28$, $P < 0.001$, Fig. 1) was:

$$\Delta\delta^{13}C = -9.43 + 2.69 * C{:}N_{bulk} \quad (2)$$

The $\delta^{13}C$ values corrected using Eq. 2 averaged −18.4 ± 0.8 ‰ and were significantly different from values predicted by the generalized lipid correction equation by Post et al. (2007), which averaged −18.6 ± 0.8 ‰ ($t_{30} = 2.31$, $P = 0.028$, Fig. 1).

Fractionation values

Fractionation values from whole *Aurelia* spp. to the available diet under controlled conditions were ~4 ‰ for C and ~0.1 ‰ for N (Table 2). Because the majority of medusa body was comprised of bell and the stable isotope composition of bell and whole body of *Aurelia* sp. were similar (Table 1), we opted to limit the determination of fractionation values to bell and whole body. Diet shift response models for bell and whole body were similar (Table 2), and therefore, we show only the stable isotope shifts in whole *Aurelia* spp. in response to the shift in the diet associated with laboratory rearing (Fig. 2). We did not include growth in the models because the difference in mean bell diameters of medusae at the beginning (29.1 ± 1.7 cm) and the end of the experiment

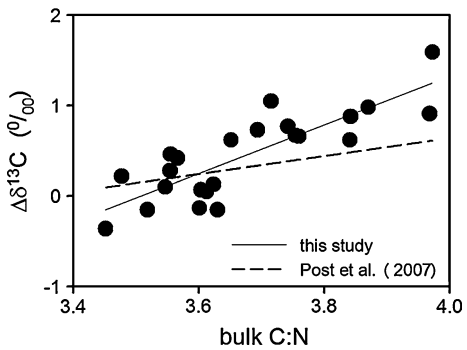

Fig. 1 Relationships between bulk C:N and $\Delta\delta^{13}C$ (the difference between lipid-extracted and bulk $\delta^{13}C$) derived from lipid-extracted samples ($N = 31$) of *Aurelia* spp. from the northern Gulf of Mexico (*continuous line*) and calculated using a non-specific correction equation (Post et al. 2007; *dashed line*)

Table 2 Parameters and fractionation values (Δ) (mean ± SD) of carbon and nitrogen estimated using the sigmoid function fitting $\delta^{13}C$ and $\delta^{15}N$ in bell and whole *Aurelia* spp. as a function of the days medusae were fed a diet of known stable isotope composition in the laboratory

	a (‰)	*b* (‰ d^{-1})	t_{50} (d)	y_0 (‰)	*P*	Δ (‰)
Carbon						
Bell	0.9 ± 0.3	(−)1.3 ± 2.0	10.8 ± 2.4	−18.3 ± 0.2	0.004	4.3 ± 0.2
Whole body	0.8 ± 0.4	(−)1.2 ± 2.2	9.7 ± 3.1	−18.5 ± 0.3	0.016	4.2 ± 0.3
Nitrogen						
Bell	1.4 ± 1.2	2.2 ± 1.6	9.7 ± 3.1	11.1 ± 0.9	0.033	0.1 ± 0.2
Whole body	1.8 ± 0.6	1.7 ± 1.9	10.3 ± 2.1	11.1 ± 0.4	0.001	0.1 ± 0.2

Parameters of the sigmoid function are absolute difference between initial and final δ values (*a*), isotopic turnover rate (*b*), isotopic half–life (t_{50}), and significance of the model (*P*)

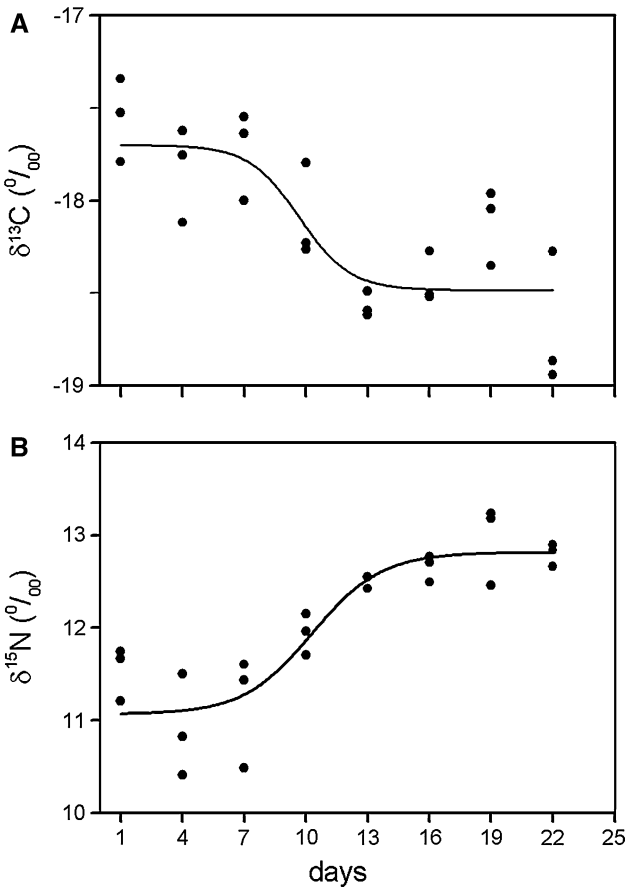

Fig. 2 Changes in A) $\delta^{13}C$ and B) $\delta^{15}N$ in whole-body samples of *Aurelia* spp. as a function of days they were maintained on a diet of constant stable isotope composition in the laboratory

(29.4 ± 1.8 cm) did not differ significantly ($t_{39} = -0.50$, $P = 0.62$). Hence, the calculated rates of stable isotope turnover (~1 ‰ day^{-1} for C and 2 ‰ day^{-1} for N) reflect basal tissue turnover rather than addition of tissue and resulted in *Aurelia* tissues reaching stable isotopic steady state with laboratory diet at 18–20 days (based on a half-life of ~9–10 days).

Discussion

Application of SIA has provided valuable insight into jellyfish trophic ecology (Brodeur et al. 2002; Pitt et al. 2008; Frost et al. 2012), but has been limited by a lack of standardized analytical protocols and potential bias in determination and interpretation of stable isotope values (Pitt et al. 2009a; Fleming et al. 2011). Our results provide data to improve the accuracy and determination of $\delta^{13}C$ and $\delta^{15}N$ in *Aurelia* sp. Unlike some other species in which tissues of different stable isotope composition comprise a large portion of the whole body and can confound trophic analyses (Lorrain et al. 2002; Miller 2006), stable isotope composition, turnover rates, and fractionation values in *Aurelia* sp. are dominated by bell, and therefore are similar between bell and whole-body samples. These similarities imply that assessment of diet composition, trophic level, and their temporal resolution can be inferred from analysis of either the bell or whole *Aurelia* sp. These findings suggest that processing *Aurelia* spp. for SIA can be simplified by using the bell (or a portion of it) for analysis, avoiding the need to dry whole bodies of often very large scyphomedusae and eliminating meticulous cleaning of entangled zooplankton from oral arms. Additionally, determining the stable isotope composition of the bell rather than other body parts of medusa potentially reduces the bias due to lipid content of tissues (Table 1). Given the importance of accurately defining trophic level and food web linkages for jellyfish (Pauly et al. 2009), these findings facilitate future SI-based studies in jellyfish trophic ecology.

Differences in stable isotope composition of tissues may be due to lipid content (Lorrain et al. 2002) and turnover rates of tissues (Miller 2006). Gonads in wild-caught and laboratory-fed medusae had higher C:N values compared to bell, oral arms, and whole body, and had lower $\delta^{13}C$ as expected from the relationship between $\delta^{13}C$ and C:N (Post et al. 2007). Similarly, the higher lipid content may explain the low $\delta^{13}C$ of oral arms compared to the other body parts and whole laboratory-fed medusae. To

unfold the difference in $\delta^{15}N$, we suggest turnover rates and fractionation values in oral arms may be different from other body parts and whole *Aurelia* sp. This pattern could be confounded in wild-caught medusae, which is the reason why laboratory-fed organisms are preferred for inter-tissue comparisons (Lorrain et al. 2002; Miller 2006). Regardless of the mechanism driving the differences in tissues, inter-tissue comparisons of wild-caught and laboratory-fed medusae after reaching steady state with their diet suggest the bell is the most suitable body part to accurately determine stable isotope composition in *Aurelia* sp.

The significant increase in $\delta^{13}C$ values after lipid removal suggested lipid extraction is an important step for accurate $\delta^{13}C$ determination in jellyfish. Accordingly, the C:N in most samples was >3.5, a value suggested to indicate correction of $\delta^{13}C$ values is needed (Post et al. 2007). Because the x-intercept of the correction equation proposed in this study is at C:N = 3.5, this value appears to be a cutoff also for *Aurelia* spp. Overall the C:N values we measured in *Aurelia* spp. in this study are consistent with previously reported dominance of proteins in the organic content of jellyfish (Arai 1997; Pitt et al. 2009b). Although an increase in $\delta^{13}C \sim 0.7$ ‰ may appear biologically insignificant, inaccurate $\delta^{13}C$ values may bias food source partitioning using mixing models (Parnell et al. 2010). Given that our C:N values were in the range reported for *Aurelia* spp. from different locations (Arai 1997), our data suggest lipid extraction will likely be necessary and should be evaluated in all cases before applying $\delta^{13}C$ values in jellyfish trophic ecology.

Because protocols to remove lipids are time consuming, they can be replaced by mathematical normalization (Post et al. 2007). Our data and others demonstrate, however, that generalized corrections may be inaccurate for specific datasets (Logan et al. 2008). Application of a non-specific lipid correction yielded potentially unfeasible diet compositions for jellyfish in Dogger Bank, North Sea (Frost et al. 2012). Differences between the equation derived in this study and the generalized correction equation were remarkable at C:N > 3.6. Because C:N > 3.6 appear to be common among *Aurelia* spp. from different locations (Arai 1997), we propose a correction in this study to refine determination of $\delta^{13}C$ and trophic relationships in *Aurelia* spp. and other species of similar biochemical composition.

Fractionation values determined in this study are important to define *Aurelia* sp. trophic linkages. In the absence of controlled laboratory studies, determination of assimilated diet based on stable isotope values in jellyfish relied on fractionation values derived from other species (Malej et al. 1993; Pitt et al. 2008, Frost et al. 2012). Laboratory determinations in this study indicated that fractionation values generally applied in SIA (McCutchan et al. 2003; Vanderklift and Ponsard 2003) may be inaccurate to define trophic shifts from *Aurelia* spp. to available prey. Our results indicated *Aurelia* spp. show tissue fractionation values much greater than typical for C (~4 ‰ in *Aurelia* sp. compared to +1 ‰ in many other species; McCutchan et al. 2003) and less than typical for N (<1 ‰ in *Aurelia* sp. compared to a range of 2–4 ‰ for other species; Vanderklift and Ponsard 2003). Stable isotope ratios of jellyfish in the northern Gulf of Mexico (D'Ambra 2012) and Dogger Bank, North Sea (Frost et al. 2012) differed from those of their potential prey by values similar to the fractionation values determined in this study. The similarity between laboratory determinations and field observations suggests jellyfish may have atypical fractionation values compared to other species, but studies are needed to prove whether the pattern observed in *Aurelia* spp. is common to all gelatinous species.

It is difficult to explain the unusual fractionation values observed in *Aurelia* sp. because of the gaps in the knowledge of scyphozoan metabolic processes. It is unknown what proportions of lipids, carbohydrates, or proteins are oxidized in scyphozoan respiration (Arai 1997). Because scyphozoan body is mostly made of proteins, it is likely that they provide the substrate for respiration in scyphomedusae, but this mechanism has not been described (Arai 1997). Whether lipids or proteins are used in respiration may affect discrimination of C and N stable isotopes, and consequently fractionation values from medusae to their diet. Although excretion rates have been determined in some scyphozoan species (revised in Arai 1997), the proportion of ammonium compared to other nitrogenous compounds excreted is unknown. Because the revision of fractionation values determined under controlled conditions in 134 animals highlighted that ammonotelic animals had smaller trophic enrichment compared to their diet than ureotelic organisms (Vanderklift and Ponsard 2003), the dominance of ammonium among excretion products may partially explain the unusually small fractionation values for N observed in *Aurelia* sp. Hence, given the close relationship between physiological processes and fractionation values in animals (Peterson and Fry 1987), laboratory studies are needed to define metabolic pathways in *Aurelia* spp. and their effect on stable isotope fractionation from medusae to their prey.

In addition to physiological processes, diet quality and feeding rates may explain the unusual fractionation values observed in this study. We provided the experimental organisms a diet with high content in lipids and low in proteins (C:N > 5.5), which may explain the large fractionation values for C and small for N (Peterson and Fry 1987). Additionally, we fed experimental medusae ad libitum. Focken (2001) and Gaye-Siesseger et al. (2003, 2004)

found that feeding levels affected fractionation values in the Nile tilapia, *Oreochromis niloticus*, likely because at low feeding rates, anabolic processes dominate the metabolism, while at high feeding levels, catabolic processes may predominate. Our findings are a first attempt to define fractionation values of jellyfish in controlled conditions; hence, additional experimental work is needed to refine fractionation values for *Aurelia* sp. and other jellyfish species.

Determination of fractionation values can be difficult because of the challenges in selecting a diet of stable isotope composition sufficiently different from the stable isotope composition of the consumer to clearly indicate when the consumer has reached the steady state with the laboratory diet. We provided our experimental medusae frozen *Cyclops* spp. because of their fairly consistent stable isotope composition. However, we have to remark that stable isotope spacing for N was reduced between consumer and prey, which makes the determination of the steady state between consumer and diet not completely unambiguous. This uncertainty has implications for the definition of fractionation values in this study. Despite this *caveat*, our results provide a baseline to interpret the trophic shift from *Aurelia* spp. to their prey. Future experiments to refine fractionation values in *Aurelia* sp. and other jellyfish species should make use of a diet with sufficient end member spacing to unambiguously define the steady state between jellyfish and their diet.

Although jellyfish trophic ecology has been studied in different areas, dietary composition and trophic position remain poorly defined for most species and systems (Pauly et al. 2009). Application of SIA can help better define dietary composition and trophic position for jellyfish throughout their range, but protocols to determine and interpret stable isotope values need to be consistent and yield accurate results. Our data inform an unbiased determination and interpretation of $\delta^{13}C$ and $\delta^{15}N$ in the cosmopolitan *Aurelia* spp. to aid future SI-based studies, better define trophic ecology for jellyfish in general, and better define their potentially significant role in marine food webs.

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