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δ13C, δ15N, and C:N ratios as nutrition indicators of zooxanthellate jellyfishes: insights from an experimental approach

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Abstract :

Some jellyfish host zooxanthellae in their tissues (mostly from the family Symbiodiniaceae; Dinophyceae) and supplement their heterotrophic nutrition with their symbiont's photosynthates. The mixotrophy of zooxanthellate jellyfishes (as holobionts) renders the study of their nutrition, growth, and population dynamics complicated. Here, we used an experimental approach to assess how carbon and nitrogen stable isotopes (δ13C and δ15N) as well as the elemental composition (C:N ratios) of zooxanthellate jellyfishes are affected by variations in nutrition sources: i. e. predation (heterotrophic) versus photosynthesis (autotrophic). Our laboratory experiment, conducted on the zooxanthellate jellyfish Cassiopea sp. medusae (including symbionts) in the presence or absence of light and prey during 24 days, showed conclusive results. Presence of light decreased δ15N, increased δ13C and C:N ratios, whereas presence of prey increased δ15N, and decreased δ13C and C:N ratios. The medusae incubated with both light and prey had intermediate δ15N, δ13C and C:N ratios. Variations in zooxanthellate jellyfishes' nutrition sources (autotrophy vs. heterotrophy) are thus reflected by their isotopic and elemental composition. By disentangling the effects of autotrophy and of heterotrophy on zooxanthellate jellyfish isotopic and elemental compositions, these results would help to interpret the values of δ13C, δ15N and C:N ratios that can be observed on these organisms in fieldwork studies.

Highlights

► First experimental study on the effect of nutrition sources on $\delta^{13}C$, $\delta^{15}N$ and C:N ratios in zooxanthellate jellyfishes. ► $\delta^{13}C$ values were higher in light. ► $\delta^{15}N$ values were lower in light and higher with prey. ► C:N ratios were higher in light and lower with prey. ► $\delta^{13}C$, $\delta^{15}N$ and C:N ratios could be valuable indicators of the nutrition of zooxanthellate jellyfishes in the field.

Keywords : Stable isotopes, C:N ratios, Photosymbiosis, Mixotrophy, Scyphozoa, Zooxanthellae

1. Introduction

Jellyfishes are increasingly acknowledged as an important component of marine ecosystems. Population dynamics of the pelagic life stages are often characterized by important fluctuations with dramatic biomass increases followed by sudden collapses (Lucas and Dawson 2014, Pitt et al. 2014). These fluctuations can have important consequences for pelagic community dynamics and nutrient cycling (Pitt et al. 2009a), or for human activities (Purcell et al. 2007). One of the key factors controlling jellyfish population dynamics, is nutrition (e. g. Lucas and Dawson 2014, Pitt et al. 2014). One way to study jellyfish nutrition is to use their stable isotopes signatures (mainly $\delta^{13}C$ and $\delta^{15}N$, see Pitt et al. 2009b). Many recent studies have focused on jellyfish stable isotopes and have provided precisions of their diets as well as competition relationships (e. g. Fleming et al. 2015, Javidpour et al. 2016, Vansteenbrugge et al. 2016, D'Ambra et al. 2018, Milisenda et al. 2018). Most of these studies have strictly focused on heterotrophic jellyfishes. However, some jellyfishes are known to live in symbiosis with zooxanthellae. Comparatively, the zooxanthellate jellyfishes have received less interest (see however Freeman et al. 2017, Zeman et al. 2018).

Zooxanthellate jellyfishes (mostly Rhizostomeae, Scyphozoa, see Djeghri et al. 2019) are characterized by their photosymbiotic relationship with zooxanthellae (generally from the family Symbiodiniaceae, Dinophyceae; LaJeunesse 2001, LaJeunesse et al. 2018). This symbiotic relationship is thought to be similar to the one well known in corals with the zooxanthellae providing their host with photosynthates while recycling the host's respiration and excretion products (see Davy et al. 2012). In such symbiosis, both carbon and nitrogen can be obtained via heterotrophy and autotrophy and are recycled between the host and its zooxanthellae. Carbon dioxide (CO_2) and nitrogen from host respiration and excretion are used and metabolized by the zooxanthellae. Simultaneously, complex molecules (including carbohydrates, lipids and amino acids) are transferred from one partner to the other (Davy et al. 2012). Zooxanthellate jellyfishes, as holobionts (host + symbionts), are thus mixotrophs, deriving their nutrition from both predation and zooxanthellae's photosynthesis (Kremer 2005, Welsh et al. 2009). Generally, the symbiosis provides most if not all of the carbon needed for respiration (Kremer et al. 1990, Kikinger 1992, McCloskey et al. 1994, Verde and McCloskey 1998) while predation is still needed to meet nitrogen and phosphorus requirements (Kremer 2005, Welsh et al. 2009). However, the relative contribution to nutrition of the predation versus the photosynthesis might be variable across species, populations, environments, or during growth (see e. g. Sugiura 1969, McCloskey et al. 1994, Verde and McCloskey

1998, Bolton and Graham 2004, reviewed in Djeghri et al. 2019). Studies using stable isotopes, in this context might be valuable tools to understand these variations.

Numerous studies on other photosymbiotic cnidarians (mainly corals), have shown that variations of nutrition affect the isotopic and elemental composition (see e. g. Muscatine et al. 1989a, Muscatine and Kaplan 1994, Alamaru et al. 2009, Reynaud et al. 2009, Ferrier-Pagès et al. 2011, reviewed by Ferrier-Pagès and Leal 2018). Similar effects can be expected in zooxanthellate jellyfishes. To date, only few fieldwork studies have focused on the isotopic composition of zooxanthellate jellyfishes (see Freeman et al. 2017, Zeman et al. 2018). The conclusions of these studies have, however, been limited due to the lack of data on the interplay between autotrophy and heterotrophy of zooxanthellate jellyfishes as reflected in their isotopic and elemental composition (Zeman et al. 2018). To better understand this, controlled experiments are needed where the resources for heterotrophy (prey), and for autotrophy (light) can be manipulated and their effect on stable isotopes signatures and elemental composition can be assessed. In this study, we aim to provide experimental insights on how isotopic and elemental composition of zooxanthellate jellyfishes are affected by different food regimes, and, more specifically, by variations of the relative importance of autotrophy and heterotrophy. In order to achieve this, we assessed the changes in the $\delta^{13}C$, $\delta^{15}N$, and C:N ratios in young specimens of zooxanthellate *Cassiopea* sp. medusae (Scyphozoa: Rhizostomeae) over a period of 24 days and in the presence or absence of prey and light.

2. Materials and Methods

2.1. Experimental set-up

Small specimens of *Cassiopea* sp. medusae (ca. 6 mm in bell diameter and 1 month old) were acquired from the Trocadéro Aquarium (Paris, France). In this aquarium, the medusae were kept at 25 °C with a daily light cycle, and were fed *Artemia* sp. nauplii twice a day. After their arrival to the laboratory the medusae were acclimatized to local heated (25-26 °C) filtered (1 µm pore size) sea-water during one day. In the following day, five medusae were randomly sampled to represent the initial state and then, the experiment was set up. A total of 72 medusae specimens were individually placed in 75 ml glass flasks filled with 50 mL of filtered sea-water. The flasks were then randomly assigned to one of the four experimental treatments (18 medusae per treatment). The experimental treatments were as follows: (1) fed and in light, (2) fed and in the dark, (3) starved and in light, and (4) starved and in the dark. The goal

of these different treatments was to target respectively: mixotrophy, heterotrophy, and autotrophy, the fourth treatment being a control. The flasks containing the medusae were kept in water baths, which regulated a fixed temperature (25-26 °C). Two water baths were used, one for the medusae kept in light, and the other for the medusae kept in the dark. The temperature changed little during the experiment and between the two water baths (25.6 ± 0.4 °C and 25.3 ± 0.4 °C respectively in the lighted and darkened water baths; mean ± s.d.). The light was provided by a fluorescent lamp on a 12:12 hours day:night cycle at an intensity of ca. 110 µmol photons.m^{-2}.s^{-1}. Food consisted of 2 h of feeding *ad libitum* every two days on *Artemia* sp. young nauplii (< 24 h after hatching). The medusae full guts and numerous remaining prey in the flasks confirmed a proper *ad libitum* feeding. Every two days, and after the feeding, the incubation water of the medusae was entirely changed. During the latter process, the flasks were also washed to prevent fouling.

Every four days, and before the feeding (ensuring empty guts), three medusae were randomly sampled from each treatment. Thus the sampling occurred at the following days: 4, 8, 12, 16, 20 and 24; plus the initial state being represented by the five medusae sampled before setting the treatments.

2.2. Processing of medusae

Immediately after sampling, presence and physiological state of the symbionts were assessed. For this purpose, the medusae were put in the dark for at least 20 minutes allowing the opening of the photosystem reactive centers of zooxanthellae. The photosynthetic parameters of the zooxanthellae were assessed by pulse amplitude modulatory (PAM) fluorometry using the “OJIP protocol” of an AquaPen-C-AP-C100 (® Photon Systems Instruments, PSI, Brno, Czech Republic), at a 450 nm excitation wavelength. This protocol measures the fluorescence emitted after a flash of saturating light. This yields the estimation of several variables among which the maximum photosystem II quantum yield (F_v/F_m), which is a proxy of the photosystem II efficiency. With F_m the maximum fluorescence under saturating light, and $F_v = F_m - F_0$ with F_0 the initial fluorescence (Strasser et al. 2000). The measures were performed on whole medusae specimens. Prior to the measurement, it was ensured that the medusae were settled at the cuvette bottom to insure proper exposition to the saturating flash. Additionally, along each medusae measure, a blank was realized by using the “OJIP protocol” on incubation water without medusae (75 blanks total).

Preparation for elemental and isotopic analysis started first by quickly rinsing the medusae in deionized water in order to remove the sea salt. The whole medusae were then placed in pre-weighted tin capsules (10.5 × 9 mm EMAL technology, United Kingdom) and oven-dried at 60 °C for ca. 48 h. After drying, the tin capsules containing the medusae were locked and weighted again to obtain the medusae dry mass, which varied between 0.2 and 3.2 mg (0.9 ± 0.6 mg; mean ± s.d.). In the preparation procedure, the medusae were unpreserved before the isotopic analysis, following the recommendations of Fleming et al. (2011). Due to the small size of the medusae specimens, it was not possible in this experiment to follow the recommendations of MacKenzie et al. (2017) by dissecting, washing and rubbing the mesoglea before conservation. In addition, due to their small size, it was not possible to separate the animal tissue from the zooxanthellae. This would have resulted to acquiring an insufficient biomass for the isotopic analysis. Thus, the results presented here are measures on the holobiont (animal host + zooxanthellae).

2.3. Processing of prey nauplii

In order to assess the isotopic and elemental composition of the *Artemia* sp. nauplii given as food for the medusae, we sampled them two times during the experiment (at days 8 and 14) plus a sampling at day 28, slightly after the end of the experiment (protocol unchanged). At each sampling, concentrated nauplii were divided in five aliquots, and oven-dried at 60 °C for ca. 48 h in clean glass flasks. The dried nauplii were then scratched from the flasks and ground into a powder. Finally, between 0.5 and 1.5 mg of the powder were inserted and locked in tin capsules (10.5 × 9 mm EMAL technology, United Kingdom).

2.4. Elemental and stable isotopes composition

The analyses of medusae and nauplii samples were performed using an Elemental analyzer (Thermo Scientific EA Flash 2000), coupled to a Mass Spectrometer (Thermo Scientific DELTA V Plus) at the Stable Isotopes Laboratory of the “Pôle Spectrométrie Océan” (PSO-IUEM, Plouzané, France). The nitrogen and carbon mass of medusae samples ranged respectively from 15 to 109 µgN (35 ± 22 µgN; mean ± s.d.) and from 60 to 543 µgC (175 ± 120 µgC; mean ± s.d.). As the whole medusae were inserted in the tin capsules, these values are representative of their total weights. The nitrogen and carbon mass of nauplii samples ranged respectively from 40 to 105 µgN (64 ± 18 µgN; mean ± s.d.) and from 200 to 522 µgC (326 ± 90 µgC; mean ± s.d.). The samples were calibrated for mass bias using casein (IVA-33802155,

Analysentechnik, Germany) as the elemental standard (range: 5-108 µgN; 16-377 µgC). Some material-rich samples were automatically diluted during the analysis process (Thermo Scientific ConFlo IV).

Stable isotopes values are expressed as permil (‰) using the δ notation (normalized to Vienna Pee Dee Belemnite and atmospheric N_2 for respectively carbon and nitrogen):

$$\delta X = \left(\frac{X^H_{sample} \,/\, X^L_{sample}}{X^H_{std} \,/\, X^L_{std}} - 1 \right) \times 1000$$

With X the element measured, X^H the amount of the heavy isotope and X^L the amount of the light isotope from the samples (X_{sample}) and the standard (X_{std}).

As some of our samples had a low (< 20 µgN) nitrogen mass, we analyzed five replicates of casein standards with a low nitrogen mass (13.4 ± 1.9 µgN; mean ± s.d.) to check whether this low mass have led to uncertainties in our measures. We found only a low variability on the obtained $\delta^{15}N$ measures (0.04 ‰ s.d., n=5) indicating that our measures were consistent even at low biomass levels.

Unless indicated otherwise, all C:N ratios are expressed by mass (following Ikeda 2014 and Molina-Ramírez et al. 2015). As the C:N ratios of both the medusae and their prey were higher than 3.5, a normalization of the $\delta^{13}C$ for lipid content was advisable (Post et al. 2007). For the nauplii, we used the general normalization for aquatic animals given by Post et al. (2007) and for the medusae, we used the normalization specific to scyphozoans proposed by D'Ambra et al. (2014). For comparison, raw data is still presented as supplementary material (see discussion).

2.5. Statistics

The data collected during the experiment (carbon masses, $\delta^{13}C$, $\delta^{15}N$, C:N ratios and F_v/F_m) were analyzed using linear mixed-effects models (LME) (e. g. Crawley 2012). The presence or absence of prey and light were considered as fixed effects while time was considered as random effect. Model assumptions (mean of residuals = 0, linearity and normality) were checked using model-checking plots. If the model assumptions were not met, the data were log transformed. If the fixed effects affected significantly the results (if p-value < 0.05), subsequent Tukey post-hoc tests were performed on least-square means (i. e. means adjusted for the effect of time) to assess which combination of the fixed effects (light and prey) led to different responses.

One-way ANOVAs were used to assess possible variations in prey $\delta^{13}C$, $\delta^{15}N$, and C:N ratios over time. Normality and homoscedasticity assumptions were checked using the Shapiro-Wilk normality test and Bartlett homogeneity of variance test (threshold: $\alpha = 0.05$). If the assumptions were not met, the data were Box-Cox transformed. All statistical analyses were performed in R (R Core Team 2017).

3. Results

3.1. Mass variation in medusae

The carbon mass of the medusae (Fig. 1) was significantly affected by light alone (LME, t-value = 7.5, p-value < 0.001) and by the interaction between light and prey (LME, t-value = 3.4, p-value < 0.01). At the beginning of the experiment, the carbon mass of *Cassiopea* sp. medusae was of 115.1 ± 27.0 µg C (mean ± s.d.). Only the carbon mass of the medusae in the treatment with both light and prey did noticeably increased, reaching 449.3 ± 68.2 µg C (mean ± s.d.) at the end of the experiment. The carbon mass of the medusae in the treatment with only light did not increased significantly reaching a carbon mass value of 133.9 ± 27.9 µg C (mean ± s.d.) at the end of the experiment. The carbon mass values of the medusae in the treatments with only prey or without resources tended to decrease, dropping to respectively 88.0 ± 26.1 µg C and 71.1 ± 15.7 µg C (mean ± s.d.) at the end of the experiment. In the treatment without resources, one medusae specimen died. Thus for this treatment, there remained two replicates instead of three at day 24.

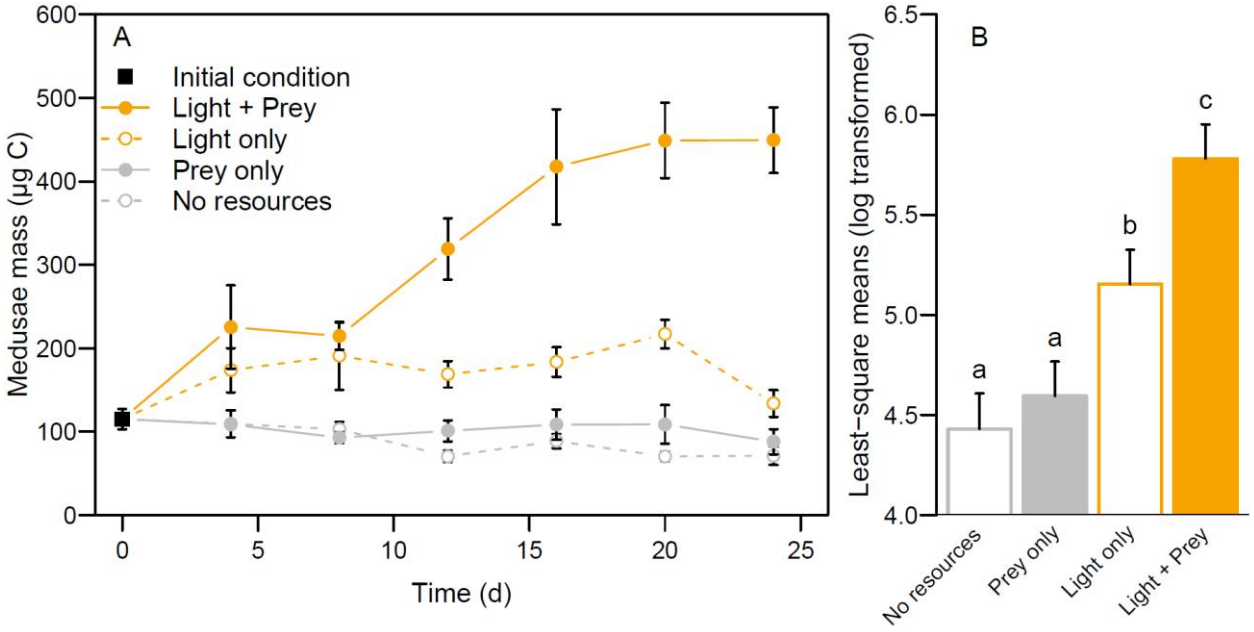

Fig. 1. (A) Changes in the *Cassiopea* sp. medusae carbon mass (µg C) (means ± s.e.m.) over the course of the experiment as a function of the experimental conditions. (B) Comparison of least-square means obtained from each treatment (± 95 % C. I.). The letters (a, b, and c) indicate statistically different treatments (Tukey post hoc test, p-value < 0.05).

3.2. $\delta^{13}C$ and $\delta^{15}N$

The medusae $\delta^{13}C$ values (Fig. 2 A, B) were significantly affected by light (LME, t-value = 22.5, p-value < 0.001) and by the interaction of light and prey (LME, t-value = -6.2, p-value < 0.001). At the beginning of the experiment $\delta^{13}C$ values of the medusae were of -18.7 ± 0.9 ‰ (mean ± s.d.). In the treatment with both light and prey the $\delta^{13}C$ values of the medusae increased quickly (in less than four days) reaching ca. -15 ‰. This trend was even more pronounced in the treatment with only light were the $\delta^{13}C$ values of the medusae reached ca. -13 ‰. Conversely, in the treatments with only prey or without resources the $\delta^{13}C$ values of the medusae remained similar or decreased slightly throughout the experiment (generally comprised between -19 ‰ and -21 ‰). It should be noted that the distinction between the medusae from the treatments with light alone and with light and prey is not as distinct with data not normalized for lipids (Fig. S1).

The medusae $\delta^{15}N$ values (Fig. 2 C, D) were significantly affected by both light (LME, t-value = -8.7, p-value < 0.001) and prey (LME, t-value = 2.2, p-value < 0.05), but not by their interaction. At the beginning of the experiment, the $\delta^{15}N$ values of medusae was of 8.9 ± 1.1 ‰ (mean ± s.d.). These values decreased slightly in the treatment with light and prey reaching 8.0 ± 0.3 ‰ (mean ± s.d.) at the end of the experiment. The decrease was more pronounced in the treatment with light only which reached 5.5 ± 0.4 ‰ at the end of the experiment. Finally, $\delta^{15}N$ values did not change in the treatments with only prey or without resources (values at the end of the experiment of 9.0 ± 0.3 ‰ and 8.8 ± 1.0 ‰ respectively; mean ± s.d.).

The $\delta^{13}C$ and $\delta^{15}N$ obtained in *Artemia* sp. nauplii prey did not vary significantly during the experiment (ANOVAs, p-values > 0.05) averaging respectively -19.4 ± 0.2 ‰ and 10.7 ± 0.5 ‰ (mean ± s.d.) (Fig. 2 A, C).

225 *3.3. C:N ratios*

226 The C:N ratios obtained in the medusae (Fig. 3) were significantly affected by light (LME, t-value = 27.5,
227 p-value < 0.001) and by the interaction of light and prey (LME, t-value = -6.6, p-value < 0.001). At the
228 beginning of the experiment, the C:N ratios of medusae were of 4.5 ± 0.1. These values increased in the
229 treatment with both light and prey (4.8 ± 0.1 at the end of experiment; mean ± s.d.). A similar, but more
230 pronounced increased was seen in the C:N ratios of the medusae exposed to only light (5.9 ± 0.2 at the
231 end of experiment; mean ± s.d.). By opposition, C:N ratios decreased slightly in the treatments with only
232 prey or without resources (respectively reaching 4.1 ± 0.1 and 3.9 ± 0.1 at the end of the experiment;
233 mean ± s.d.).

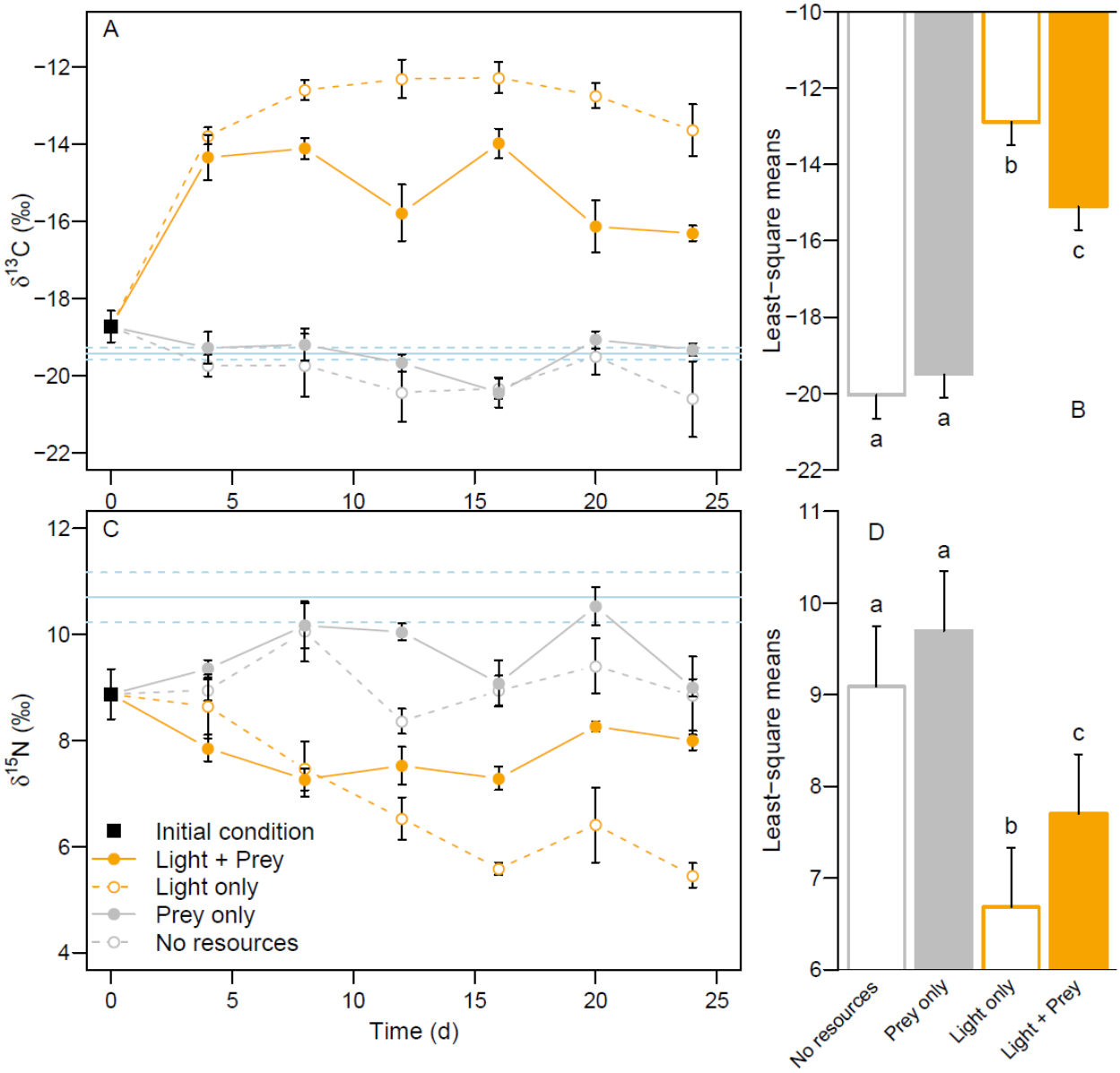

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Fig. 2. Changes in the *Cassiopea* sp. medusae $\delta^{13}C$ (‰) (A) and $\delta^{15}N$ (‰) (C) (means ± s.e.m.) over the course of the experiment as a function of the experimental conditions. Solid and dashed blue lines represent the mean ± s.d. of the isotopic signatures of *Artemia* sp. nauplii used as prey in fed treatments. (B and D) Comparison of least-square means obtained for each treatment (± 95 % C. I.). The letters (a, b, and c) indicate statistically different treatments (Tukey post hoc test, p-value < 0.05). $\delta^{13}C$ values of have been normalized for lipid content according to Post et al. (2007) for nauplii, and D'Ambra et al. (2014) for *Cassiopea* sp. medusae.

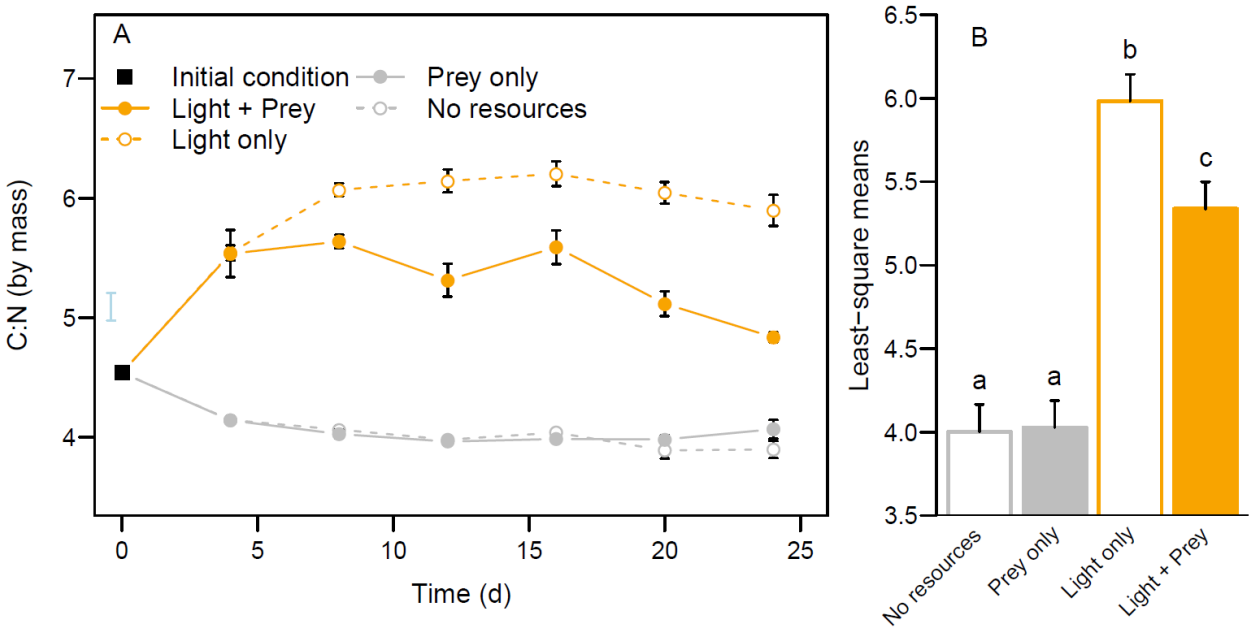

Fig. 3. (A) Changes in the *Cassiopea* sp. medusae mass C:N ratios (means ± s.e.m.) over the course of the experiment as a function of the experimental conditions. The blue error bar indicate the range of C:N ratios in nauplii prey measured in the course of the experiment (see text). (B) Comparison of the least-square means obtained for each treatment (± 95 % C. I.). The letters (a, b, and c) indicate statistically different treatments (Tukey post hoc test, p-value < 0.05).

The C:N ratios obtained in *Artemia* sp. nauplii did vary significantly during the experiment (ANOVA, F = 25.9, p-value < 0.001). However, these variations were of small amplitudes (minimum: 4.97, maximum: 5.20) compared to the variationsobserved in *Cassiopea* sp. medusae following the different experimental treatments (Fig. 3 A). Therefore, these small variations of the C:N ratios of the prey are unlikely to have

254 significantly affected the outcome of the experiment. Throughout the experiment, C:N ratios in *Artemia*
255 sp. nauplii averaged 5.1 ± 0.1 (mean ± s.d.).

256

257 *3.4. PAM parameters of zooxanthellae*

258 The blanks always yielded low values of F_0 (90 ± 9; mean ± s.e.m.) as compared to the F_0 values of the
259 medusae (5270 ± 630; mean ± s.e.m.). This equates to a signal-to-noise ratio of ca. 60, which is sufficient
260 to have a reliable estimate of photosynthetic activity. Two outliers were removed from the medusae's
261 PAM data (F_v/F_m below 0.4, similar to a blank, most likely due to a lack of exposition of the medusae to
262 the saturating flash). With the exception of this two outliers, the F_v/F_m of medusae remained very stable
263 in all conditions and during the whole experiment averaging an overall value of 0.70 ± 0.06 (mean ± s.d.;
264 Fig. S2). The LME models did not indicate any effect of presence or absence of prey and light on the
265 zooxanthellae F_v/F_m. Independently of this lack of effect of the experimental treatments on PAM
266 parameters (discussed in Supplementary Material 2), high F_0 values as compared to the blanks
267 demonstrate the presence of zooxanthellae in the *Cassiopea* sp. medusae in all treatments and during
268 the whole experiment.

269

270 **4. Discussion**

271

272 *4.1. Isotopic composition*

273 In this study, the $\delta^{13}C$ values obtained in the medusae were the highest in the treatment with light only,
274 lowest in the treatments with only prey and without resources, and intermediate in the treatment with
275 both prey and light (Fig. 2A, B). Similar effects of heterotrophic feeding on $\delta^{13}C$ values have been
276 reported for corals (e. g. Reynaud et al. 2002, Ferrier-Pagès et al. 2011). As in this study, the $\delta^{13}C$ of the
277 predator tended towards the $\delta^{13}C$ of the prey when fed. However, some caution should be taken when
278 interpreting the results of this study concerning the effects of heterotrophic feeding on zooxanthellate
279 jellyfish's $\delta^{13}C$. Indeed, it is unsure that the lipid normalization used here can be applied to a
280 photosymbiotic holobiont as it has been derived from the heterotrophic *Aurelia* sp. (D'Ambra et al.
281 2014). Without this normalization, the effect of heterotrophic feeding on $\delta^{13}C$ is less clear (Fig. S1). Thus,
282 albeit an effect of heterotrophic feeding on zooxanthellate jellyfishes' $\delta^{13}C$ is likely, our results should be

taken with caution regarding this point. In contrast, light had a clear positive effect on $\delta^{13}C$ of zooxanthellate jellyfishes whether the normalization for lipid content is made or not (Fig. 2A, B; Fig. S1). These conclusions are consistent with the previous findings on corals (e. g. Muscatine et al. 1989a, Swart et al. 2005, Alamaru et al. 2009, Ferrier-Pagès et al. 2011).

In the experiment, $\delta^{15}N$ values were the lowest in the medusae exposed to only light, compared to the other treatments (Fig. 2C, D). This is different from what is known in tropical scleractinian corals in which photosynthesis tend to increase, or have little effect on $\delta^{15}N$ rather than decrease it, like seen here (see Muscatine and Kaplan 1994, Alamaru et al. 2009, Reynaud et al. 2009). Our results are more comparable to what is observed in more heterotrophic temperate corals (Ferrier-Pagès et al. 2011). The treatment with no resources and the treatment with only prey presented the same $\delta^{15}N$ (Fig. 2C, D). However, the effect of predation is clear as the $\delta^{15}N$ values in the treatment with prey and light were intermediate between those of the treatment with only light, and the treatment with only prey (Fig. 2C, D). The similitude between the treatment without resources and the treatment with only prey would thus be explained by the initial condition (i. e. at day 0, medusae already had high $\delta^{15}N$). Thus, overall, predation would have led to higher $\delta^{15}N$ of medusae (Fig. 2C, D). Interestingly however, the $\delta^{15}N$ values in medusae were never higher than those obtained on prey (Fig. 2C), which suggests that no measurable fractionation occurred between the holobiont and their prey. This is most likely due to high recycling of nitrogen between the host and its symbionts (see also Reynaud et al. 2009).

These patterns seen in $\delta^{13}C$ and $\delta^{15}N$ can be explained through two main processes affecting stable isotopes signatures in photosymbiotic organisms (reviewed in Ferrier-Pagès and Leal 2018):

The first process is the mixing of carbon or nitrogen coming from two contrasted sources; autotrophic uptake of dissolved inorganic nutrients, on one hand, and heterotrophic predation—mainly on zooplankton—on the other hand (Reynaud et al. 2002, Alamaru et al. 2009, Ferrier-Pagès et al. 2011, Ferrier-Pagès and Leal 2018). The uptake of dissolved inorganic carbon by zooxanthellae generally leads to higher $\delta^{13}C$ values (typically -10 ‰ to -14 ‰) than those of typical oceanic particulate organic matter and plankton (ca. -20 ‰; Muscatine et al. 1989a, Ferrier-Pagès et al. 2011, Ferrier-Pagès and Leal 2018). Thus, $\delta^{13}C$ values obtained through zooxanthellae's autotrophy would be higher than those obtained through predation on zooplankton (Fig. 4A). For nitrogen, the pattern is reversed; zooxanthellae take up dissolved inorganic nitrogen with a low $\delta^{15}N$ value (ca. 5 ‰ Ferrier-Pagès and Leal 2018) while predation leads the uptake of nitrogen with higher $\delta^{15}N$ values due to fractionation through the food web (Post

2002, Ferrier-Pagès et al. 2011, Ferrier-Pagès and Leal 2018, Fig. 4B). For both carbon and nitrogen, the isotopic signature of the two sources (inorganic nutrient uptake, and predation) is then exchanged and recycled between the zooxanthellae and the host (e. g. Reynaud et al. 2009).

The second process involves the depletion of *in-hospite* nutrient pools due to photosynthesis. Zooxanthellae tend to take up preferentially inorganic nutrients with light isotopes resulting in fractionation (Ferrier-Pagès and Leal 2018). However, at high photosynthesis rates, the host's pool of inorganic nutrients can get depleted. Thus, to meet their photosynthetic requirements, zooxanthellae take up more heavy isotopes, reducing fractionation ("depletion-diffusion hypothesis", see Muscatine et al. 1989a, Fig. 4A and B). This results in a tendency for isotopic signature to correlate with photosynthesis levels. The higher the photosynthesis, the higher the $\delta^{13}C$ (Muscatine et al. 1989a, Swart et al. 2005, Alamaru et al. 2009) or the $\delta^{15}N$ values (Muscatine and Kaplan 1994, Baker et al. 2011; reviewed in Ferrier-Pagès and Leal 2018).

It is important to notice that these two processes—mixing of the heterotrophic and autotrophic sources, and reduced fractionation at high photosynthesis levels—would have similar consequences on $\delta^{13}C$, but not on $\delta^{15}N$. For $\delta^{13}C$, a predominantly autotrophic nutrition would imply a decreased fractionation *in-hospite* due to high photosynthesis and a predominant uptake of dissolved inorganic nutrients. Both of these processes result in high $\delta^{13}C$ (and *vice-versa* for a predominantly heterotrophic nutrition, Fig. 4A). By opposition, for $\delta^{15}N$, reduced fractionation due to high photosynthesis levels would result in high $\delta^{15}N$, but high uptake of dissolved inorganic nutrients would result in low $\delta^{15}N$ (and *vice-versa* for a predominantly heterotrophic nutrition, Fig. 4B). Thus, to understand how $\delta^{15}N$ would react to change in holobiont nutrition, it is important to know which of the above-mentioned processes controls its dynamics.

In the context of this study, the increase in $\delta^{13}C$ due to light, could be explained by both increased uptake of CO_2 and reduced fractionation at higher photosynthesis (and conversely in the dark, Fig. 4A). The results obtain on $\delta^{15}N$ values in this study, suggest that, in zooxanthellate jellyfishes, of the two processes above-mentioned—mixing of autotrophic and heterotrophic sources, and reduced fractionation at high photosynthesis—the former is the dominant one (Fig. 4B). Hence, in zooxanthellate jellyfishes, a predominantly autotrophic nutrition would imply that most nitrogen comes from the

fixation of dissolved inorganic nitrogen (see e. g. Muscatine and Marian 1982, Wilkerson and Kremer 1992, Freeman et al. 2016), and would result in low $\delta^{15}N$. On the contrary, a predominantly heterotrophic nutrition would imply that more nitrogen comes from predation (mainly on zooplankton) resulting in a comparatively higher $\delta^{15}N$. Thus, values of $\delta^{15}N$ can be considered as a good indicator of the relative importance of autotrophy and heterotrophy in zooxanthellate jellyfishes.

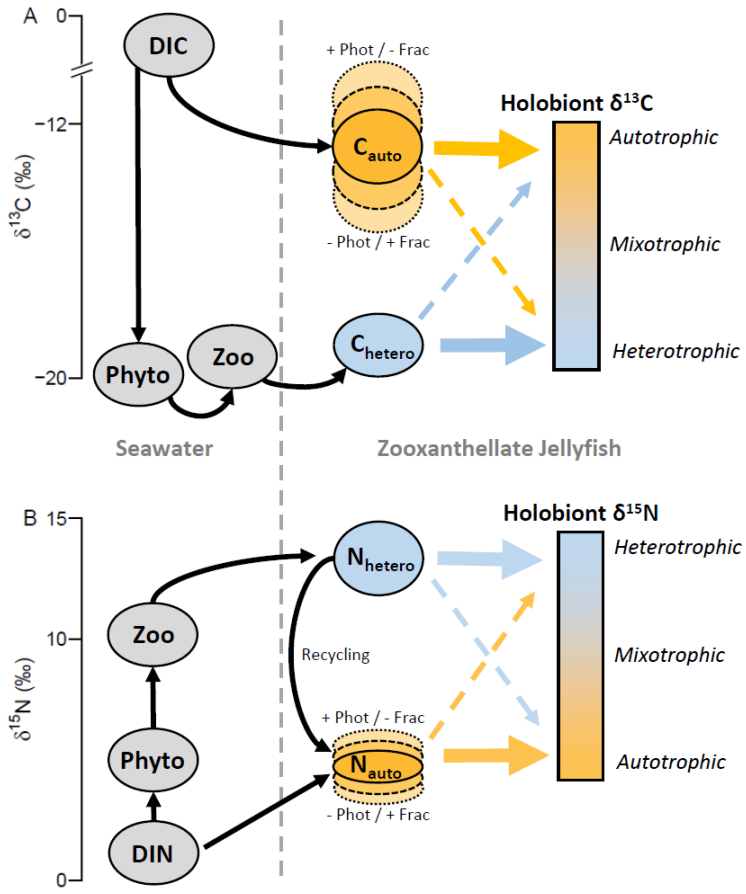

Fig. 4. Conceptual diagram illustrating how $\delta^{13}C$ (A) and $\delta^{15}N$ (B) are affected by the relative importance of heterotrophic (blue circles and arrows, C_{hetero} and N_{hetero}) and autotrophic (orange circles and arrows, C_{auto} and N_{auto}) nutrition pathways in zooxanthellate jellyfishes. DIC = dissolved inorganic carbon, DIN = dissolved inorganic nitrogen, Phyto = phytoplankton, Zoo = zooplankton, Phot = photosynthesis, Frac = fractionation (more photosynthesis tend to decrease fractionation, this effect, in zooxanthellate

357 jellyfishes, is unlikely to be significant for nitrogen, see text). Values on the axes are provided for
358 illustrative purpose only. See also Ferrier-Pagès and Leal (2018).

359

360 *4.2. C:N ratios*

361 In our experiment, light increased C:N ratios of the *Cassiopea* sp. holobionts whereas prey generally
362 decreased it, with intermediate C:N ratios in the treatment with both light and prey (Fig. 3A, B). As with
363 $\delta^{15}N$, C:N ratios of the treatment with no resources and with prey only were similar. This similarity may
364 be again explained by the initial condition. Another potential explanation would be that starved
365 zooxanthellate jellyfishes use first reserves accumulated by photosynthesis (generally carbon rich, e. g.
366 Muller-Parker et al. 1996). Such preferential degradation would make their C:N ratios decrease and get
367 similar to the ones typically reported for non-zooxanthellate jellyfishes (Ikeda 2014, Molina-Ramírez et
368 al. 2015). Independently of the treatment with no resources, our results suggest that predation would
369 tend to decrease C:N ratios (Fig. 3A, B). Such a decrease of C:N ratios due to predation have already
370 been reported for the zooxanthellae of a sea anemone (Cook et al. 1988). However, other studies have
371 pointed out that a similar decrease of C:N ratios can also be due to an enrichment by dissolved inorganic
372 nitrogen (e. g. Muscatine et al. 1989b, Belda et al. 1993). As zooxanthellate jellyfishes are able to take up
373 dissolved inorganic nutrients via their symbionts (see e. g. Muscatine and Marian 1982, Wilkerson and
374 Kremer 1992, Pitt et al. 2005, Welsh et al. 2009, Freeman et al. 2016, see Pitt et al. 2009a for a review), it
375 is likely that their C:N ratios would react to nutrient enrichment too. This suggests that C:N ratios of
376 zooxanthellate jellyfishes might be impacted by nitrogen availability (either as prey or as dissolved
377 inorganic nitrogen).

378

379 *4.3. Remarks on tissue turnover*

380 One of the advantages of the study of the elemental and isotopic composition over e.g. gut content
381 analyses, is that it provides a more time-integrated information (Pitt et al. 2009b). The time frame
382 represented by isotopic composition is, however, dependent on tissue turnover which can be variable as
383 function of, e. g. taxonomy, organ, body size, or temperature (Thomas and Crowther 2015, Vander
384 Zanden et al. 2015). In another scyphozoan jellyfish, *Aurelia* sp., the isotopic half-life was determined to
385 be ca. 10 days for both carbon and nitrogen (D'Ambra et al. 2014). In the present experiment, changes of
386 $\delta^{13}C$ and C:N ratios occurred very fast (within the first four days, Figs. 2A, 3). This was apparently less

true for the $\delta^{15}N$ values which may have experienced slower changes (Fig. 2B). These fast changes may have several explanations: First, the medusae used here were of small size and, additionally, some of them grew (Fig. 1, see also Supplementary Material 2) which can explain the fast changes (Fry and Arnold 1982, Thomas and Crowther 2015, Vander Zanden et al. 2015). In its natural environment, *Cassiopea* sp. can grow up to ca. 20-25 cm in bell diameter (see e. g. Morandini et al. 2017). It is unlikely that such large specimens would display such fast change in composition. Another aspect that could explain the fast change in elemental and isotopic composition observed here, is that *Cassiopea* sp. is zooxanthellate. The zooxanthellae are also likely to impact residence time of elements within the holobiont, possibly differently for nitrogen and carbon, due to recycling (Reynaud et al. 2009).

4.4. Implications for fieldwork studies

One of the challenges to understand the nutrition of zooxanthellate jellyfishes in their natural environments relates to their mixotrophy. As zooxanthellate jellyfishes obtain their nutrition from predation and photosynthesis (Kremer 2005, Welsh et al. 2009), both processes must be investigated. Ideally, predation, photosynthesis, respiration, nutrient uptake and excretion have all to be measured which may represent an intensive amount of work rarely carried out in its entirety (see however, Kremer et al. 1990, Kremer 2005). Studies of stable isotopes and elemental composition are comparatively easier and have the advantage of providing more time-integrated information (Pitt et al. 2009b). The findings of this study provide baseline information on how C:N ratios, $\delta^{13}C$ and $\delta^{15}N$ can be interpreted in fieldwork studies focusing on the nutrition sources of zooxanthellate jellyfishes.

To summarize, our results suggest that both $\delta^{13}C$ and $\delta^{15}N$ vary with the relative balance of autotrophy vs. heterotrophy (although, the effect of heterotrophy on $\delta^{13}C$ is less well supported by our results). It would be expected that, if dominantly heterotrophic, zooxanthellate jellyfishes would have $\delta^{15}N$ values close to those of their prey (see e. g. Zeman et al. 2018). By opposition, if dominantly autotrophic, zooxanthellate jellyfishes would have $\delta^{15}N$ values close (or lower) than those of primary producers (see e. g. Freeman et al. 2017). The $\delta^{13}C$ values would display opposite trends. Finally, C:N ratios may be indicators of the efficiency of nitrogen supplies. Future fieldwork studies would be able to build on these results to better characterize zooxanthellate jellyfishes' nutrition.

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Supplementary Material 1: Raw $\delta^{13}C$

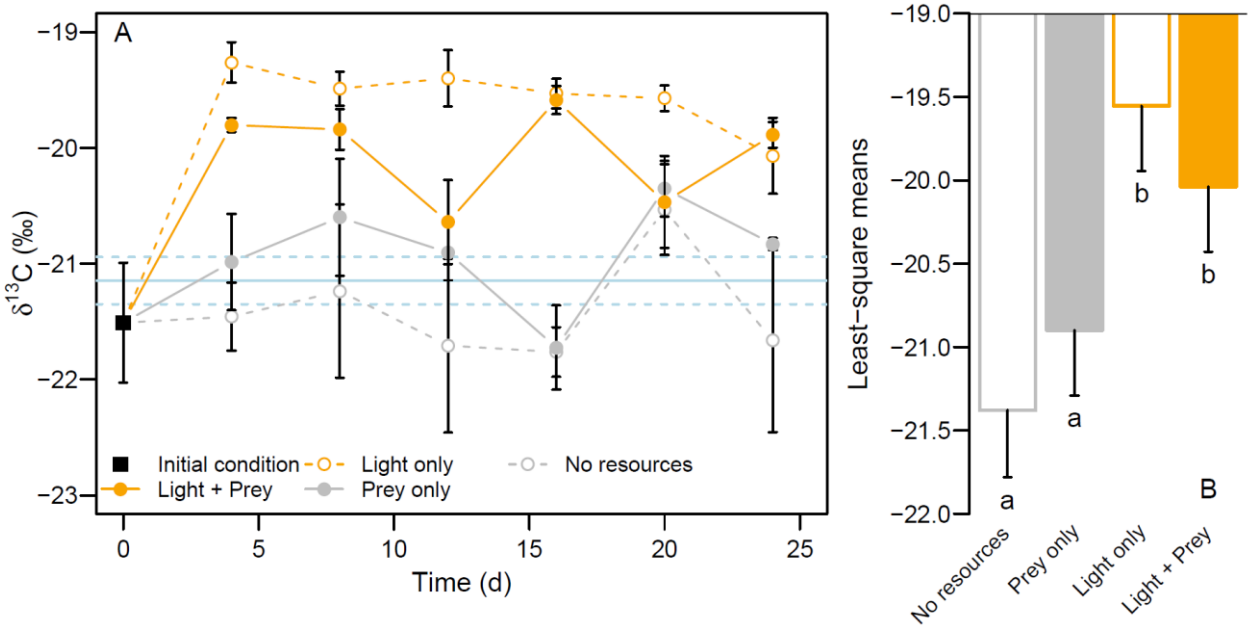

Fig. S1. (A) Changes in the *Cassiopea* sp. medusae $\delta^{13}C$ (‰) without normalization for lipids (means ± s.e.m.) over the course of the experiment as a function of the experimental conditions. Solid and dashed blue lines represent the mean ± s.d. of the isotopic signatures of *Artemia* sp. nauplii used as prey in fed treatments. (B) Comparison of least-square means obtained for each treatment (± 95 % C. I.). The letters (a and b) indicate statistically different treatments (Tukey post hoc test, p-value < 0.05).

Supplementary Material 2: Notes on growth of medusae and PAM parameters

Medusae grew only in the treatment with both light and prey (i. e. increase in carbon mass of the holobiont, Fig. 1 in main text; and increase in medusae bell diameter from ca. 6 mm to 8-10 mm, data not shown). The absence of carbon mass increase (Fig. 1 in main text) in the other treatments confirmed previous findings stipulating that both predation and zooxanthellae's photosynthates are necessary for some zooxanthellate jellyfishes (Kremer 2005, Welsh et al. 2009). The F_v/F_m ratio can be used as a proxy of photosynthetic organism's performance (e. g. Strasser et al. 2000, Long et al. 2018), and was constantly high (0.70, higher or equal to values typically reported for coral zooxanthellae: e. g. Iglesias-Prieto et al. 2004, Roth et al. 2012) in our experiment, independently of treatments (Fig. S2). The absence of decreasing F_v/F_m ratio in the treatments kept in the dark, suggests that zooxanthellae within their *Cassiopea* sp. host stayed photochemically competent for several days without light. We hypothesize that, in such conditions, as their nutrition can only be provided by the host (e. g. in the form of fatty acids; Imbs et al. 2014), zooxanthellae were heterotrophic (see Steen 1986, Jeong et al. 2012).

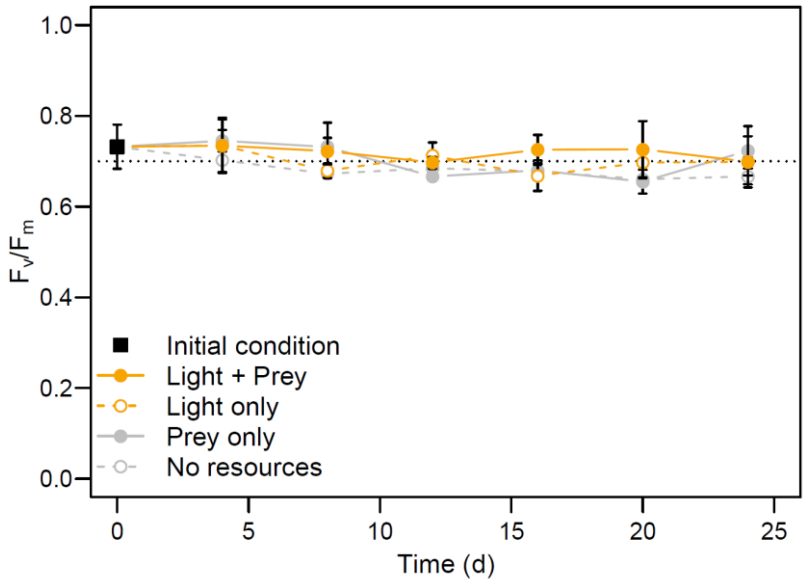

Fig. S2. F_v/F_m ratios of the whole *Cassiopea* sp. medusae (including symbionts) over the course of the experiment and as a function of the experimental treatments (means ± s.e.m.). The dotted line represents the mean of all points.

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