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Diet-dependent $\delta^{13}C$ and $\delta^{15}N$ fractionation among sea urchin *Lytechinus variegatus* tissues: implications for food web models

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ABSTRACT: Consumer-diet discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$) are often applied without corroboration from laboratory experiments. Deviations in $\Delta^{15}N$ and $\Delta^{13}C$ that may occur from different diet types were quantified by raising 120 sea urchins *Lytechinus variegatus* in laboratory tanks on 3 different diets: seagrass *Thalassia testudinum*, red foliose macroalgae (*Grauteloupia* sp. and *Palmaria palmata*) and a mixed diet specifically formulated for *L. variegatus*. Patterns of $\delta^{13}C$ and $\delta^{15}N$ and resulting fractionation factors were then determined in muscle, gonad, gut, test organic matrix and whole individuals. Tissue $\delta^{13}C$ values showed a strong positive association with $\delta^{13}C$ of diets and estimates of absorption efficiency ($R^2 = 0.81$). The seagrass diet consistently resulted in negative $\Delta^{13}C$ values in all tissues (from −0.86‰ in muscle to −1.63‰ in gonad) and whole individuals (−1.19‰), whereas macroalgal and formulated diets showed positive values (0.11 and 0.19‰, respectively). Only individuals on the formulated diet clearly reached isotopic equilibrium for $\delta^{13}C$, suggesting that other lower quality diets may have resulted in more continuous reallocation of internal resources. $\delta^{15}N$ values increased as the nitrogen content of these diets decreased (3.18, 1.21 and 0.82‰ for seagrass, macroalgae and formulated diets, respectively). Overall differences in the biochemical composition of diets and a robust relationship between $\delta^{13}C$ and $\delta^{15}N$ signatures suggest that protein quantity and quality could be central in driving isotope fractionation. The influence of macrophyte material type in the diet can be stronger than that of trophic level for both $\delta^{13}C$ and $\delta^{15}N$, so further compound-specific isotope analyses are necessary to determine reliable values of $\Delta^{13}C$ and $\Delta^{15}N$ for ecological applications.

KEY WORDS: Seagrass · Macroalgae · Omnivory · Diet quality · Absorption efficiency

INTRODUCTION

Stable isotope analyses are increasingly used to answer multiple types of ecological questions, such as identifying trophic position and food web linkages, foraging ranges, or dietary reconstructions (reviewed by Fry 2006). The implementation of stable isotope techniques has aided dietetic studies based on irregular foraging observations, long-term evaluation of assimilation rates, and complex interpretations of gut and fecal contents (Afik & Karasov 1995). Accurate interpretation of diet and trophic relation-

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ships, however, requires distinct stable isotope signatures among diet sources (DeNiro & Epstein 1978) and the availability of accurate estimates of consumer-diet fractionation (see Gannes et al. 1997). The theoretical $\Delta^{15}N$ (i.e. the increase in $\delta^{15}N$ between consumers and their diets) is considered to be around 3.4‰ (Peterson et al. 1985, Kwak & Zedler 1997), but in practice it ranges between 2 and 5‰ (DeNiro & Epstein 1981). For $\delta^{13}C$, animals are generally considered to be enriched (i.e. $\Delta^{13}C$) by +1‰ and to resemble the $\delta^{13}C$ value of the diet (Vander Zanden & Rasmussen 2001, Post 2002).

Diet nutritional quality may mediate the magnitude of isotopic fractionation (Hobson et al. 1993, Adams & Sterner 2000, Oelbermann & Scheu 2002, Gaye-Siessegger et al. 2003, Pearson et al. 2003). A meta-analysis across taxa from terrestrial and aquatic systems found that $\Delta^{15}N$ increased in diets of low nutritional quality (Vanderklift & Ponsard 2003). Robbins et al. (2005) also pointed out that $\Delta^{15}N$ in mammals and birds was lower for carnivores than for herbivores, presumably because carnivores feed on protein of higher nutritional value that is more similar to that of their own tissues. Similarly, animals fed on high-quality prey have shown smaller $\Delta^{13}C$ values (<0.37‰) than those starved or reared on low quality diets (Hobson et al. 1993, Oelbermann & Scheu 2002), thus supporting the view that the $\delta^{13}C$ of predators resembles that of their diet (Minagawa & Wada 1984). Therefore, isotope fractionation in omnivorous animals may vary depending on the availability of plant and animal resources.

The type of plant resource may also have large effects on isotope fractionation, possibly as a result of differences in qualitative characteristics influencing food and/or absorption efficiency by consumers. Crawley et al. (2007) found $\Delta^{13}C$ values of −9 to −10‰ for amphipods fed on seagrass diets but only −2 to −4‰ for those fed on macroalgae. Since these results provide a large deviation from theoretical values, they suggest that the importance of seagrasses in the diet of marine herbivores could have been previously underestimated (e.g. Lepoint et al. 2000, Vizzini et al. 2002, Tomas et al. 2006). Nutrient absorption may be enhanced owing to the action of symbiotic microbes in consumers' guts (Lawrence & Klinger 2001) but low quality resources are mostly thought to be compensated for by enhanced consumption (Valentine & Heck 2001). Hence, the low values of $\Delta^{13}C$ reported for seagrass diet by Crawley et al. (2007) could be explained as a result of the assimilation of specific components of the plant material with lower $\delta^{13}C$ than the bulk tissue, most of which is not metabolized.

The type of body tissue considered is another important influence on isotope signatures and fractionation factors (e.g. Roth & Hobson 2000, Pearson et al. 2003, Sweeting et al. 2007). Differences in $\delta^{15}N$ among tissues have been related to rates of protein synthesis or degradation and to the relative requirements of essential versus non-essential amino acids subjected to additional metabolic pathways (Fogel & Tuross 2003, Schmidt et al. 2004, O'Brien et al. 2005). $\Delta^{15}N$ is usually higher in tissues with enhanced protein turnover such as muscle, and lower in other tissues such as heart and liver (Sweeting et al. 2007). Additional variability (up to 1–2‰ of the bulk $\delta^{15}N$ of tissues) may be induced by the relative composition of amino acids, which may vary in $\delta^{15}N$ ratios from 0.8 to 6.4‰ among body tissues (Schmidt et al. 2004). For $\delta^{13}C$, values across tissues appear to be strongly related to metabolic rates; more metabolically active tissues such as fat and liver have more rapid carbon turnover rates and lower signatures than less metabolically active tissues such as blood or muscle (Tieszen et al. 1983, Hobson & Clark 1992) and may interact with characteristics of the dietary regime such as protein abundance and/ or amino-acid composition (Fantle et al. 1999, Fogel & Tuross 2003, O'Brien et al. 2005). Lipid formation — usually the most depleted biochemical component due to discrimination against ^{13}C during lipid synthesis (DeNiro & Epstein 1977) — also depends on the type of diet and is unevenly stored within tissues. White muscle is characterized by low fat and usually displays the lowest ranges of isotope variability, which makes it the most frequently measured in ecological studies (Pinnegar & Polunin 1999). Invertebrates, however, may only have a small proportion of muscle tissue in their bodies and, on its own, this muscle may not adequately represent dietary contributions. In addition, some taxa such as sea urchins are capable of accumulating reserve compounds other than lipids, such as carbohydrates or proteins within guts, gonads and body wall (Giese 1961, Moss & Lawrence 1972), although the effect of diet type in tissues' signatures remains unexplored. Other factors such as growth rates or developmental stage may also influence metabolic rate and the isotopic turnover of tissues, though such effects appear to be somewhat inconsistent (Roth & Hobson 2000, Oelbermann & Scheu 2002).

Sea urchins are widespread and play important roles in shallow benthic communities (Prado et al. 2007, Watts et al. 2007). Specifically, the variegated sea urchin *Lytechinus variegatus* is recognized as the main seagrass consumer in the northern Gulf of Mex-

ico (Valentine & Heck 2001), but it also feeds on a variety of macroalgae and benthic animals such as mussels, crustaceans and epibionts (Watts et al. 2007) when the availability of macroflora becomes limited (Cobb & Lawrence 2005). Therefore, the variegated sea urchin offers an excellent model to investigate the effects of macrophyte type (seagrass vs. macroalgae), trophic position (herbivore vs. omnivore diets) and tissue-type on consumer-diet fractionation.

In the present study we examine patterns of $\delta^{13}C$ and $\delta^{15}N$ dietary acquisition in the sea urchin *Lytechinus variegatus* growing from juvenile, sexually immature individuals (~2 cm diameter and >6 mo of age; Beddingfield & McClintock 1998) to adult stages (sizes > 4 cm and 1 yr of age; Moore et al. 1963) and fed 2 common types of macrophyte diets, the seagrass *Thalassia testudinum* and red foliose macroalgae (*Grauteloupia* sp. and *Palmaria palmata*), and a diet formulated for *L. variegatus* with high nutritional and energy content. We assess the variability in $\Delta^{15}N$ and $\Delta^{13}C$ across diets, tissues (muscle, gonad, gut and the organic matrix of tests) and whole individuals and conclude with recommendations for future interpretations of stable isotope values from seagrass meadows' food webs.

MATERIALS AND METHODS

Collection of individuals and initial acclimation

Small *Lytechinus variegatus* (1.5 to 2 cm; n = 150) were collected in mid February 2009 from a shallow seagrass bed of *Thalassia testudinum* located at the end of the Tyndall peninsula in the Saint Andrew's Bay estuary, FL (30° 7′ 20.57″ N, 85° 41′ 16.32″ W). The urchins were transported to the laboratory within an aerated cooler and, once there, transferred to a large tank (500 l) within the wet lab facilities at the Dauphin Island Sea Lab. Sea water was pumped from Mobile Bay, filtered and recirculated in an environmentally-controlled lab. Experimental conditions were salinity 32 ppt, 22 to 23°C (median annual temperature in the field), alkalinity >200 ppt, pH 8.1 to 8.3, and low dissolved nutrients (NO_3: <10 mg l^{-1}; NO_2 and NH_4: 0 to 0.2 mg l^{-1}), and the aquarium room was maintained on a 12 h light: 12 h dark photoperiod (see Hammer et al. 2004). Nutrient and alkalinity levels in the experimental sea water were monitored daily and water changes made and appropriate quantities of sodium bicarbonate (Arm and Hammer®) added when necessary to maintain the initial levels.

To standardize the stable isotope composition of field-collected individuals during the initial acclimation period, sea urchins were fed a uniform diet from mid February until the end of March. The diet during the acclimation period included thalli of the green alga *Ulva* sp. that were collected from rocks in Mobile Bay, AL, dried, ground and mixed with water and agar (10 g of algal powder plus 2 g of agar per 100 ml of seawater) to produce storable food blocks that were prepared once a week. The tank was cleaned daily to remove excess food and feces.

Experimental design and diet treatments

Ten 75 l tanks connected to the same feeding system of recirculating water (ca. 2000 l) were deployed within a room at the wet-lab facility. Sea water and photoperiod conditions were the same as during the initial acclimation period. Within each tank, 12 sea urchins were placed in individual containers and 4 individuals randomly assigned to one of the diets (seagrass, red macroalgae, or a formulated diet) for a total of 120 individuals in the experiment. The containers consisted of a 50 × 10 cm rigid plastic mesh cylinder (3 mm hole-size) attached to a PVC base that kept the cylinder upright. Each container was fitted with an aerator.

Natural diets

Sea urchins were fed with green leaves of the seagrass *Thalassia testudinum* and 2 types of foliose red algae (*Grauteloupia* sp. from April to mid June, and *Palmaria palmata* from mid June to July). Shoots of *T. testudinum* and fronds of the red alga *Grauteloupia* sp. were collected weekly from a site in Big Lagoon, FL (30° 18′ 32.47″ N, 87° 22′ 55.54″ W) and from a rocky area adjacent to the Mobile Bay ferry landing at Fort Morgan, AL (30° 13′ 54.44″ N, 88° 0′ 55.70″ W). They were then kept alive in aerated tanks in the wet lab and replaced at the end of each week, at which time samples were collected and preserved (–20°C) for later examination of stable isotope contents and nutritional composition (n_{Thal} = 16; once a week from April to July). The population of *Grauteloupia* sp. sampled is possibly a first invasive settlement of *G. turuturu* (J. M. Lopez-Bautista, University of Alabama, pers. comm.), and although no sea urchins were observed in the area, it seems to lack defenses against urchin grazing (Rothäusler et al. 2005, Jacquin et al. 2006) and was

consumed at high rates by *Lytechinus variegatus* (P. Prado pers. obs.). Compared to detached clumps of drift macroalgae within seagrass beds (commonly *Laurencia* sp., *Polysiphonia* sp., *Chondria* sp., *Hypnea* sp., and *Gracilaria* sp.; P. Prado pers. obs.), the thick fronds of *Grauteloupia* sp. provided a proxy for red macroalgae that does not easily fragment when manipulated and reduced the risk of contamination across dietary treatments. The alga's local life-cycle was, however, unknown and it disappeared suddenly in mid June, necessitating its replacement with *Palmaria palmata* from a commercial supplier (Atlantic Mariculture) during the last 6 wk of the experiment. Therefore, the number of samples were n_{Gra} = 10 for *Grauteloupia* sp., collected once a week over 2.5 mo and n_{Pal} = 5 for *P. palmata* collected once from the received bag. We selected *P. palmata* because it also has a sheet-like morphology that is easy to manipulate and has been indicated to have a similar protein and carbohydrate content to *Grauteloupia* sp (Morgan et al. 1980, present study) that enhances roe development and growth in other species of sea urchins from the Atlantic coast (Vadas et al. 2000, Cook & Kelly 2007). *P. palmata* was received sun-dried and recovered fresh texture when hydrated. Epiphytes were carefully removed from *T. testudinum* leaves and *Grauteloupia* sp. fronds (no epiphytes were present on *P. palmata*) before offering these diets to the urchins.

Formulated diet

This consisted of an extruded, nutrient-dense diet formulated with vegetal and animal ingredients designed to maximize sea urchin growth (see Hammer et al. 2012 for details on composition), which was embedded in agar (10 g of pellets plus 2 g of agar per 100 ml of seawater) to minimize disintegration and cross-contamination across treatments. The formulated diet was prepared when necessary and in the same manner throughout the experiment. Samples for later examination of stable isotopes and nutritional composition were collected once from the material remaining at the end of the experiment (n_{For} = 5).

All diets were supplied ad libitum for 1 d and replaced after 24 h, after careful removal of food remains and feces from tanks and containers. At the end of each experimental month, the amount of food ingested and absorbed per individual was monitored during 5 consecutive days.

Characterization of diets

Diet samples of seagrass, red macroalgae and formulated diet were dried to a constant weight at 70°C and ground to a fine powder with a mortar and pestle. Total organic matter (% OM) was calculated by subtraction after combustion of dry samples at 500°C for 5 h. For calorimetry, dried dietary materials and feces (see next subsection) were ground and pelletized. Pellets were mixed with benzoic acid to ensure a complete combustion and then burned in a Parr 6725 Semimicro calorimeter. Gross energy values were expressed in cal g dry wt^{-1}. Total lipids (%) were extracted from dried diet samples by direct elution with chloroform and methanol, using the methods described by Folch et al. (1957). Total carbohydrates (%) in diets were determined with the phenol–sulfuric acid assay of Dubois et al. (1956) based on colorimetric absorbance at 490 nm. Total crude protein analyses were carried out by combustion at the Eurofins Scientific facility in Des Moines, IA. Only 1 protein sample was analyzed per diet type, which for seagrass and *Grauteloupia* sp. consisted of pooled dried material available from the different collection times.

Production efficiency and energetics

At the end of each month, changes in sea urchin sizes were investigated by weighing individuals to the nearest 0.1 g. Growth rates were estimated as the difference in g wet wt (WW) between urchin weights at the beginning and at the end of each month of the experiment and used to calculate food efficiency rates: growth rate/proportion of OM in diet. Absorption rates of each dietary material (seagrass, red macroalgae and formulated diet) were estimated as the decline in caloric content (cal g dry wt^{-1}) from food to feces (Fernandez 1997).

Stable isotope analyses

Sample preparation

Ten individuals from the initial acclimation period (n = 5 per tissue and 5 for whole-individual analyses) were stored in a −20°C freezer for dissection and determination of stable isotope contents. During later months, 3 individuals from each tank, 1 on each experimental diet, were separated and stored. Individuals from Tanks 1 to 5 were assigned for stable

isotope analyses of specific tissues (i.e. muscle, gonad, gut and test), whereas whole individuals from Tanks 6 to 10 were analyzed. Samples of epiphyte-free seagrass and *Grauteloupia* sp. were taken weekly for stable isotope analyses and stored at −20°C. For the *Palmaria palmata* and the nutrient-dense formulated diet, 5 replicate samples were collected throughout the experimental period.

After they were defrosted, individual sea urchins from Tanks 1 to 5 (n = 60) were dissected into different tissues, rinsed with ultrapure water to eliminate food pellet remains, dried, weighed and ground to fine powder using a glass bar (muscle and guts) or a mortar and pestle (gonads and tests). Whole sea urchin individuals from Tanks 6 to 10 (n = 60) were ground in a blender that was acid-washed after each sample. The powder was placed in pre-weighed vials. To avoid small losses of nitrogen or possible deviations of isotopic signatures due to removal of inorganic carbon with acidification methods (Carmichael et al. 2008), test and whole sea urchin samples were divided in 2 equal parts that were analyzed separately for $\delta^{15}N$ and $\delta^{13}C$ signatures. Inorganic carbon in tests and whole individuals was removed following low stringency methods indicated in Carmichael et al. (2008) for shell material of *Mercenaria mercenaria*. Briefly, 2 series of 2 ml of 1% $PtCl_2$ in 1 N HCl were carefully pipetted into scintillation vials containing ca. 100 mg dry wt of pre-weighed sample. Samples were stirred after each acid addition and left to rest until no further bubbling was observed (24 h). Then, samples were filtered through pre-combusted 0.7 µm glass fiber filters (Whatman) and ultrapure water added thoroughly to minimize residual acid in filtered material. Filters were dried at 60°C and ca. 1 mg of dried organic material was carefully removed from the filter surface. All samples were checked under the microscope to ensure no glass fiber fragments from the filter were included. Finally they were packed into tin capsules for nutrient content and stable isotope analyses.

In total, 130 sea urchins were analyzed for stable isotope composition: 10 during the initial acclimation period and then 60 for tissue and 60 for whole-individual samples (5 tanks × 4 mo × 3 diets) during the rest of the experiment. In contrast, the number of samples from each diet type depended on the need to account for possible temporal variability and availability of the diet: $n_{Ulva} = 5$ (once in March); $n_{Thal} = 16$ (once per week from April to July); $n_{Gra} = 10$ (once per week from April to mid June); $n_{Pal} = 5$ (once from the same package); $n_{For} = 5$ (once at the end of the experiment). All samples were weighed (ca. 2 to 2.5 mg for vegetal tissues and ca. 1 to 1.3 mg for urchin tissues and the nutrient-dense formulated diet), packed into tin capsules and stored in a desiccator.

Water samples (n = 15) were randomly collected from different tanks during the experiment to assess the possible contribution of particulate organic matter (POM) to sea urchin diets. Water was filtered through pre-combusted 0.7 µm Whatman glass fiber filters (4 h, 495°C) until clogging (ca. 1500 to 2500 ml), dried at 60°C, packed and stored with the other samples.

Analyses

Samples were analyzed with a Thermo Finnigan Delta V advantage mass spectrometer connected to a Costech 4010 elemental analyzer through a Thermo Finnigan Conflo III interface at the Biochemical, Mass spectral, Stable Isotope Analytical Facility at University of Alabama (Tuscaloosa). Isotope ratios in samples were calculated from linear calibration curves constructed with standard reference materials of known composition and a blank correction. The difference in isotopic composition between the sample and reference material ($\delta_{sample-standard}$, expressed in per mille, ‰) is determined with the equation:

$$\delta_{sample-standard} = [(R_{sample} - R_{standard})/(R_{standard})] \times 1000 \quad (1)$$

where R_{sample} is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio in the sample, and $R_{standard}$ is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio in the calibration material and $\delta_{sample-standard}$ is the difference in isotopic composition of the sample relative to that of the reference (Vienna Peedee Belemnite and atmospheric nitrogen for carbon and nitrogen, respectively). Experimental precision (based on the standard deviation of replicates of an atropine standard and/or IAEA or USGS intercomparison materials) was very good (±0.15‰ for ^{13}C and ±0.11‰ for ^{15}N).

Data analyses

The possible variability in $\delta^{13}C$ signatures introduced by uneven storage of lipids (typically depleted ^{13}C) across tissues was corrected using the equations provided by Post et al. (2007). The threshold for the application of this correction was an increase in corrected signatures of at least 0.1‰, which occurred at lipid contents of ca. 5% of tissue weights. $\delta^{13}C$ signatures of diets were normalized using the relationship

with available lipid contents (see Table 1), whereas for tissues we used the relationship with C:N ratios indicated for aquatic animals. Whole individuals, however, presented high levels of carbon (ca. 42 to 54%) that prevented the correction of $\delta^{13}C$ signatures using C:N ratios. Instead, percent lipid content in the carbonate-free body fraction (i.e. dry wt of whole individuals minus dry wt of $CaCO_3$ content) was estimated from dry weights and C:N ratios within individual tissues (per diet and experimental month).

$\Delta^{15}N$ and $\Delta^{13}C$ per diet, tissue and for whole individuals on each diet were calculated using $\delta^{15}N$ and normalized $\delta^{13}C$ signatures of diets and urchins during the last 2 mo of the experiment to ensure that they reflected the incorporation of contemporary dietary material as much as possible. In fact, for the 2 natural diets, $\delta^{13}C$ in tissues was not clearly at equilibrium and $\Delta^{13}C$ represents mean values within a possible range. In the particular case of individuals raised on macroalgae, May to June values were used instead due to the unexpected need to replace algal species and the higher $\delta^{13}C$ signature of *Palmaria palmata* compared to *Grauteloupia* sp. (see Fig. 1), which may have obscured equilibrium patterns.

Differences in stable isotope composition across Diet (3 levels, fixed factor), Tissue (4 levels, fixed factor) and Month (4 levels, fixed factor) were investigated with a 3-way factorial analysis of variance (ANOVA) and Student-Newman-Keuls (SNK) post-hoc analyses. Month and not growing stage (juveniles vs. adults) was considered as a factor in the analyses to circumvent variability in the size at an age among diet treatments. Patterns of isotope composition in whole individuals were assessed with a 2-way factorial ANOVA (Diet and Month, fixed factors). We did not consider *Grauteloupia* sp. and *Palmaria palmata* as different diet levels within the ANOVA because one of the primary objectives of the study was to compare macroalgae to seagrass diets, and possible differences resulting from the change are reflected in the temporal trends. Isotope fractionation ($\Delta^{15}N$ and $\Delta^{13}C$) across tissues was investigated with a 2-way factorial ANOVA (Diet and Tissue, fixed factors) and with a 1-way ANOVA for whole individuals (Diet, fixed factor). ANOVA assumptions of normality (Chi-square test) and homogeneity of variances (Cochran's test) were not always achieved by transformation and in those instances, the significance level was fixed to $p < 0.01$ to minimize the risk of making a Type I error. The associations between the $\delta^{15}N$ and normalized $\delta^{13}C$ signatures of sea urchin tissues and whole individuals (n = 65 each when including individuals fed on the initial *Ulva* diet), $\delta^{15}N$ and normalized $\delta^{13}C$ signatures of the diets, and %C, %N of the diets (4 mean monthly values for the seagrass and red macroalgae diets and a single value for the formulated diet and the initial *Ulva* diet, respectively) were investigated with correlation and regression analyses. The association between absorption, rates of body growth and food efficiency, and $\delta^{13}C$ and $\delta^{15}N$ on those same whole individuals (n = 60; initial time not measured) was investigated with regression analyses. All statistical analyses were performed using Statistica version 7 software.

RESULTS

Sea urchin growth, energetics and survival

Urchins fed on the seagrass diet showed less than half the growth of those fed on red macroalgae (mean ± SE, 4.04 ± 0.31 and 8.96 ± 0.27 g wet wt mo^{-1}, respectively) and 3 times less growth than those fed on the formulated diet (12.85 ± 0.43 g wet wt mo^{-1}). Individuals on the seagrass diet also displayed the lowest food absorption and efficiency rates (22.7 ± 1.28% and 5.33 ± 0.30 g wet wt mo^{-1} %OM $diet^{-1}$, respectively), whereas those fed on macroalgae showed intermediate values (46.58 ± 0.49% and 12.76 ± 0.38 g wet wt mo^{-1} %OM $diet^{-1}$) and those fed on the formulated diet the highest values (58.12 ± 0.39% and 18.85 ± 0.63 g wet wt mo^{-1}. No mortality or starvation was observed for individuals fed any of the diets during the 5 mo experiment.

Lipid contents and corrections

Mean lipid contents (estimated from C:N ratios; Post et al. 2007) were higher in individuals fed the formulated diet, from 10.7 in April to 20.7% in July) than those fed red macroalgae (3.5 to 19.2%) and seagrass diets (2.3 to 6.3%), and lipid correction (Post et al. 2007) increased $\delta^{13}C$ signatures by 1.82 ± 0.2, 0.92 ± 0.2, and 0.06 ± 0.04‰, respectively for each diet. Differences in lipid contents among sea urchin tissues were also large, with higher values in gonad (23%), followed by gut (14.1%), muscle (5.2%) and negligible values in test. Lipid correction also increased $\delta^{13}C$ signatures by 2.42 ± 0.13, 1.43 ± 0.06, and 0.20 ± 0.02‰, for gonad, gut and muscle, respectively for each tissue.

Table 1. Mean (±SE) nutritional features of the different diets used to feed sea urchins during the experiment. *Ulva* sp. was used throughout March 2009 as a homogenization diet. *Grateloupia* sp. was used to feed individuals on the red algae treatment from April to mid-June and *Palmaria palmata* from mid-June to the end of July following the exhaustion of *Grateloupia* sp. in the field. Seagrass (*Thalassia testudinum*) and nutrient-dense formulated diets were used constantly from April until the end of the experiment

Diet type	Species in diet	Nitrogen (%)	Carbon (%)	Organic matter (%)	Calories (cal g^{-1})	Protein (% dry wt)	Lipid (% dry wt)	Carbohydrate (% dry wt)
Homogenization	*Ulva* sp.	1.95 ± 0.02	31.67 ± 0.18					
Seagrass	*T. testudinum*	2.47 ± 0.10	34.37 ± 0.35	75.72 ± 0.99	2889 ± 29.8	15.70	4.3 ± 0.25	8.86 ± 0.58
Red macroalgal	*Grateloupia* sp.	2.57 ± 0.14	27.44 ± 0.43	70.22 ± 0.82	3017.7 ± 14.9	16.34	1.6 ± 0.1	34.71 ± 1.76
	P. palmata	2.84 ± 0.01	35.82 ± 0.14	73.5 ± 0.76	3027 ± 15	18.92	3.5 ± 0.26	40.90 ± 1.64
Formulated		3.55 ± 0.09	31.96 ± 0.76	68.14 ± 1.00	3405.3 ± 8.5	23.40	9.6 ± 0.62	19.75 ± 0.32

In diets, lipid correction increased $\delta^{13}C$ signatures by 0.87‰ for the formulated diet, followed by 0.5‰ for the seagrass diet, and 0.31 and 0.2‰ for the red macroalgae and *Ulva* diets, respectively.

Diet characterization

Nutritional characterization of diets showed lower nitrogen, protein, and caloric contents in seagrass and magroalgae than in the formulated diet (Table 1). Lipids were higher in the nutrient-rich formulated diet despite higher carbohydrate content in macroalgae. Lipid-corrected $\delta^{13}C$ signatures remained lighter in the lipid-rich formulated diet (−21.58 ± 0.05‰) and the macroalgae *Grateloupia* sp. (−20.53 ± 0.53‰) and *Ulva* sp. (pre-treatment diet for signature homogenization; −18.86 ± 0.02‰) than in the macroalgae *Palmaria palmata* (−13.10 ± 0.06‰) and the seagrass *Thalassia testudinum* (−10.21 ± 0.13‰). $\delta^{15}N$ signatures were lighter in the formulated (5.15 ± 0.12‰) and seagrass diets (4.77 ± 0.37‰) than in macroalgae (6.77 ± 0.06‰ in *Ulva* sp.; 6.77 ± 0.21‰ in *Grateloupia* sp.; and 5.56 ± 0.05‰ in *P. palmata*). For the 2 natural diets collected weekly (*T. testudinum* and *Grateloupia* sp.), there was substantial temporal variation (April to July; Fig. 1). For example, seagrass increased $\delta^{15}N$ signatures by 2.4‰ and declined $\delta^{13}C$ by 0.24‰, while *Grateloupia* sp. increased both $\delta^{15}N$ and $\delta^{13}C$ signatures by 0.9 and 1.6‰, respectively. The preserved *P. palmata* showed little variation. Particulate organic matter in the water displayed signatures of −18.45 ± 0.45 and 5.93 ± 0.26‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively, which were closer to those of macroalgae than to seagrass or formulated diets.

Stable isotope composition in tissues and whole individuals

$\delta^{13}C$ signatures (corrected for lipid content) were different across all diet treatments (Table 2), with significantly higher values in individuals fed seagrass (−12.17 ± 0.29‰) than in those fed red macro-

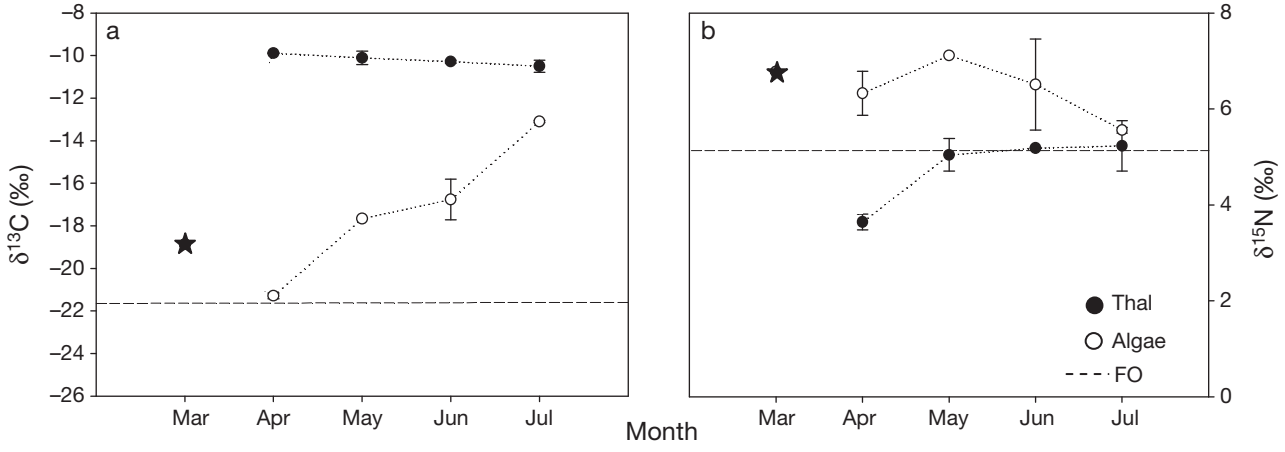

Fig. 1. Mean (±SE) stable isotope signatures of (a) $\delta^{13}C$ and (b) $\delta^{15}N$ of diets throughout the experiment. Seagrass diet (Thal) was *Thalassia testudinum* throughout. Red macroalgae diet (Algae) was *Grauteloupia* sp. in April and May, mixed *Grauteloupia* sp. (Days 1 to 15) and *Palmaria palmata* (Days 16 to 30) in June and only *P. palmata* in July. (---) Formulated diet (FO) signatures were consistent over the course of the study. (★) *Ulva* sp. diet during urchin acclimation period

algae (–16.32 ± 0.28‰) and the formulated diet (–20.83 ± 0.11‰). Differences among tissues depended on the diet (i.e. significant Diet × Tissue interaction) but displayed higher values in muscle and gut (–15.86 ± 0.40 and –16.18 ± 0.48‰, respectively), intermediate in test (–16.52 ± 0.60‰), and lowest in gonad (–17.40 ± 0.47‰). Signatures were similar during the 2 first mo of the experiment and then increased significantly in June and July, particularly for the red macroalgae and the seagrass diets and for gonad tissue (i.e. a significant Diet × Month and Tissue × Month interactions; Table 2, Fig. 2). In contrast, the formulated diet seemed to reach isotopic equilibrium after the first month of the experiment for test and gut and at the third month for muscle and gonad (Fig. 2).

$\delta^{15}N$ signatures were significantly higher in individuals fed the formulated diet (5.62 ± 0.07‰) than in those fed seagrass (7.72 ± 0.13‰) and red macroalgae (7.74 ± 0.09‰) (Table 2). Differences among tissues were variable across diets (i.e. Diet × Tissue interaction; Table 2) but overall values were higher in muscle (8.04 ± 0.22‰) and test (7.25 ± 0.14‰) than in gut (6.44 ± 0.14‰) and gonads (6.38 ± 0.12‰), which were not significantly different. Temporal variability in $\delta^{15}N$ was also significant across diets and tissues (i.e. Diet × Month and Tissue × Month interactions; Table 2) but low compared to that displayed by $\delta^{13}C$ (Fig. 2).

In whole individuals, $\delta^{13}C$ values were also significantly higher for urchins fed seagrass (–12.77 ± 0.32‰), than for those fed macroalgae (–16.57 ± 0.60‰) and formulated diet (–21.39 ± 0.21‰). For the 2 natural diets, values were low during the first 2 mo of the experiment and then increased in June and July, whereas the formulated diet showed similar values through time (Table 3, Fig. 2). $\delta^{15}N$ signatures were higher in individuals fed seagrass (8.05 ± 0.07‰) than for those on macroalgae (7.79 ± 0.10‰) and the nutrient-dense formulated diet (5.9 ± 0.16‰) with no significant time effects (Table 3, Fig. 2).

Isotope fractionation in tissues and in whole individuals

$\Delta^{13}C$ values were consistently negative in individuals fed *Thalassia testudinum* (–1.13 ± 0.29‰), and slightly positive in individuals fed red macroalgae and formulated diets (0.89 ± 0.28 and 0.83 ± 0.15‰, respectively). However, for individuals fed red macroalgae, the change from *Grauteloupia* sp. to *Palmaria palmata* in June introduced a certain degree of uncertainty in the estimations and possibly masked further differences among diets. In contrast, differences among tissues were not significant, although they tended to be highest in muscle and lowest in the gonad (Table 4).

For $\Delta^{15}N$, significantly higher values were observed in individuals fed seagrass (2.85 ± 0.23‰), followed by those fed macroalgae (1.10 ± 0.21‰), and the formulated diet (0.43 ± 0.14‰). There were also

Table 2. *Lytechinus variegates.* (a) Three-way analysis of variance (ANOVA) and (b) post-hoc Student-Newman-Keuls (SNK) results showing the effects of diet with factors seagrass (SG), red macroalgae (RM) and formulated (FO), tissue type with factors muscle (MU), gonad (GO), gut (GU) and test (TE) and month with factors Apr through Jul in the patterns of $\delta^{13}C$ (corrected for lipid content) and $\delta^{15}N$ composition. Significant values in **bold** at $p < 0.01$ for non-transformable data. ns: not significant

Source of variation	df	$\delta^{13}C$			$\delta^{15}N$		
		MS	*F*	p	MS	*F*	p
(a) ANOVA							
Diet	2	1254.96	1120.93	**0.0000**	11841.25	47477.39	**0.0000**
Tissue	3	97.59	87.16	**0.0000**	118.69	475.88	**0.0000**
Month	3	17.03	15.21	**0.0000**	0.01	0.05	0.9834
Diet × Tissue	6	28.36	25.34	**0.0000**	36.40	145.93	**0.0000**
Diet × Month	6	9.49	8.48	**0.0000**	3.18	12.74	**0.0000**
Tissue × Month	9	10.25	9.15	**0.0000**	1.99	7.98	**0.0000**
Diet × Tissue × Month	18	2.16	1.93	0.0155	0.66	2.64	0.0066
Error	192	1.12			0.53		
Cochran's *C*			**0.52**			0.29 (ns)	
Transformation			None			None	
(b) SNK							
Diet		FO < RM < SG			FO < RM = SG		
Tissue		GO < TE < GU = MU			GO = GU < TE < MU		
Month		Apr = May < Jun < Jul					

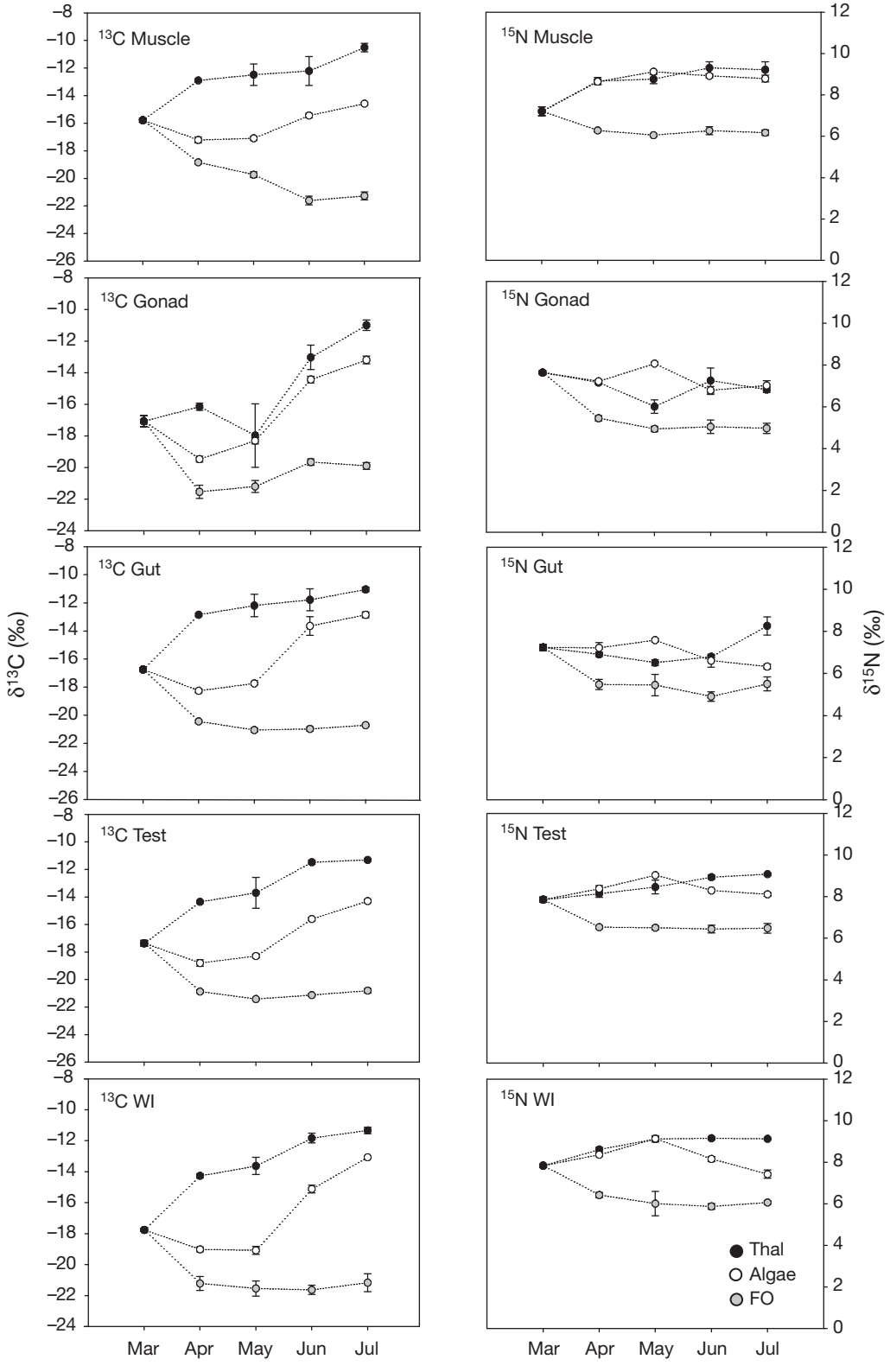

Fig. 2. *Lytechinus variegates.* Mean (±SE) (left column) δ^{13}C and (right column) δ^{15}N stable isotope composition of sea urchin tissues (muscle, gonad, gut and test) and whole individuals fed on seagrass (Thal), macroalgae (Algae) and nutrient-dense formulated diet (FO) at each month of the experiment. WI: whole individual

Table 3. *Lytechinus variegates.* (a) Two-way analysis of variance (ANOVA) and (b) post-hoc Student-Newman-Keuls (SNK) results showing the effects of each diet (seagrass, red macroalgae and the nutrient-dense formulated diet) and month in the patterns of $\delta^{13}C$ and $\delta^{15}N$ signatures of whole individuals (WI). Significant values in **bold** at $p < 0.01$ for non-transformable data. Abbreviations as in Table 2

Source of variation	df	$\delta^{13}C$ MS	$\delta^{13}C$ *F*	$\delta^{13}C$ p	$\delta^{15}N$ MS	$\delta^{15}N$ *F*	$\delta^{15}N$ p
(a) ANOVA							
Diet	2	373.66	602.57	**0.0000**	28.167	134.72	**0.0000**
Month	3	32.19	51.91	**0.0000**	0.664	3.18	0.0324
Diet × Month	6	11.20	18.05	**0.0000**	0.680	3.25	0.0091
Error	48	0.62			0.209		
Cochran's *C*			0.22 (ns)			**0.68**	
Transformation			None			None	
(b) SNK							
Diet			FO < RM < SG			FO < RM < SG	
Month			Apr = May < Jun < Jul				

significant differences across tissues, with higher values in muscle and test and lower values in gonad and gut, which were not significantly different (Table 4a,b). Test represented the mean $\Delta^{15}N$ consistently better than all other tissues and whole individuals on each diet (Table 4c).

In whole individuals, dietary effects were similar to those observed in tissues. $\Delta^{13}C$ values were only significantly lower in individuals fed the seagrass diet (–1.19 ± 0.21‰; $F = 4.135$, df = 27, $p < 0.05$) although the switch of macroalgae species may have partially masked the differences. In contrast, all diets were significantly different in $\Delta^{15}N$ ($F = 225.13$, df = 27, $p < 0.0001$) with highest values in seagrass and lowest in the formulated diet (Table 4b).

Table 4. (a) Two-way analysis of variance (ANOVA) and (b) post-hoc Student-Newman-Keuls (SNK) results showing the effects of diet and tissue type in the values of $\Delta^{13}C$ and $\Delta^{15}N$. Significant values in **bold** at $p < 0.01$ for non-transformable data. (c) Mean (±SE) $\Delta^{13}C$ and $\Delta^{15}N$ in sea urchin tissues and whole individuals (WI) fed on each experimental diet. Calculations were conducted using isotope data for the last 2 mo of the experiment (i.e. individuals were fed seagrass, red macroalgae and the formulated diet during 3 to 4 mo prior). Abbreviations as in Table 2

Source of variation	df	$\Delta^{13}C$ MS	$\Delta^{13}C$ *F*	$\Delta^{13}C$ p	$\Delta^{15}N$ MS	$\Delta^{15}N$ *F*	$\Delta^{15}N$ p
(a) ANOVA							
Diet	2	52.74	37.28	**0.0000**	62.58	183.19	**0.0000**
Tissue	3	4.596	3.249	0.0246	19.25	56.35	**0.0000**
Diet × Tissue	6	2.079	1.469	0.1953	0.888	2.59	0.0215
Error	108	1.414			0.341		
Cochran's *C*			**0.22**			**0.24**	
Transformation			None			None	
(b) SNK							
Diet			FO = RM < SG			FO < RM < SG	
Tissue						GO = GU < TE < MU	

(c) Means	$\Delta^{13}C$			$\Delta^{15}N$		
Diet type	Seagrass	Red macroalgae	Formulated	Seagrass	Red macroalgae	Formulated
Muscle	–0.86 ± 0.61	0.94 ± 0.16	1.81 ± 0.11	4.22 ± 0.20	2.21 ± 0.09	1.07 ± 0.11
Gonad	–1.63 ± 0.54	0.84 ± 0.51	0.15 ± 0.21	1.83 ± 0.31	0.62 ±0.15	–0.14 ± 0.19
Gut	–1.03 ± 0.41	1.52 ± 0.62	0.74 ± 0.09	2.32 ± 0.31	0.28 ± 0.16	0.05 ±0.22
Test	–1.01 ± 0.10	0.26 ± 0.31	0.61 ± 0.10	3.06 ± 0.08	1.29 ± 0.05	0.74 ± 0.15
WI	–1.19 ± 0.21	0.11 ± 0.54	0.19 ± 0.32	3.18 ± 0.08	1.21 ± 0.09	0.82 ± 0.08

Relationships among variables

Sea urchin isotope signatures with diet composition

$\delta^{13}C$ signatures in tissues were positively associated (df = 64, from $R^2 = 0.56$ in gonad to $R^2 = 0.86$ in gut and test) to $\delta^{13}C$ signatures of diets (i.e. means per month of natural diets; for further details see 'Materials and methods'). Significant but lower positive associations were also observed with monthly values of %C in diets (Table 5). For sea urchin tissues, $\delta^{13}C$ was positively correlated to $\delta^{15}N$ whereas relationships with %C in tissues were inconsistent.

Conversely, $\delta^{15}N$ in tissues was negatively associated to %N content in the diet (df = 64, from $R^2 = 0.49$ in muscle to $R^2 = 0.67$ in test) and only some tissues showed a weak relationship to $\delta^{15}N$ signatures of diets (Table 5). The relationship between $\delta^{15}N$ and %N of tissues was only significant for tests and whole individuals.

Sea urchin isotope signatures with production and energetic variables

Absorption efficiency, body growth rates, and food efficiency also displayed positive associations with $\delta^{13}C$ in those same whole individuals (df = 59, R^2 = 0.81, 0.59 and 0.58, respectively; $p < 0.0001$). For $\delta^{15}N$, similar positive relationships were observed (df = 59, R^2 = 0.48, 0.38 and 0.40 for absorption efficiency, body growth, and food efficiency, respectively; $p < 0.0001$), albeit weaker compared with those observed for $\delta^{13}C$.

DISCUSSION

To our knowledge this is the first time that the effect of diets with different plant and animal ingredients on isotopic fractionation has been investigated in a marine omnivore. Mean $\Delta^{13}C$ in whole individuals was similar for individuals fed on macroalgae and on the agar-incorporated formulated diet (0.11 and 0.19‰, respectively) and negative for individuals fed on the seagrass diet (–1.19‰). However, only individuals on the formulated diet were clearly in isotopic equilibrium for $\delta^{13}C$ during the experiment, suggesting that urchins fed on less balanced diets, such as seagrass, may constantly reallocate internal resources. Yet, there is likely uncertainty for macroalgae because the species switch in June may have prevented isotopic equilibrium and altered $\Delta^{13}C$ values. On the other hand, our results also suggest that other metabolic aspects such as digestibility and food assimilation may be important factors influencing $\Delta^{13}C$ in seagrass systems, which does not adequately reflect carbon sources. Individuals fed on the seagrass diet, which contained the lowest protein content, also had higher $\Delta^{15}N$ (3.18‰) than those fed on diets richer in protein (1.21 and 0.82‰ for macroalgae and formulated diet, respectively), which attained values that were lower than the 1.3 to 5.3‰ increase per trophic level indicated by Minagawa & Wada (1984). Our results highlight the problems in using theoretical levels of fractionation across species and ecosystems but also suggest that nutritional and metabolic variables could be used to adjust these values for mixing models.

Table 5. *Lytechinus variegates.* Correlation coefficients (r) between lipid-corrected $\delta^{13}C$ and $\delta^{15}N$ in tissues and whole individuals (WI); and determination coefficients (R^2) between stable isotope contents in tissues and whole individuals (WI) and lipid-corrected $\delta^{13}C$ and $\delta^{15}N$ in all diets, and %C and %N in tissues and diets. $^{*}p < 0.01$, $^{**}p < 0.001$, $^{***}p < 0.0001$

	$\delta^{15}N$ tissue	$\delta^{13}C$ diet	%C tissue	%C diet
$\delta^{13}C$ muscle	0.78***	0.84***	0.0007	0.24***
$\delta^{13}C$ gonad	0.61***	0.56***	0.07*	0.20***
$\delta^{13}C$ gut	0.55***	0.86***	0.001	0.31***
$\delta^{13}C$ test	0.77***	0.86***	0.32***	0.29***
$\delta^{13}C$ WI	0.70***	0.85***	0.49***	0.33**
		$\delta^{15}N$ diet	%N tissue	%N diet
$\delta^{15}N$ muscle		0.02	0.09*	0.49***
$\delta^{15}N$ gonad		0.17**	0.07	0.58***
$\delta^{15}N$ gut		0.10*	0.14	0.64***
$\delta^{15}N$ test		0.09	0.17	0.67***
$\delta^{15}N$ WI		0.04	0.38***	0.61***

Dietary effects

Differences in $\delta^{13}C$ values across individuals strongly reflected patterns of $\delta^{13}C$ within diets. However, the association between dietary and $\delta^{13}C$ signatures in tissues was lower for urchins fed on seagrass (R^2 from 0.2 in tests to 0.4 in gonad) than for those fed on macroalgae (R^2 from 0.67 in gut to 0.79 in test and gonad), suggesting that carbon components are not incorporated equally into tissues from each diet. In fact, $\delta^{13}C$ signatures in tissues and whole individuals were strongly associated to overall differences in body growth, absorption, and food efficiency of diets. Sea-

grass absorption efficiency is usually low among its consumers, e.g. 19% of organic matter in *Lytechinus variegatus* (Lowe & Lawrence 1976), 40% in Trochoid gastropods (Peduzzi 1987) and 18 to 38% in fish (Klumpp & Nichols 1983, Velimirov 1984), and uneven across biochemical components (Klumpp & Nichols 1983). The seagrass 'juice' (i.e. water with soluble compounds) is assimilated up to 100% and contains the largest part of the leaf protein fraction (ca. 67% according to Klumpp & Nichols 1983). The carbon isotope ratio of plant amino acids reflects both photosynthetic physiology and subsequent isotopic fractionation during amino acid synthesis (Abelson & Hoering 1961). Despite large variability across plant species (by ca. 20‰), $\delta^{13}C$ values of non-essential amino acids tend to be more enriched in $\delta^{13}C$ than essential amino acids (Fogel & Tuross 2003, O'Brien et al. 2005). Therefore, enhanced absorption of essential amino acids and/or other biochemical compounds in seagrass leaf 'juice' with a $\delta^{13}C$ signature that is lower than bulk tissue could partly explain negative $\Delta^{13}C$ values for the seagrass diet (see also Fantle et al. 1999). *L. variegatus* absorbs 67% of the turtlegrass lipid content and 13% of carbohydrates (Lowe & Lawrence 1976). Carbohydrate content in seagrass was small compared to protein, and lipid content in urchins fed the seagrass diet was also the lowest. In addition, significant positive associations between $\delta^{13}C$ and $\delta^{15}N$ in test ($R^2 = 0.64$) and whole individuals ($R^2 = 0.47$) suggests incorporation from seagrass protein pools.

The low nutritional quality of the seagrass diet in terms of biochemical composition and absorption efficiency may have also slowed down (or prevented) $\delta^{13}C$ equilibrium in tissues compared to the rapid stabilization observed in individuals fed the nutrient-rich formulated diet. Isotopic equilibrium is a meaningful paradigm in some biological processes such as shell formation that commonly precipitates $\delta^{18}O$ and $\delta^{13}C$ in equilibrium with ambient waters (see McConnaughey 1989). This, however, may be unrealistic for a trophic model, as the quality of a single resource may be insufficient to warrant the whole nutritional needs of a consumer (Westoby 1974), and the stable isotope composition of natural diets can also vary through time (Boon & Bunn 1994, present study). We found little monthly variation in seagrass $\delta^{13}C$ (0.61‰ decline) compared to $\delta^{15}N$ (1.54‰ increase) whereas monthly variability in sea urchin tissues was much higher for carbon (up to 5.15‰ increase in gonad $\delta^{13}C$ and a 0.35‰ decrease in $\delta^{15}N$) and suggests the influence of nutritional aspects in the isotopic equilibrium of tissues. For red macroalgae, unfortunately it is also possible that variability of $\delta^{13}C$ signatures within tissues is due to the species switch during the experiment, and the effect of nutritional quality in non-equilibrium patterns cannot be fully discerned.

For nitrogen, elemental dietary content, rather than $\delta^{15}N$ signatures, was the most important factor determining patterns of $\delta^{15}N$ and $\Delta^{15}N$ in sea urchins. $\delta^{15}N$ in *Thalassia* was very similar to that of the formulated diet (except in April) whereas values of macroalgae were significantly higher. In contrast, $\delta^{15}N$ in sea urchins was similar for plant diets (7.72 and 7.74‰ for seagrass and macroalgae, respectively) and significantly lower for the formulated diet (5.62‰). Higher $\delta^{15}N$ signatures in individuals fed herbivorous diets show that, on their own, they may not be accurate descriptors of the trophic position of sea urchins (Prado et al. 2010, Wangensteen et al. 2011) and require further support from fractionation rates. Yet, strong positive relationships with dietary N content were observed in all tissues, confirming the influence of N intake on $\Delta^{15}N$ (e.g. Adams & Sterner 2000, Vanderklift & Ponsard 2003). Higher fractionation rates in nitrogen-poor vegetal diets (2.47% N and 2.57 to 2.84% N in seagrass and macroalgae) compared to the nutrient-dense formulated diet (3.55% N) could be partly explained by concentration-dependent enzymatic discrimination against the heavy isotope. For instance, nutrient concentration is a key factor influencing $\Delta^{15}N$ in soil–plant systems (see review by Högberg 1997). $\Delta^{15}N$ increases at low availability of NO_3^- (when it is reduced to NO_2^-; Mariotti et al. 1982) or NH_4^+ in the medium (during assimilation of ammonia; Yoneyama et al. 1991), an analogous pattern to that observed here for nutrient-poor diets. In animals, the effect of enhanced protein quantity may also cause increased fractionation because protein catabolism and excretion increase when protein intake exceeds animal requirements (Robinson et al. 2006), although this does not seem to be the case here.

Our results are also in agreement with the Roth & Hobson (2000) hypothesis, which predicts decreasing $\Delta^{15}N$ with increasing protein quality (expressed as biological value). In line with this hypothesis, Robbins et al. (2005) found that $\Delta^{15}N$ decreased as protein quality increased with trophic level (i.e. herbivores to carnivores), with this trend accounting for up to 72% of the variation in discrimination factors across 5 major diet groupings of mammals and birds. Similar results were also reported for fish by Gaye-Siessegger et al. (2003), who found higher absolute values of trophic shift in tilapia *Oreochromis niloticus*

fed a wheat-based diet than in tilapia fed a fish-based diet. Because animals generally have very consistent amino acid profiles (Robbins 1993), one would expect that herbivores would have to undergo higher rates of transamination than carnivores to meet structural and metabolic requirements and that the gathering of ^{15}N through metabolic reactions would lead to enhanced $\Delta^{15}N$. Additionally, $\Delta^{15}N$ variability may result from differences in $\delta^{15}N$ signals among dietary groups because individual amino acids also differ in their stable isotopic signatures (Schmidt et al. 2004). Thus, we propose that both dietary protein quality and quantity may be important factors influencing patterns of $\Delta^{15}N$.

Tissue specificity

Mean values of $\Delta^{13}C$ tended to be higher in muscle (0.63‰) and gut (0.41‰) than in gonad (–0.21‰) and test (–0.04‰), possibly as a result of metabolic differences in rates of carbon turn-over within tissues (Tieszen et al. 1983, Hobson & Clark 1992). Tieszen et al. (1983) found shorter half-lives of carbon components (from 6.4 d in liver to 47.5 d in hair) and lower $\delta^{13}C$ signatures in more metabolically active tissues. Indirect evidence that muscle had the lowest carbon turnover rates compared to storage organs (i.e. gut, gonads and body wall; Giese 1961, Moss & Lawrence 1972) is provided by a lower increase in $\delta^{13}C$ in muscle when the red macroalgae diet was changed from *Grauteopia* sp. to *Palmaria palmata* in June, a change that may have influenced observed differences among tissues to an uncertain state. Muscle was also the tissue with slowest growth throughout the experiment (ca. 61 to 63% increase from initial weight), whereas gonads increased by 78 to 97% and gut and body wall showed intermediate values. Additional differences may also arise from variability in major biochemical fractions of tissues (DeNiro & Epstein 1978). Mean lipid contents estimated from C:N ratios (Post et al. 2007) were larger in the gonad (23%), followed by gut (14.1%) and muscle (5.2%), and the use of lipid corrections (Post el al. 2007) increased $\delta^{13}C$ signatures from 2.4‰ in gonad to 0.20‰ in muscle. However, since a correction was used, observed variability among tissues must be due to differences in the accumulation of other biochemical compounds rather than lipids. Guts and gonads may attain comparable protein levels and mainly differ in carbohydrate storage (0.3 to 6% in gut and up to ca. 35% in gonad; Moss & Lawrence 1972). Carbohydrate levels may also partly account for $\delta^{13}C$ differences in protein-rich tissues such as muscle (ca. 1 to 2% glycogen) and the body wall (ca. 15 to 20%; Swift et al. 1986). Carbohydrate storage may be promoted by protein-rich diets (Fernandez 1997), which may explain the lack of association between $\delta^{13}C$ and $\delta^{15}N$ in tissues of individuals fed the formulated diet.

There were significant differences in mean $\Delta^{15}N$ among tissues, with higher values in muscle (2.49‰) and test (1.69‰) and similar low values in gut (0.89‰) and gonad (0.77‰) with no influence of age or body size (see also Minagawa & Wada 1984). In fact, several authors have also found differences among vertebrate tissues (e.g. DeNiro & Epstein 1981, Hobson & Clark 1992, Sweeting et al. 2007), albeit with contrasting results that are possibly related to taxonomic differences. In birds, Hobson & Clark (1992) found that only muscle tissue had significantly higher values than blood, liver, collagen and feathers. In fish, Sweeting et al. (2007) also found higher rates of $\Delta^{15}N$ in muscle and heart compared to liver for the 2 types of diets tested. Our results show similar patterns for sea urchin muscle, with the differences found among tissues being possibly influenced by the balance between protein levels and turn-over rates across tissues. For instance, gut and gonads, which often contain similar protein levels (ca. 20 to 35%; Giese 1961, Moss & Lawrence 1972), displayed comparable values of $\Delta^{15}N$. In contrast, the organic portion of sea urchin tests, which may be made up to 81–85% of protein (Swift et al. 1986), and the lantern muscle itself displayed the highest fractionation values. Patterns of whole individuals reflected that of the test (i.e. the major body constituent); therefore, for sea urchins, test tissue may provide more realistic patterns of $\delta^{15}N$ and $\Delta^{15}N$ than those based on traditional muscle values.

Our results provide new insights into the interpretation of results from previous isotope studies on seagrass food-webs (e.g. Lepoint et al. 2000, Vizzini et al. 2002, Tomas et al. 2006). Specifically, our data suggest that certain compounds may be preferentially transferred into secondary production by seagrass consumers, which may explain why herbivores and seagrasses can have poor isotopic correspondence despite high levels of consumption (e.g. Valentine & Heck 2001, Prado et al. 2007). Here we provide evidence that absorption efficiency may influence the stable isotope signatures of consumers, particularly for aquatic herbivores feeding on plant material with low digestibility such as seagrasses (e.g. Lowe & Lawrence 1976, Klumpp & Nichols 1983). The negative values of $\Delta^{13}C$ for the sea urchin studied here and other herbivores (Crawley et al.

2007) may result from selective assimilation of certain compounds. Theoretical fractionation values cannot be applied with impunity and trophic models for seagrass beds based on isotope analyses require further investigation and precision. Differences in $\Delta^{13}C$ and $\Delta^{15}N$ between seagrass and algae were higher than those between trophic levels. In addition to knowledge of absorption efficiencies, the robust relationship between $\delta^{13}C$ and $\delta^{15}N$ signatures suggests that protein quantity and quality could be important to defining stable isotope fractionation, although for $\Delta^{15}N$, effects may be variable among organisms or groups of organisms depending on the biochemical form of nitrogen excretion (Vanderklift & Ponsard 2003). The causes, nature and implications of variability in fractionation deserve more research to improve mixing models and species-specific estimates of trophic positions.

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