Effect of temperature on an alga-grazer trophic transfer: A dual stable isotope (13C, 15N) labeling experiment

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Abstract

In this study, we examined the impact of temperature on the carbon and nitrogen trophic transfers from a macroalga to a macro-grazer by the use of dual ^{13}C- and ^{15}N-labeling. Using an experimental approach in mesocosms, individuals of the urchin *Psammechinus miliaris* were maintained for 1 month at 17°C (mean summer temperature in the Bay of Brest) and at 20°C (maximum summer temperature) and fed with ^{13}C- and ^{15}N-labeled *Solieria chordalis*. The results showed that the urchins' ^{13}C uptake was 0.30 $\mu g^{13}C$ g dry weight $(DW)^{-1}$ at 17°C and 0.14 $\mu g^{13}C$ g DW^{-1} at 20°C at the end of the experiment. The lower uptake at the higher temperature may be attributed to a decrease in metabolic activity at 20°C, involving lower feeding and/or respiration rates. Conversely, no significant effect of temperature was detected on ^{15}N uptake. At the end of the experiment, the urchins' ^{15}N uptake was 0.04 $\mu g^{15}N$ g DW^{-1} at 17°C and 0.03 $\mu g^{15}N$ g DW^{-1} at 20°C. This suggests that temperature may affect carbon and nitrogen trophic fluxes differently. The use of dual isotope labeling offers interesting prospects and needs to be further extended in order to better understand trophic interactions in marine communities and the consequences of current environmental changes, such as global warming.

KEYWORDS
carbon, macroalga, nitrogen, stable isotope labeling, temperature, trophic interaction, uptake, urchin

1 | INTRODUCTION

Temperature is one of the most important environmental factors affecting biological processes in marine ectothermic species (Nguyen et al., 2011; Uthicke, Liddy, Nguyen, & Byrne, 2014; Zuo, Moses, West, Hou, & Brown, 2012). Several studies demonstrated the effect of temperature on physiological processes, including feeding rates (Frey & Gagnon, 2015; Gooding, Harley, & Tang, 2009; Thomas, Crear, & Hart, 2000). However, the response to changes in temperature in terms of assimilation rates remains poorly documented, despite the importance of this process in species ecophysiology. In benthic ecosystems, urchins are considered as strong drivers of community structure, mainly as a result of their ability to control macroalgal biomass (Steneck, 2013). Within maerl beds of the Bay of Brest (Britany, France), the purple-tipped sea urchin, *Psammechinus miliaris* (Müller, 1771), can reach high densities and is known to be a major factor regulating macroalgal biomass (Guillou, Grall, & Connan, 2002).

Several studies have already evidenced the usefulness of stable isotope labeling for quantifying the importance of microalgal or bacterial production as food source for grazers (Herman, Middelburg, Widdows, Lucas, & Heip, 2000; Leroy et al., 2012; Middelburg et al., 2000). However, studies using dual stable isotope labeling (^{13}C, ^{15}N) to examine trophic transfers from macroalgae to macro-grazers under the influence of environmental factors, such as temperature, are still lacking.

The present study aimed to investigate whether temperature can influence macroalgal-derived carbon and nitrogen assimilation into grazer tissues. Carbon and nitrogen trophic transfers from the red seaweed *Solieria chordalis* (J. Agardh, 1842) to the urchin *P. miliaris* were experimentally examined using dual ^{13}C- and ^{15}N-labeling.

In the Bay of Brest, the alga *S. chordalis* is abundant (Hily, Potin, & Floch, 1992) and reaches the highest biomass in the summer (Guillou et al., 2002). Therefore, trophic transfers were investigated at 17°C (mean summer temperature, recorded by Service d'Observation en Milieu LITtoral, SOMLIT) and at 20°C (maximum summer temperature, Martin, Clavier, Chauvaud, & Thouzeau, 2007).

2 | MATERIAL AND METHODS

2.1 | Species collection

Individuals of *Psammechinus miliaris* were collected from a maerl bed in the bay of Brest, France (48°17′18″N, 04°23′46″W) in April 2016 using a naturalist's dredge (width: 1 m, height: 0.2 m, net: 1.5 m long). Organisms were then transported in seawater tanks to the Roscoff Marine Station. To mitigate the stress experienced by the species during sampling and transport, organisms were maintained in open-flow aquaria pending experiments. Thalli of the seaweed *Solieria chordalis* were collected from maerl beds in the Bay of Brest (48°19′56″N, 04°19′56″W) in early June 2016 and transported to the Roscoff Marine Station.

2.2 | Experimental design

In May 2016, 36 *Psammechinus miliaris* individuals of medium size (26 ± 3 mm) were randomly assigned to 10 15-l aquaria (Supporting Information Fig. S1). In all aquaria, the temperature was gradually increased by 0.5°C per day over 4 days to reach 17°C, corresponding to the mean summer temperature in the Bay of Brest (SOMLIT). Then, in five aquaria, the temperature was increased again for 6 days to reach 20°C, the mean maximum summer temperature in the Bay of Brest (Martin et al., 2007). The temperature was controlled in two 100-l tanks, continuously supplied with filtered (5 µm) open natural seawater, with a high water flow rate of 150 L/hr per tank. The temperature was maintained by an offline feedback system (IKS Aquastar, Karlsbad, Germany) that activated or stopped heaters, controlling the temperature in the tanks. Each 100-L tank provided seawater to five aquaria. During the experiment, the temperature was at 17.2 ± 0.3 and at 19.9 ± 0.2°C, according to the treatments (17 and 20°C). For both temperature treatments, urchins were starved 10 days before the feeding experiment to empty their gut. During the experiment, the irradiance was 90–100 µmol photons m^{-2} s^{-1}, corresponding to the mean in situ summer irradiance at 5 m depth on maerl beds in the Bay of Brest (Martin, Castets, & Clavier, 2006).

2.3 | Seaweed labeling

Thalli of *Solieria chordalis* were cleaned of epiphytes, rinsed with filtered seawater (on 0.7 µm GF/F glass microfiber filters) and placed in a 9-L closed-circuit aquarium. Five liters of filtered (0.7 µm) seawater was added in the aquarium. A solution of ^{13}C-HCO_3^- at 1 g/L was prepared from $NaH^{13}CO_3$ (99 atom% ^{13}C) and 1 L of filtered 0.7 µm GF/F seawater. Similarly, a solution of ^{15}N-NH_4 at 0.25 g/L was prepared by diluting $^{15}NH_4Cl$ (99 atom% ^{15}N) in filtered seawater. The seaweeds were fertilized with the solutions of $NaH^{13}CO_3$ and $^{15}NH_4Cl$ for a period of 7 days. Each day, 10 ml of a solution of phosphate (PO_4^{3-}) at 1 g/L was also added to ensure that P was not limiting. A circulation pump ensured nutrient homogeneity in the aquarium. At the end of the labeling period, some of the thalli were rinsed with filtered seawater and immediately frozen at −20°C until isotope measurements. In order to provide sufficient food for urchins for the duration of the experiment, a second stock of *S. chordalis* was labeled after 8 days, following the same protocol.

2.4 | Feeding experiment

For each temperature treatment, three urchins were collected at day 0 (T0) to measure the initial isotopic signature. Labeled thalli of *Solieria chordalis* from the same labeled stock were provided every 2 days to urchins of eight aquaria (four per condition) at an amount of 1 g of fresh weight per urchin. In one control aquarium per temperature treatment, urchins were fed with non-labeled thalli at the same amount (1 g per urchin). After 6 days, one urchin was collected per aquarium. A second urchin was collected after 15 days and the last urchin after 30 days (T30). Just before collection, urchins were starved for 48 hr to allow the assimilation of ingested algae and to allow evacuation of their digestive content. All samples were immediately frozen at −20°C pending isotope measurement.

2.5 | Isotope analysis

For each individual, the digestive system was removed and samples were acidified under a magnifying glass to remove calcareous structures (Kamp & Witte, 2005). Urchin soft tissues and algae were dried in an oven for 48 hr at 60°C, then ground to a fine powder. Subsamples were weighed into tin cups for isotope analyses. Stable isotope ratios for carbon ($R = {}^{13}C/{}^{12}C$) and nitrogen ($R = {}^{15}N/{}^{14}N$) were determined using a carbon, hydrogen and nitrogen (CHN) analyser (ThermoFinnigan 1112 Series) interfaced with a mass spectrometer (ThermoFinnigan MAT Deltaplus) via a Conflow III open split interface.

2.6 | Uptake of ^{13}C and ^{15}N by urchins

Carbon and nitrogen ratios were used to calculate δ-values (in ‰):

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1{,}000$$

where X is ^{13}C or ^{15}N. Sample ratios (R_{sample}) were reported relative to international standards ($R_{standard}$) Vienna Pee Dee Belemnite ($R_{VPDB} = 0.0112372$) for carbon and atmospheric N_2 ($R_{air\ N2} = 0.0036765$) for nitrogen.

Incorporation of ^{13}C or ^{15}N labels in urchins was calculated as excess ^{13}C or ^{15}N, expressed in terms of specific uptake (i.e., $\Delta\delta^{13}C = \delta^{13}C_{T30} - \delta^{13}C_{T0}$ and $\Delta\delta^{15}N = \delta^{15}N_{T30} - \delta^{15}N_{T0}$) and total uptake (I), in accordance with previous studies (Herman et al., 2000;

Leroy et al., 2012). I corresponds to the product of excess ^{13}C or ^{15}N (E) and carbon and nitrogen biomass (B), with I in $\mu g^{13}C$ or ^{15}N, B in μg C or N. E is calculated as the difference between the fraction ^{13}C or ^{15}N of the initial value (F_{T0}) and the final value (F_{T30}):

$$E = F_{T30} - F_{T0}$$

with $F = {}^{13}C/({}^{13}C + {}^{12}C) = R/(R + 1)$ for ^{13}C and $F = {}^{15}N/({}^{15}N + {}^{14}N) = R/(R + 1)$ for ^{15}N. The carbon and nitrogen isotopic ratios (R) were obtained from the measured $\delta^{13}C$ and $\delta^{15}N$, respectively, as:

$$R = (\delta^{13}C/1{,}000 + 1) \times R_{VPDB}$$

with R_{VPDB} the Vienna Pee Dee Belemnite-limestone standard, and,

$$R = (\delta^{15}N/1{,}000 + 1) \times R_{airN2}$$

with $R_{air\ N2}$ the atmospheric dinitrogen standard.

Total carbon and nitrogen transfer (^{12}C and ^{13}C; ^{14}N and ^{15}N) from algae to grazers was obtained from ^{13}C and ^{15}N incorporation, dividing $I^{13}C$ or $I^{15}N$ of urchins by $F^{13}C$ or $F^{15}N$ of the source.

2.7 | Data analysis

All data were expressed as the mean ± *SE*. Statistical analysis was completed using the statistical software R version 3.2.2 (R Core Team, 2014). $\delta^{13}C$ and $\delta^{15}N$ of labeled *Solieria chordalis* were compared between the beginning (0 day) and the end (8 days) using the non-parametric Wilcoxon–Mann–Whitney test, as assumptions of normality (Shapiro test) and homogeneity of variances (Bartlett test) were not met. The differences in $\delta^{13}C$ and $\delta^{15}N$ between labeled and non-labeled urchins were also examined using the Wilcoxon–Mann–Whitney test. The effects of temperature and time on $\delta^{13}C$ and $\delta^{15}N$ of urchins supplied with labeled *S. chordalis* were examined using the non-parametric Scheirer–Ray–Hare test, as assumptions of normality and homogeneity of variances (Bartlett test) were not met. Similarly, the Scheirer–Ray–Hare test was performed to examine the effect of increased temperature and time on urchin ^{13}C and ^{15}N uptake.

3 | RESULTS

After 8 days, stocks of *Solieria chordalis* thalli were labeled at a level of $\delta^{13}C$ = 1152 ± 106‰, (*n* = 3) and 2131 ± 617‰, (*n* = 3) for stocks 1 and 2, respectively. These levels were significantly higher than non-labeled thalli ($\delta^{13}C$ = −17.6 ± 0.4‰; Wilcoxon–Mann–Whitney test, *p* = .005). Similarly, labeled *S. chordalis* were significantly ^{15}N-enriched ($\delta^{15}N$ = 5191 ± 701‰, *n* = 3 and 4604 ± 319‰, *n* = 3, for stocks 1 and 2, respectively) compared with non-labeled seaweeds ($\delta^{15}N$ = 16.2 ± 0.3‰; Wilcoxon–Mann–Whitney test, *p* = .005).

At the beginning of the experiment, *Psammechinus miliaris* exhibited an initial $\delta^{13}C$ level of −17.2 ± 0.7‰ (*n* = 3) and −18.4 ± 0.3‰ (*n* = 3) at 17 and 20°C, respectively. In urchins supplied with labeled *S. chordalis*, $\delta^{13}C$ reached 941 ± 203‰ (*n* = 4) at 17°C and 623 ± 118‰ (*n* = 4) at 20°C after 30 days (Figure 1a). This enrichment was significant compared with the initial isotopic signatures (Wilcoxon–Mann–Whitney, *p* < .001). Throughout the experiment, higher $\delta^{13}C$ values were observed at 17 than at 20°C, whereas no significant effect of time was observed (Table 1, Figure 1a).

Psammechinus miliaris initial $\delta^{15}N$ values were 5.3 ± 0.8‰ (*n* = 3) and 4.4 ± 0.3‰ (*n* = 3) at 17 and 20°C, respectively. After 30 days, $\delta^{15}N$ in urchins supplied with labeled *S. chordalis* reached 1405 ± 109‰ (*n* = 4) at 17°C and 1514 ± 335‰ (*n* = 4) at 20°C (Figure 1b). The ^{15}N enrichment after 30 days was significant in comparison with initial isotopic values (Wilcoxon–Mann–Whitney, *p* < .001; Figure 1b). Temperature and time had no significant effect on urchins' $\delta^{15}N$ (Table 1).

At 17°C, mean ^{13}C specific uptake in urchins supplied with labeled *S. chordalis* ranged from 0.22 $\mu g^{13}C$ (day 15) to 0.36 $\mu g^{13}C$ (day 6) and reached 0.30 $\mu g^{13}C$ after 30 days (Figure 2a). At 20°C, ^{13}C uptake ranged from 0.14 $\mu g^{13}C$ (day 30) to 0.19 $\mu g^{13}C$ (day 6). ^{13}C uptake was significantly higher at 17 than at 20°C (Table 2), whereas no significant effect of time was evidenced (Table 2). *Psammechinus*

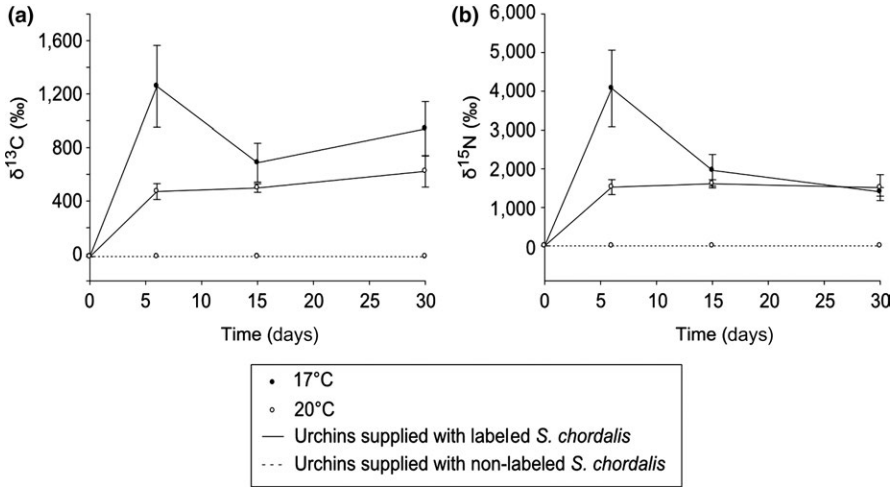

FIGURE 1 Variations in $\delta^{13}C$ (a) and $\delta^{15}N$ (b; mean ± *SE*) in *Psammechinus miliaris* maintained for 6, 15 and 30 days at 17°C (black dots) and at 20°C (white dots) and supplied with labeled (full lines) and non-labeled (dotted lines) *Solieria chordalis* (*n* = 4)

TABLE 1 Results of Scheirer–Ray–Hare tests to assess the effect of temperature (17 and 20°C) and time (days 6, 15 and 30) and their combined effect on the $\delta^{13}C$ and $\delta^{15}N$ in *Psammechinus miliaris* supplied with labeled *Solieria chordalis* (*n* = 4)

		$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)	
Factor	***df***	**H**	***p***	**H**	***p***
Temperature	1	7.3	**.007****	1.2	.27
Time	2	1.3	.53	4.6	.10
Temperature × time	2	1.0	.60	2.2	.32

Significant *p*-values are in bold.
*p < .05; **p < .01; ***p < .001.

TABLE 2 Results of Scheirer–Ray–Hare tests on the effect of temperature (17 and 20°C) and time (days 6, 15 and 30) and their combined effect on ^{13}C and ^{15}N uptake (I ^{13}C and I ^{15}N, respectively) in *Psammechinus miliaris* supplied with labeled *Solieria chordalis* (*n* = 4)

		I ^{13}Cµg^{13}C g DW^{-1}		I ^{15}Nµg^{15}N g DW^{-1}	
Factor	***df***	**H**	***p***	**H**	***p***
Temperature	1	5.8	**.016***	0.74	.39
Time	2	2.3	.32	11.9	**.003****
Temperature × time	2	1.0	.62	1.5	.47

Significant *p*-values are in bold.
*p < .05; **p < .01; ***p < .001.
DW, dry weight.

miliaris supplied with labeled *S. chordalis* exhibited ^{15}N specific uptake values ranging from 0.04 µg^{15}N (day 30) to 0.11 µg^{15}N (day 6) at 17°C (Figure 2b). At 20°C, ^{15}N uptake varied between 0.03 µg^{15}N (day 30) and 0.05 µg^{15}N (day 6). Temperature had no significant effect on ^{15}N specific uptake in *P. miliaris*, whereas ^{15}N uptake decreased significantly over time (Table 2).

4 | DISCUSSION

In this experiment, the urchin *Psammechinus miliaris* consumed substantial amounts of the alga *Solieria chordalis* at 17 and 20°C. The gut transit time of *P. miliaris* varies according to the food source ingested (Bedford & Moore, 1985) and may reach 42 hr in small individuals (Otero-Villanueva, 2004). The present feeding experiment was performed over 30 days, which is sufficient to measure carbon and nitrogen assimilation rates in *P. miliaris*.

At 17°C, an increase in $\delta^{13}C$ and $\delta^{15}N$ occurred during the first six days, corresponding to a significant ^{13}C and ^{15}N uptake. This may be a result of a high level of consumption of the labeled *S. chordalis* following the starving period that occurred before the experiments, as recently observed by Rubilar et al. (2016). In addition, several studies have reported a link between feeding and other metabolic processes in echinoids, such as respiration or excretion (Brockington & Peck, 2001; Carr & Bruno, 2013). These physiological processes can influence the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios. Among the physiological processes that can influence the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios, the increase in respiration rates induced by higher levels of feeding may contribute to ^{13}C enrichment due to a preferential loss of ^{12}C (Rau et al., 1983), and higher ^{15}N enrichment may be due to an increase in ^{15}N depleted nitrogen excretion (Minagawa & Wada, 1984) in the form of urea (Basuyaux & Mathieu, 1999). At 20°C, although the food source was identical, the increases in $\delta^{13}C$ and $\delta^{15}N$ of *P. miliaris* during the first 6 days were less pronounced as compared to those at 17°C. Hence, from the present study, temperature appears to play an important role in the trophic fluxes from algae to grazers, especially regarding carbon uptake.

^{13}C uptake was significantly higher at 17 than at 20°C throughout the experiment. At the end of the experiment, urchins supplied with labeled ^{13}C showed an assimilation of 10.1 and 4.9 ng ^{13}C day^{-1} at 17 and 20°C, respectively. The effect of temperature increase on the ^{13}C uptake of *P. miliaris* appeared to significantly alter the total carbon uptake, as estimated total carbon fluxes after 30 days were 0.29 µg C g dry weight (DW)$^{-1}$ day^{-1} at 17°C and 0.14 µg C g DW^{-1} day^{-1} at 20°C. These differences in carbon uptake observed between the two temperatures may be attributed to the higher metabolic activity at 17 than at 20°C, involving higher feeding and respiration rates. The lower metabolism at 20°C contrasts with the findings of several studies on diverse marine taxa (e.g., bivalves, gastropods, echinoderms), which showed higher feeding and respiration rates as the temperature increases (Cáceres-Puig,

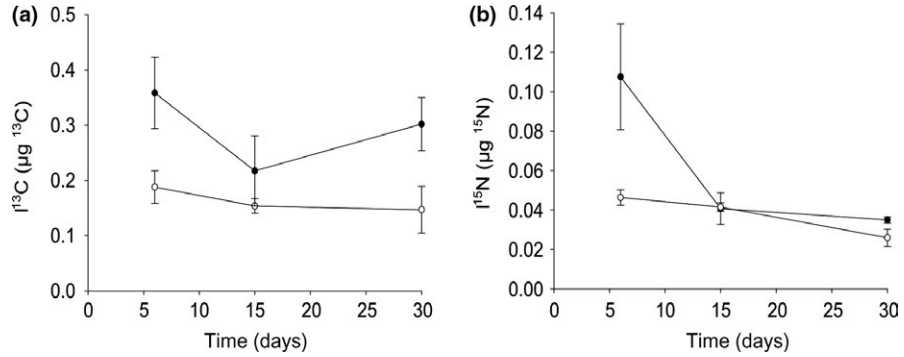

FIGURE 2 Variations in ^{13}C (a) and ^{15}N (b) uptake (I ^{13}C and I ^{15}N, respectively; mean ± *SE*) in *Psammechinus miliaris* maintained for 6, 15 and 30 days at 17°C (black dots) and at 20°C (white dots) and supplied with labeled *Solieria chordalis* (*n* = 4)

Abasolo-Pacheco, Mazón-Suastegui, Maeda-Martínez, & Saucedo, 2007; Carr & Bruno, 2013; Gooding et al., 2009; Noisette et al., 2014). However, a previous experiment on the high-latitude urchin *Strongylocentrotus droebachiensis* evidenced a decrease in feeding rates above 15°C, which is close to the maximum summer temperature (16°C, Frey & Gagnon, 2015). Similarly in the lobster *Jasus edwardsii*, lower feeding and respiratory rates were observed above the upper thermal limit at 24°C (Thomas et al., 2000).

Interestingly, ^{13}C and ^{15}N uptake responded differently during the experiment. Indeed, conversely to ^{13}C uptake, no effect of temperature increase was observed on ^{15}N uptake in *P. miliaris*. At the end of this feeding experiment, ^{15}N incorporation was 1.2 ng ^{15}N day^{-1} at 17°C and 0.8 ng ^{15}N day^{-1} at 20°C, which corresponded to estimated total nitrogen fluxes of 17.8 and 13.2 ng N g DW^{-1} day^{-1}, respectively. This suggests an effect of temperature increase on carbon and nitrogen stoichiometry in *P. miliaris*. Although heterotrophs are considered as more homeostatic than autotrophs (Persson et al., 2010), several biological and environmental factors have been evidenced to influence stoichiometric homeostasis. Some studies have showen that changes in elemental composition of food sources may induce imbalance in consumer elemental homeostasis (Malzahn & Boersma, 2012; Prado, Heck, & Cebrian, 2014). Moreover, in the bacteria *Pseudomonas fluorescens*, Chrzanowski and Grover (2008) demonstrated that temperature can influence their elemental content regardless of their resource nutrient content, which appears consistent with the present results. This imbalance in carbon and nitrogen fluxes in *P. miliaris* between 17 and 20°C may have major implications for physiological processes, such as growth and reproduction (Frost, Xenopoulos, & Larson, 2004; Heflin et al., 2012).

Some studies have shown that temperature increase may alter macroalgal palatability to grazers (Poore et al., ,), through changes in nutritional qualities or levels of deterrent secondary metabolites (Staehr & Wernberg, 2009; Sudatti, Fujii, Rodrigues, Turra, & Pereira, 2011). In this context, further labeling experiments should be conducted to examine the trophic transfer from macroalgae to grazers at different temperatures. In addition, these results highlight that quantitative estimation of trophic transfers between the two first trophic levels should not be based solely on carbon fluxes as is often the case.

In conclusion, the use of dual stable isotope labeling shows promise for testing the influence of various environmental factors on major macroalga–grazer trophic interactions within marine habitats, including their response to climate change. Further similar experimental studies should also couple stable isotope data related to assimilated nutrients with other physiological processes associated with feeding, such as ingestion, excretion or respiration.

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