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Moderate stoichiometric homeostasis in the sea urchin *Lytechinus variegatus*: effects of diet and growth on C:N:P ratios

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Abstract The influence of dietary elemental contents on consumer stoichiometry was investigated in selected and combined soft tissues (as a proxy of the whole individual) of the omnivorous sea urchin, *Lytechinus variegatus*. We raised urchins for 4 months in controlled seawater tanks using three different diets with different nutritional contents (from lower to higher: seagrass, red macroalgae, and a formulated diet). Individuals fed the different diets varied an average of 19.7, 19.4, and 38 % in C:N, C:P, and N:P ratios, respectively, with stronger temporal variability for C:P and N:P ratios across tissues and whole individuals. This resulted in homeostasis parameters (1/H) of −0.45, 0.09, and 0.38, respectively, for C:N, C:P, and N:P, indicative of homeostatic to weakly homeostatic organisms, at least for C:P and N:P ratios. Individuals fed the nutrient-rich formulated diet had higher growth rates (14 ± 0.83 g WW month^{-1}) than those fed macroalgae or seagrass (9.3 ± 0.57 and 3.4 ± 0.33 g WW month^{-1}, respectively). However, rapid body increments in more nutritional diets caused both a decrease in the %N and an increase in the %P of soft tissues, which resulted in significant but opposite effects of diet stoichiometry and growth

in sea urchin C:N ($R = -0.74$ and $R = 0.93$, for diet and growth effects, respectively) and N:P ratios ($R = 0.60$ and $R = -0.63$, also, respectively, for diet and growth effects). Among potential compensatory mechanisms helping to preserve certain levels of homeostasis, ingestion rates (g WW diet per g WW of urchin) were higher for seagrass and macroalgae diets than for the nutrient-rich formulated diet. In contrast, absorption and growth efficiencies displayed significant negative associations with nutrient contents in diets and did not exhibit nutritional compensation. Overall, our results suggest that resource stoichiometry strongly determines the growth rate of individuals ($R = 0.88$, $P < 0.01$), and moderate variability in C:N:P ratios of sea urchins possibly arise from differences in the allocation of proteins and RNA to body components, similarly to what has been proposed by the growth rate hypothesis.

Introduction

Elemental homeostasis, defined as the persistence of a relatively constant elemental composition of consumer body tissues, despite large differences in the elemental composition of food resources (Persson et al. 2010), is a central concept of ecological stoichiometry. It relates to potential imbalances in the supply of elements between organisms and their food resources that can scale up to all levels of organization within a system (Sterner and Elser 2002). For instance, nutritional imbalances between consumer needs and the composition of their diets can strongly constrain consumer growth and reproduction (Frost et al. 2005; Heflin et al. 2012) and ultimately impact ecosystem-level processes such as energy flow and material cycling (Elser et al. 2000; Cebrian and Lartigue 2004; Sterner and Elser 2002). In addition, growth rates and environmental factors

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such as temperature can shape the elemental content of organisms independently of variation in resource nutrient content (Chrzanowski and Grover 2008). Only organisms able to maintain invariant nutrient levels regardless of fluctuations in food resource elemental composition are strictly homeostatic, whereas those that change composition following variation in food resources are non-homeostatic (Sterner and Elser 2002). Typically, the primary focus of stoichiometric studies is on nitrogen (N) and phosphorus (P) concentrations, since these major nutrients control important ecosystem features such as the fate of primary productivity and trophic structure (Cebrian et al. 2009).

Persson et al. (2010) showed that autotrophic and heterotrophic organisms constitute two distinct groups in regard to nutritional contents and homeostatic regulation. On average, the N and P contents (as percentages of DW) of autotrophs are 10–20 times lower than those of herbivores, depending on differences in nutrient availability, animal size, and taxonomic affiliation (Mattson 1980; Elser et al. 2000). In addition, studies to date indicate that changes in the stoichiometric composition of autotrophs in response to environmental and nutrient fluctuations are often large when compared to changes in the composition of heterotrophs (Prado et al. 2010; Baggett et al. 2013). On this basis, heterotrophs are usually regarded as more homeostatic than autotrophs. Yet, controlled experiments with zooplankton have shown that variability in the elemental composition of food resources may return distinctive responses, ranging from few to no effects (Hessen 1990; Urabe and Watanabe 1992) to considerable changes in the stoichiometry of consumers (Boersma et al. 2001; Malzahn et al. 2007; Malzahn and Boersma 2012). Persson et al. (2010) conducted a meta-analysis with 132 data sets from the literature and found a wide range of responses from strictly homeostatic to non-homeostatic, although with dominance of moderate elemental imbalances. Different mechanisms have been proposed for the preservation of homeostasis when faced with imbalanced resources. Animals can compensate for reduced nutrient levels in a number of ways. For example, they may vary food selectivity, or eat more of their usual diet to increase acquisition of the limiting nutrient (e.g., Cruz-Rivera and Hay 2000; Valentine and Heck 2001). At the ecosystem level, compensatory feeding has been associated with consumers that have low mobility and high site fidelity (Stachowicz and Hay 1999). In addition, the excess carbon acquired from imbalanced resources can be reduced through a number of mechanisms, including depressed C uptake (DeMott et al. 1998), increased lipid storage (Tessier and Goulden 1982), and increased C egestion (Fink and Von Elert 2006) or respiration (Darchambeau et al. 2003). Differential acquisition of elements post-ingestion can be accomplished by adjusting the assimilation efficiencies of each element (Frost et

al. 2004; Logan et al. 2004), thus reducing the amount of nutrients that are returned to the system as feces.

Sea urchins often play important trophic roles in shallow benthic communities (Watts et al. 2007; Prado et al. 2007), and they are also important players in the biogeochemical cycling of marine carbonates (Lebrato et al. 2010). More specifically, the variegated sea urchin (*Lytechinus variegatus*) is one of the main seagrass consumers in the northern Gulf of Mexico and to a lesser extent in the Caribbean Sea (Valentine and Heck 2001). *L. variegatus* feeds on a variety of macroalgae, as well as benthic animals such as mussels, crustaceans, and epibionts (Watts et al. 2007) when the availability of macroflora becomes limited. Here, we investigate whether *L. variegatus* shows homeostatic behavior under varying stoichiometric composition in its diet and consider the mechanisms involved in regulating the urchin's stoichiometric composition. To do this, we examined changes in the C:N:P composition of sea urchins from juvenile (~2 cm diameter and <6 months of age; Beddingfield and McClintock 1998) to adult stages (sizes > 4 cm and 1 year of age; Moore et al. 1963) that were fed three types of diets: seagrass (*Thalassia testudinum*), red foliose macroalgae (*Grauteloupia* sp. and *Palmaria palmata*), and a diet specifically formulated for *L. variegatus* that contained high nutritional and energy content. More specifically, we assessed: (1) differences in C:N:P ratios in selected (muscle, gonad, and gut) and combined sea urchin soft tissues (ST) (as a proxy of the whole individual) across diets and time, and the resulting homeostasis parameter (1/H); (2) the effect of diet in relative tissue contributions along sea urchin development; (3) the relationship between C:N:P ratios in diets and sea urchin stoichiometry and overall growth; (4) the effect of growth on C:N:P ratios of sea urchins; and (5) differences in food ingestion, absorption, and growth efficiencies of *L. variegatus* among experimental treatments and their relationship with C:N:P ratios in diets. Given the global importance of sea urchins as consumers of seagrass and macroalgae, knowledge of their capacity to maintain internal nutrient levels and the controlling mechanisms involved will contribute to a better understanding of food web interactions and the trophic functioning of seagrass ecosystems and kelp forests.

Materials and methods

Collection of individuals and initial period

Small *L. variegatus* (1.5–2 cm; $n = 150$) were collected in mid-February 2009 from a shallow seagrass bed of *T. testudinum* located at the end of the Tyndall peninsula in Saint Andrew's Bay estuary, Florida, USA (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) in the wet laboratory facilities at the Dauphin Island Sea Lab. Seawater was pumped from Mobile Bay, filtered, and recirculated in an environmentally controlled laboratory. Experimental conditions were set for the optimal growth of *L. variegatus* at 32-ppt salinity, 22–23 °C (median annual temperature in the field), alkalinity > 200 ppt, 8.1–8.3 pH, and low dissolved nutrients (NO_3: < 10 mg L^{-1}; NO_2 and NH_4: 0–0.2 mg L^{-1}), and a 12-h light: 12-h dark photoperiod (see Hammer et al. 2012). Nutrient and alkalinity levels in seawater were monitored daily, water changes made and appropriate quantities of sodium bicarbonate (Arm and Hammer brand) added when necessary to maintain optimal levels.

To standardize the internal C:N:P compositions of the collected sea urchins, we fed them a uniform diet from mid-February through the end of March. This diet included thalli of the green alga *Ulva* sp. collected in Mobile Bay, AL, that were dried, grounded, and mixed with water and agar (10 g of algae plus 2 g of agar per 100 ml of distilled water) to make storable food blocks once a week. The tank was cleaned daily to remove excess food and feces. Seawater and photoperiod during this period were the same as those maintained during the experiment.

Experimental design and diet treatments

Five 75-L tanks connected to the same recirculating-water system (ca. 2,000 L) were established within an experimental room at the wet laboratory facility. Within each tank, 12 sea urchins were placed within separate containers and four individuals randomly assigned to one of three diets: seagrass, red macroalgae, or the formulated diet. The containers consisted of 50 × 10 cm rigid plastic mesh cylinders (3-mm mesh) attached to a PVC base that kept the cylinder upright. Each container was fitted with an aerator. No mortality or starvation was observed for individuals fed any of the diets during the 5-month experiment. All diets were supplied ad libitum for a day and replaced 24 h later, after careful removal of food remains and feces from tanks and containers.

Natural diets

The natural diets consisted of green seagrass leaves (*T. testudinum*) and two types of foliose red algae, *Grauteloupia* sp. from April to mid-June and *Palmaria palmata* from mid-June to July. Seagrass shoots 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), respectively. All macrophyte samples were kept within aerated tanks in the wet laboratory. The population of *Grauteloupia*

sp. sampled appeared to be a first invasive settlement of *G. taiwanensis* (DePriest et al. 2011); nevertheless, it was consumed at high rates by *L. variegatus* (P. Prado, pers. observ.). The alga's life cycle is, however, unknown, and it disappeared suddenly in mid-June. Thus, during the last month and a half of the experiment, we replaced this alga with the red alga *P. palmata* that was purchased from a commercial supplier (Atlantic Mariculture). We selected *P. palmata* because it has similar protein and carbohydrate contents to *Grauteloupia* sp., and it has a sheet-like morphology that was easy to manipulate. The alga was received sun-dried and recovered fresh texture when hydrated. Epiphytes attached to seagrass leaves and *Grauteloupia* sp. fronds, respectively (no epiphytes were present in commercial algae), were carefully removed by scraping the macrophytes with a razor blade before offering them to the urchins.

Formulated diet

This consisted of a nutrient-dense composite of vegetal and animal ingredients designed to maximize sea urchin growth (see Hammer 2006 for details on composition). This diet was embedded in agar (10 g of pellets plus 2 g of agar per 100 ml of distilled water) to minimize disintegration and cross-contamination across treatments.

Sample preparation and C:N:P analyses

Samples of epiphyte-free seagrass ($n = 3$; April–July) and *Grauteloupia* sp. were preserved at −20 °C after weekly collection, and samples of *P. palmata* and the formulated diet were obtained only once. All diet samples were dried to a constant weight at 70 °C, grounded to a fine powder with a mortar, and stored in a desiccators for further C:N:P analyses.

For sea urchins, five individuals from the initial diet-standardization period were stored within a −20 °C freezer for dissection and determination of initial C:N:P contents within tissues. In each experimental month, three individuals from each tank, including one from each experimental diet, were collected and frozen for later dissection and C:N:P analyses of muscle, gonad, and gut. Tissues were carefully rinsed with ultrapure water to eliminate food pellet and feces remains, dried at 70 °C, weighed, and grounded to fine powder using an Eppendorf and a glass bar (muscles and guts) or a mortar and pestle (gonads). Samples were analyzed simultaneously for stable isotope values (see Prado et al. 2012) and for C and N contents (this study).

Sea urchin tissues ($n = 65$ each; 5 during the acclimation period and 60 from experimental treatments (5 individuals × 3 diets × 4 months)) and diet samples ($n_{Thal} = 12$; $n_{Gra} = 9$; $n_{Pal} = 5$; $n_{For} = 5$; i.e., 3 monthly replicates for

the natural diets and five replicates for the fixed diets not subjected to natural variation) were weighted and packed into tin capsules that were kept stored in a desiccator for C:N:P analyses. For each individual, concentrations of C, N, and P (%) in combined ST were estimated as the total of C, N, and P in tissues' weights with respect to the total weight of tissues.

C and N contents in diets, and sea urchins, 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 The University of Alabama. Phosphorus content in non-acidized sea urchin samples and diets was determined through dry oxidation and acid hydrolysis extraction followed by a colorimetric analysis (Prado and Heck 2011).

Growth, ingestion, absorption, and growth efficiency

Monthly values of sea urchin size were monitored by weighing all individuals to the nearest 0.1 g. These values were used to calculate growth increments as the difference in g WW between sea urchin weights at the beginning and at the end of each month. Measurements were conducted in the morning, typically following assessment of ingestion rates (see below).

Ingestion rates were estimated over 1 day at the end of each month as the difference between the initial wet weight of the diet offered to individuals and that remaining after 24 h per sea urchin wet weight (i.e., g WW d^{-1} g WW^{-1}) also obtained at the end of each experimental month.

At each month, absorption efficiencies of each dietary material were estimated as the percent decline in caloric content (cal g DW^{-1}) from food to feces as in Prado et al. (2012). Briefly, this was determined by forming dry pellets of diets and fecal particles (collected by placing a 100-μm mesh filter underneath the sea urchins' plastic cylinders) and measuring caloric content in a Parr 6725 Semimicro calorimeter. Results showed similar values to those indicated by Lowe and Lawrence (1976) for *L. variegatus* feeding on a variety of marine plants, including *T. testudinum*, thus confirming the validity of the method. The Gross Growth Efficiency (GGE) was calculated as the ratio of sea urchin growth (g WW d^{-1}) to dietary ingestion (g WW d^{-1}) and expressed as a percent (%).

Analyses of data

Differences in the C:N:P composition of combined ST and tissue types across diets (3 levels, fixed factor) and months (4 levels, fixed factor) were investigated with a two-way factorial ANOVA and SNK post hoc analyses. We did not consider *Grauteloupia* sp and *P. palmata* as different diet levels within the ANOVA, because our goal was to compare

macroalgal with seagrass and formulated diets, and any effect due to the change from *Grauteloupia* sp and *P. palmate* should have been captured by this analysis.

Differences in the C:N:P composition of diets and in sea urchin growth rates (g WW month^{-1}), ingestion rates (g WW g WW d^{-1}), absorption efficiency (%), and growth efficiency (%) were also investigated with a two-way factorial ANOVA with diet type (3 levels) and months (4 levels) as fixed factors. ANOVA assumptions of normality and homogeneity of variances were tested using the chi-square test and the Cochran's test, respectively, and variables transformed when necessary to correct for possible deviations.

Overall, differences in the relative weight of tissues (gut, muscle, and gonad) during the experiment were investigated with a two-way MANOVA with diet (3 levels) and month (4 levels) as fixed factors. MANOVA assumptions of normality (Levene's test) and homogeneity of variances (Cochran's test) were not always achieved by transformation, but the test is known to be robust against violations of this assumption (Sokal and Rohlf 1995). Significant factors groupings were further investigated with SNK (Student–Newman–Keuls) post hoc tests.

The homeostasis parameter (1/H) was calculated using monthly data for the three types of diet (see Sterner and Elser (2002); Persson et al. 2010). This metric is the slope of the regression between log(x) and log(y), where x is the resource nutrient stoichiometry (C:N, C:P, and N:P), and y is the organism's nutrient stoichiometry in the same units as the resource. Therefore, 1/H usually takes values between zero and one that were classified as in Pearson et al. (2010): 0 < 1/H < 0.25 "homeostatic," 0.25 < 1/H < 0.5 "weakly homeostatic," 0.5 < 1/H < 0.75 "weakly plastic," and 1/H > 0.75 "plastic."

Correlation analyses were used to investigate the relationship between: (1) mean monthly C:N:P ratios in combined sea urchin ST and mean monthly C:N:P ratios in diets; (2) mean monthly C:N:P ratios in diets and ST and mean growth rates per month; (3) monthly ingestion rates per diet and C:N:P ratios in diets; and (4) monthly values of absorption and growth efficiency and C:N:P ratios in diets. In the case of the red macroalgae, the June nutrient value was considered as the average of the monthly replicates of *Grauteloupia* sp. and those of *P. palmata*. All statistical analyses were performed using Statistica v.7 software.

Results

C:N:P contents in sea urchins and diets

Nutrient ratios in diets were highest in seagrass, lowest in the formulated diet, and intermediate in red macroalgae (Fig. 1a–c; Table 1). In addition, seagrass showed increasing
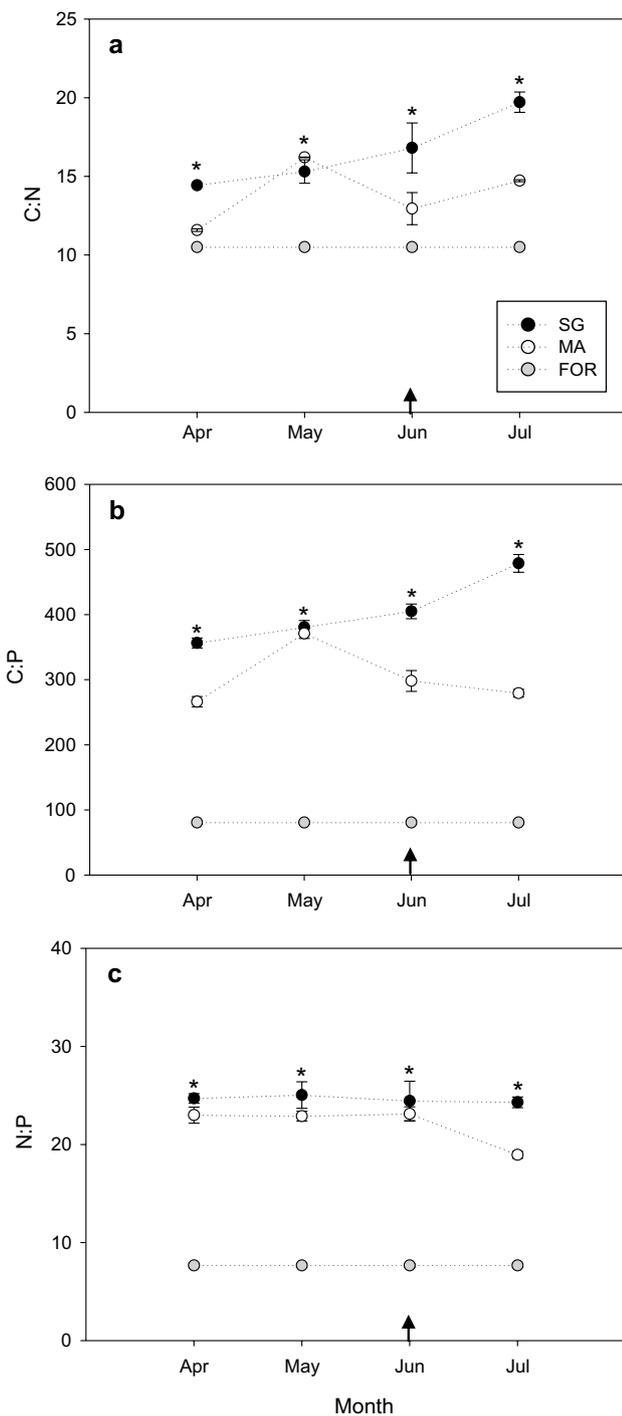
Fig. 1 Nutrient ratios of diets at each month of the experiment: **a** C:N, **b** C:P, and **c** N:P. *Error bars* are SE. *Arrows* indicate the shift in the macroalgae diet from *Grauteloupia* sp. to *Palmaria palmata* in mid-June. SG = seagrass, MG = macroalgae, and FOR = formulated diet. In SNK, the presence of a significant Di × Mo interaction is indicated with *asterisks*

monthly values of C:N and C:P, whereas in macroalgae, there was considerable temporal fluctuation, and the formulated diet remained stable throughout the experiment.

Table 1 Two-way ANOVA results for differences in nutrient contents (C: N, C: P, and N: P) among experimental diets

ANOVA	df	MS	F	P
C:N				
Diet	2	153.89	161.33	**<0.001**
Month	3	16.29	17.07	**<0.001**
Di × Mo	6	10.21	10.71	**<0.001**
Error	36	0.95		
		$C = 0.30$ (NS)		
C:P				
Diet	2	468746	2085.06	**<0.001**
Month	3	4852	21.58	**<0.001**
Di × Mo	6	7107	31.61	**<0.001**
Error	36	225		
		$C = 0.24$ (NS)		
N: P				
Diet	2	1433.94	787.14	**<0.001**
Month	3	7.24	3.97	**0.015**
Di × Mo	6	5.52	3.03	**0.017**
Error	36	1.82		
		$C = 0.34$ (NS)		

C = Cochran's C test statistic. SNK with significant Di × Mo interactions are indicated in Fig. 1

Boldfaced *p* values are statistically significant at the $P < 0.05$ level

For the combined ST, two-way ANOVA and SNK of C:N ratios showed higher values in individuals fed the nutrient-rich formulated diet (Table 2, Fig. 2a), and some significant variability in the monthly trends of each diet (i.e., a significant Di × Mo interaction). For individual tissues, the variability in C:N ratios among diets was relatively small (Fig. 2b, c), although both gut and muscle showed significantly higher values for the formulated diet compared to seagrass and macroalgae (Table 2).

In contrast, ST displayed significantly higher C:P and N:P ratios for the seagrass diet (Table 2; Fig. 2e, i) and a significant Di × Mo interaction for the C:P ratio. Differences in C:P and N:P across individual tissues were less clear than for combined ST, but guts also showed higher values in individuals fed the seagrass diet and similar patterns of temporal variability (Fig. 2f, j). For muscle, significant differences were only observed across months (Fig. 2g, k), and no effects or clear patterns were found in gonads (Table 2; Fig. 2h, l).

Homeostasis parameter

For C:N, the association between log values of diets and sea urchins resulted on a significant negative relationship ($R = 0.77$, $df = 11$, $F = 15.30$, $P < 0.01$) with a slope value (1/H) of −0.45 (Fig. 3a), which could not be included in

Table 2 Two-way ANOVA results for differences in nutrient contents (C:N, C:P, and N:P) in combined soft tissues (ST) and across tissue types

ANOVA	ST				Gut				Muscle				Gonad			
	df	MS	F	P	df	MS	F	P	df	MS	F	P	df	MS	F	P
C:N																
Diet	2	0.029	10.91	**<0.001**	2	1.43	9.57	**<0.001**	2	0.19	11.40	**<0.001**	2	2.68	2.30	0.087
Month	3	0.0025	0.92	0.43	3	1.83	12.21	**<0.001**	3	0.28	16.48	**<0.001**	3	2.09	1.79	0.17
Di × Mo	6	0.0091	3.32	**0.0075**	6	0.18	1.23	0.30	6	0.018	1.03	0.414	6	2.65	2.27	0.050
Error	48	0.0027			48	0.15			48	0.017			48	1.16		
	$C = 0.15$ (log x + 1; NS)				$C = 0.32$ (NS)				$C = 0.32$ (NS)				$C = 0.17$ (NS)			
	SNK: SG = MA < FOR				SNK: SG = MA < FOR				SNK: SG = MA < FOR							
					SNK: April = Jul = May ≤ Jun				SNK: May = April ≤ Jul = Jun							
C:P																
Diet	2	0.218	15.00	**<0.001**	2	6084	4.16	**0.021**	2	3128.7	1.57	0.21	2	1119	0.28	0.84
Month	3	0.013	0.93	0.43	3	10260	7.019	**<0.001**	3	21624.6	10.89	**<0.001**	3	797	0.19	0.82
Di × Mo	6	0.041	2.85	**0.017**	6	4538	3.10	**0.011**	6	4147.8	2.09	0.070	6	5502	1.37	0.24
Error	48	0.014			48	1462			52	1985.7			48	4008		
	$C = 0.29$ (log x + 1; NS)				$C = 0.36$ (NS)				$C = 0.20$ (NS)				$C = 0.19$ (NS)			
	SNK: FOR = MA < SG								SNK: Jul < May = Jun < April							
N:P																
Diet	2	1601.90	18.22	**<0.001**	2	340.59	6.94	**0.002**	2	443.4	3.64	0.053	2	79.87	1.017	0.39
Month	3	115.74	1.317	0.27	3	322.58	6.58	**<0.001**	3	1296.5	10.65	**<0.001**	3	14.61	0.18	0.83
Di × Mo	6	90.23	1.026	0.41	6	128.78	2.62	**0.026**	6	284.1	2.33	0.055	6	119.64	1.52	0.18
Error	48	87.89			48	49.02			52	121.7			48	78.53		
	$C = 0.26$ (NS)				$C = 0.26$ (NS)				$C = 0.23$ (NS)				$C = 0.21$ (NS)			
	SNK: FOR = MA < SG								SNK: Jul < May = Jun < April							

C = Cochran's C test statistic. Diet labels as in Fig. 1. In SNK, significant groups are indicated in the absence of significant Di × Mo interactions. Remaining SNK results are indicated in Fig. 2

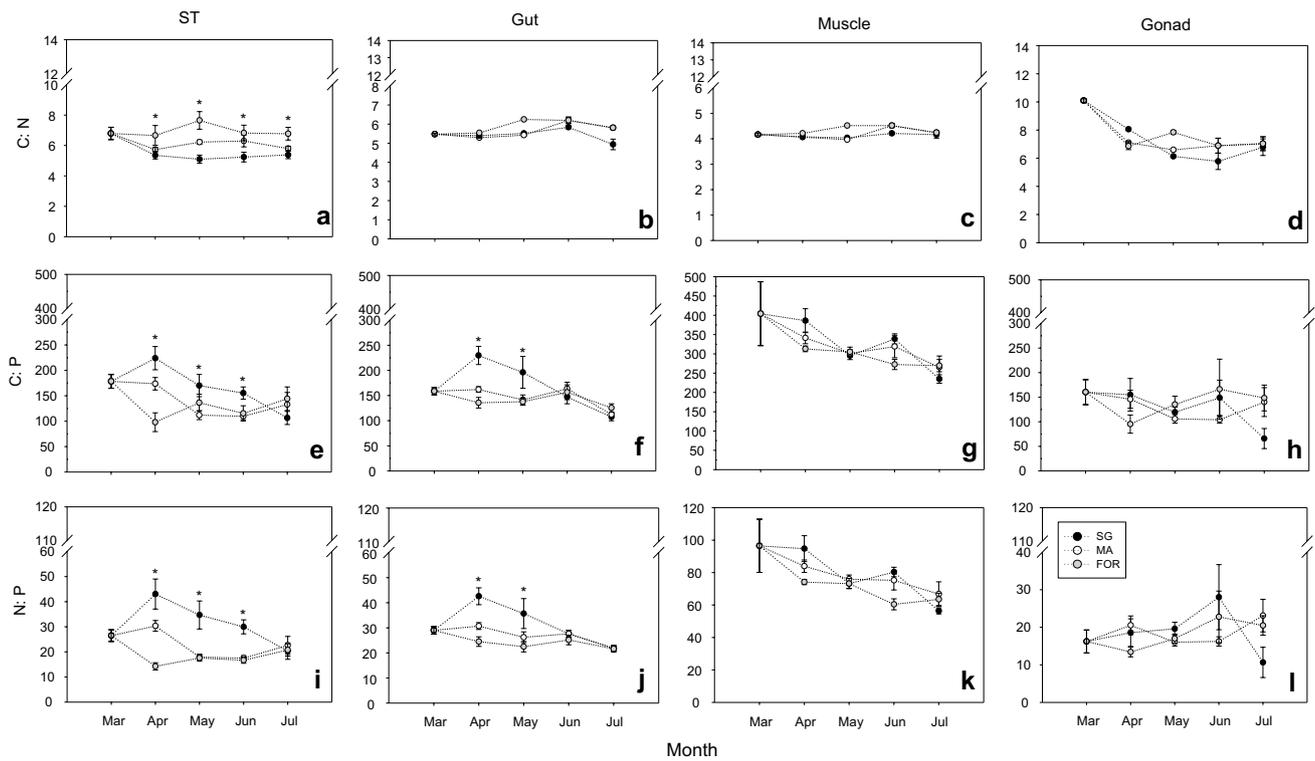Boldfaced P values are statistically significant at the $P < 0.05$ level

Fig. 2 Nutrient contents in combined sea urchin soft tissues (ST), guts, muscle, and gonads at each month of the experiment: (**a–d**) C: N ratios, (**e–h**) C: P ratios, and (**i–l**) N: P ratios. Diet labels as in Fig. 1. *Error bars* are SE. In SNK, the presence of a significant Di × Mo interaction is indicated with *asterisks*

any homeostatic category and suggest the influence of factors other than dietary contents (see later). For C:P, 1/H was 0.09, which falls in the range of homeostatic organisms (0 < 1/H < 0.25), with a nonsignificant association between variables ($R = 0.28$, $df = 11$, $F = 0.85$, $P > 0.05$; Fig. 3b). Finally, N:P ratios showed a 1/H value of 0.38, which corresponds to weakly homeostatic organisms (0.25 < 1/H < 0.5) and also displayed significant associations between log variables ($R = 0.62$, $df = 11$, $F = 6.37$, $P < 0.05$; Fig. 3c).

Relative tissue weights and individual growth rates

There were significant effects due to overall variability in the relative weights of gut, muscle, and gonad for the two factors investigated and their interaction (Table 3). Univariate results for each tissue also showed significant effects of diet and month, as well as a significant Di × Mo interaction in gut (Table 3). The relative contribution of guts to the total weight of ST was higher in urchins feed seagrass, followed by those fed macroalgae, and the formulated diet (Fig. 4; Table 3). Temporal differences were small for the seagrass diet, whereas in individuals fed macroalgae and the formulated, diet contributions from guts decreased in April–May and increased again

in the last month of the experiment (Fig. 4). For muscle, dietary patterns evidenced higher contributions under the seagrass diet compared to macroalgae and the formulated diet, which also showed a larger decline through the experiment (Fig. 4; Table 3). In contrast, the contribution of gonads was much higher in urchins fed formulated and macroalgae diets, which experienced a rapid increase in April–May, whereas those fed seagrass displayed little variation (Fig. 4; Table 3).

Individuals fed the seagrass diet showed the lowest growth rates (3.4 ± 0.33 g WW month^{-1}), whereas those fed macroalgae displayed intermediate rates (9.3 ± 0.57 g WW month^{-1}), and those fed the formulated diet had the highest rates (14 ± 0.83 g WW month^{-1}) (Table 4a). There were also significant temporal differences, with a peak of growth in May–June and a decline in July on all diets (Table 4a).

Ingestion rates

Ingestion rates expressed as g WW diet g WW of sea urchin^{-1} d^{-1} displayed lower values for the formulated diet (0.04 ± 0.002 g WW g WW d^{-1}) than for macroalgae and the seagrass (0.05 ± 0.002 g WW g WW d^{-1} in both), except for the first month of the experiment (Fig. 5b;
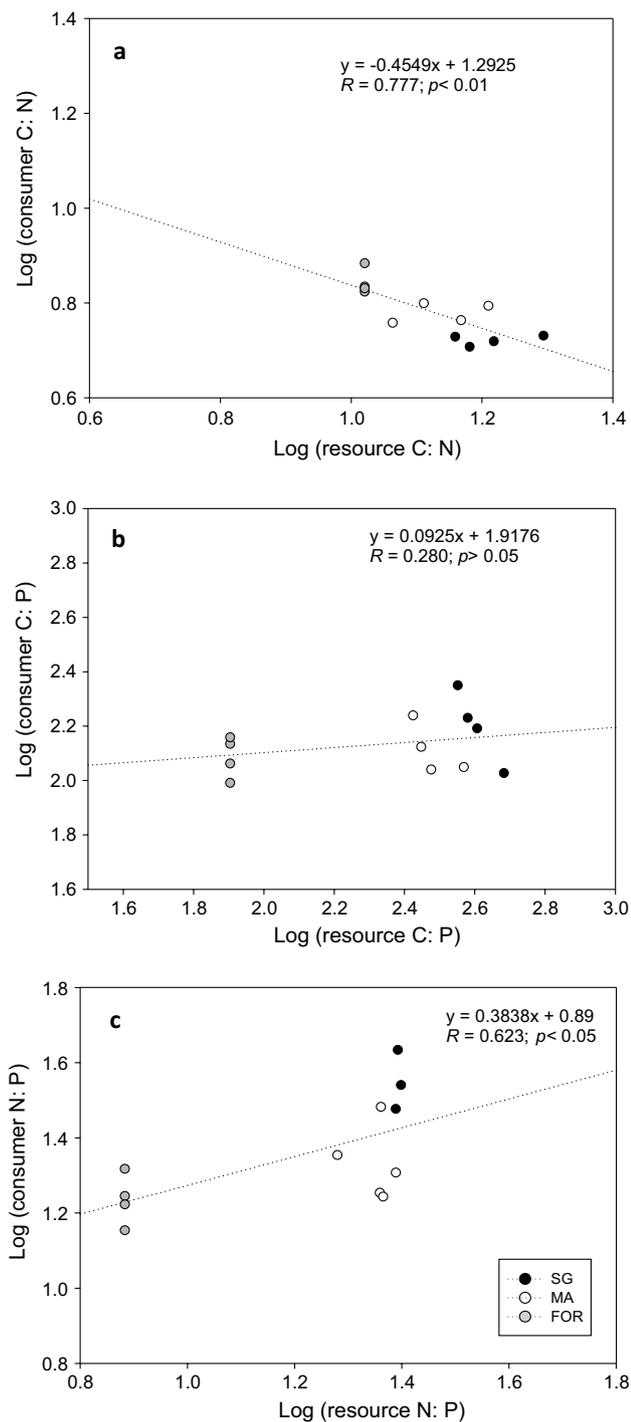
Fig. 3 Regressions between log consumer (sea urchin soft tissues) and log resource for: **a** C:N, **b** C:P, and **c** N:P, used to estimate the homeostasis coefficient (1/H). The *dotted diagonal line* shows the slope of the relationship. We considered insignificant regressions with slope close to zero (0 < 1/H < 0.25) as strictly homeostatic

Table 4b). In addition, as sea urchins grew, there was a general temporal decline in ingestion rates over the course of the experiment (Fig. 5b).

Absorption and growth efficiencies

Individuals fed seagrass displayed the lowest absorption and growth efficiencies (22.7 ± 1.28 and 18.5 ± 0.5 %, respectively), while those fed macroalgae showed intermediate values (46.58 ± 0.49 and 27.8 ± 0.9 %) and those fed the formulated diet the highest values (58.12 ± 0.39 and 37.7 ± 1 %) (Fig. 6a, b; Table 4c, d).

Absorption efficiency showed significant temporal variability with small temporal differences for individuals fed the formulated diet and increasing and decreasing temporal trends for the seagrass and the macroalgae diets, respectively (i.e., a significant Di × Mo interaction; Fig. 6a; Table 4c). For growth efficiency, the formulated diet displayed a peak in May, whereas seagrass and macroalgae diets showed little temporal variation (i.e., a significant Di × Mo interaction; Fig. 6b; Table 4d).

Relationship among variables

Significant associations were observed between C:N ratios and N:P ratios in combined ST and their diets obtained from monthly averages for each diet ($R = -0.74$ and $R = 0.60$, respectively; Table 5a), but not for specific tissues.

Dietary C:N:P ratios were strongly associated with sea urchin growth ($df = 11$, Multiple $R = 0.88$, $F = 9.57$, $P < 0.01$; see also Table 5b for separate ratios). In addition, there were also significant associations between sea urchin growth rates C:N and N:P ratios in ST throughout the experiment ($R = 0.93$ and $R = -0.63$, respectively; see Table 5c).

C:N, C:P, and N:P ratios of diets were not significantly associated with ingestion rates expressed as g WW diet g WW of sea urchin^{-1} d^{-1}. In contrast, significant negative associations were found between C:N, C:P, and N:P ratios of diets and absorption and growth efficiencies in sea urchins (Table 5e–f). For both variables, similar high values were observed for the correlation coefficients ($R = -0.78$ to -0.84, for absorption rates and $R = -0.72$ to -0.82, for growth efficiency).

Discussion

Our results show that C:N:P ratios of sea urchins behave as moderately homeostatic (Sterner and Elser 2002), with variations ranging from ca. 14.4 to 38 %, depending on the nutrient composition of the diet and the growth rates of individuals. The largest differences among diet treatments and the strongest association with nutrient levels in diets occurred at the level of combined soft tissues, whereas in discrete tissues, they varied from similar patterns in guts to no effects in

Table 3 Two-way MANOVA results testing for overall variability in relative tissue weights (gut, muscle, and gonad) among diets and experimental months

MANOVA	Diet			Month			Di x Mo		
	Wilk's λ	$F_{4, 102}$	p	Wilk's λ	$F_{6, 102}$	p	Wilk's λ	$F_{12, 102}$	P
	0.260	24.48	**0.0000**	0.556	5.786	**0.0000**	0.6249	2.251	**0.0143**
Univ. results	Gut			Muscle			Gonad		
	df	MS	F	p	MS	F	p	MS	F	P
Diet	2	0.3817	37.7416	**0.0000**	0.5488	67.7174	**0.0000**	1.8356	61.2833	**0.0000**
Month	3	0.0429	4.2465	**0.0093**	0.0367	4.5384	**0.0067**	0.1045	3.4916	**0.0219**
Di x Mo	6	0.0284	2.8141	**0.0190**	0.0102	1.2610	0.2914	0.0574	1.9184	0.0952
Error	48	0.0101			0.0081			0.0299		
Total	59									
		SNK: FOR < MA < SG			SNK: FOR < MA < SG			SNK: SG < MA < FOR		
		SNK: Jun = May ≤ Jul = April			SNK: Jul = Jun ≤ May = April			SNK: April < May = Jul = Jun		

Univariate results for each tissue are provided

Statistically significant results are indicated in bold

gonads despite being the dominant tissue in individuals fed macroalgae and formulated diets. The overall homeostasis parameter (1/H) for ST was −0.45 for C:N, 0.09 for C:P, and 0.38 for N:P, indicative of homeostatic to weakly homeostatic responses to dietary changes for C:P and N:P (Persson et al. 2010). Among potential compensatory mechanisms, ingestion rates per unit weight of animal tissue were slightly higher in low-quality vegetal diets, particularly seagrass, whereas absorption and growth efficiencies were much lower (see Fernandez and Boudouresque 2000 for similar results) and could not account for the preservation of internal nutrient levels. Overall, the strong association between nutrient ratios of diets and sea urchin growth suggests that animals tend to allocate excess nutrients into enhanced body size, thus preserving their body composition within constrained ranges (see also Heflin et al. 2012).

Individuals fed seagrass had on average 17.7 % lower C:N and 17.4 and 38 % higher C:P and N:P ratios than individuals fed the nutrient-rich formulated diet. The hypothesis of stoichiometric homeostasis has been mostly tested for zooplankton, with contrasting results, ranging from low (e.g., Hessen 1990; Urabe and Watanabe 1992) to considerable variability of nutrient ratios (Boersma et al. 2001; Malzahn and Boersma 2012), possibly associated with differences in resource quality features across studies, among other possible reasons. During this study, sea urchins were fed food items representing extreme dietary conditions (from nutrient poor indigestible seagrass to nutrient-rich and highly digestible formulated diet resembling animal resources) to provide the broadest possible information on natural ranges of stoichiometric variability. Hence, our results suggest that sea urchins faced with chronic nutritional stress for adequate body growth, reproduction, and

preservation of metabolic functions (Larson et al. 1980; Heflin et al. 2012; this study) are nonetheless capable of moderate homeostatic control. Yet, individuals feed seagrass showed a higher degree of temporal variation compared to macroalgae and the formulated diet, suggesting that in omnivorous species, the homeostatic investment decreases when food closely matches consumer requirements (Laspoumaderes et al. 2010).

The values estimated for the homeostasis parameter (1/H $_{C:P}$ and 1/H$_{N:P}$) were similar to those reported by Persson et al. (2010) for zooplankton and invertebrates. In their study, 94 % of the data sets had 1/H$_{C:P}$ values lower than 0.25; and 88.8 % had 1/H$_{N:P}$ values lower than 0.5, which includes observed ratios of 0.09 and 0.38, respectively, for C:P and N:P. In contrast, a negative value of −0.45 was observed for 1/H$_{C:N}$. Negative values have been also reported in other studies with zooplankton, but they are not regarded as a direct consequence of diet stoichiometry and are have been dismissed from further discussion (e.g., Hood II 2010; Persson et al. 2010). Although C:N:P ratios of animals can be partly influenced by the stoichiometric composition of food (e.g., Sterner and Elser 2002; this study), higher concentrations of N and P per unit C in the diet commonly trigger faster organismal growth rates (up to fourfold differences during the study, but see also review by Elser et al. 2000) that may affect the relationships between nutrient ratios of diets and animal tissues (Boersma et al. 2001; Malzahn and Boersma 2012). In our opinion, the negative 1/H$_{C:N}$ value was the result of a N decline (by ca. 14 %) at high growth rates in individuals feed the more nutritional diets, thus causing inverse relationships between C:N ratios in diets and sea urchins ($R = −0.74$, $P < 0.01$). In contrast, the P content in sea urchins increased with diet and resulted
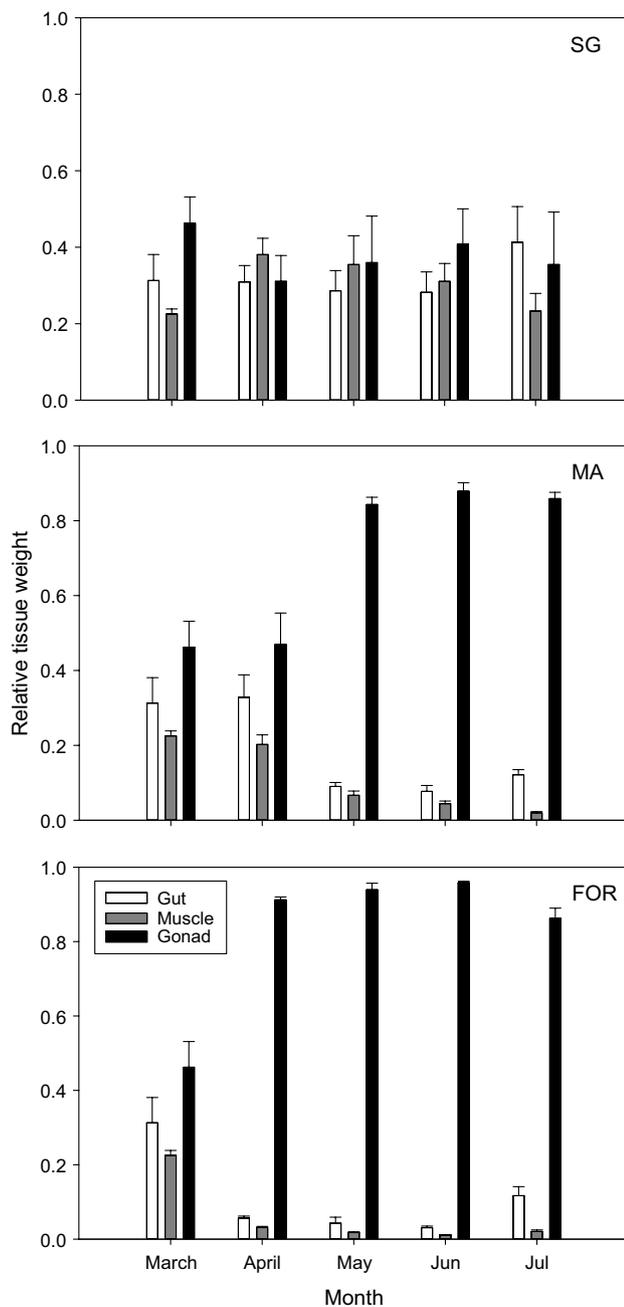
Fig. 4 Relative tissue weights (gut, muscle, and gonad) of sea urchins fed each type of diet (seagrass, macroalgae, and formulated), during the 4 months of the experiment (April to July). Initial values at the end of March were the same for each diet. Diet labels as in Fig. 1. For gut, a significant Di × Mo interaction was detected (see Table 4). *Error bars* are SE

Table 4 Two-way ANOVA results for differences in: (a) body growth; (b) ingestion rates among experimental diets (g WW g WW^{-1}); (c) absorption efficiency (%) and; (d) Gross Growth Efficiency (body growth ingestion^{-1} × 100) during the experiment

ANOVA	df	MS	F	P
(a) Body growth (g month^{-1})				
Diet	3	564.17	110.98	**<0.001**
Month	2	43.14	8.48	**<0.001**
Di x Mo	6	10.25	2.02	0.081
Error	48	5.08		
		C = 0.35 (NS)		
		SNK: SG < MA < FOR		
		SNK: April = Jul < May = Jun		
(b) Ingestion rates (g WW g WW^{-1})				
Diet	2	0.0012	8.685	**<0.001**
Month	3	0.030	219.37	**<0.001**
Di x Mo	6	0.0020	14.54	**<0.001**
Error	348	0.00013		
		C = 0.18 (NS)		
(c) Absorption (%)				
Diet	3	6843.3	2527.75	**<0.001**
Month	2	35.4	13.06	**<0.001**
Di x Mo	6	153.8	56.8	**<0.001**
Error	48	2.7		
		C = 0.40 (NS)		
(d) GGE (%)				
Diet	3	1132.2	16.75	**<0.001**
Month	2	11140.0	164.82	**<0.001**
Di x Mo	6	597.9	8.84	**<0.001**
Error	348	67.6		
		C = 0.22 (NS)		

C = Cochran's C test statistic. Diet labels as in Fig. 1. In SNK, significant groups are indicated in the absence of significant Di × Mo interactions. Remaining SNK results are indicated in Figs. 5 and 6

Boldfaced P values are statistically significant at the $P < 0.05$ level

in positive relationships in C:P and N:P ratios ($R = 0.23$ and $R = 0.60$, respectively).

According to the growth rate hypothesis (GRH), when P is not limited, there is a tight and generalizable coupling between growth rate, RNA allocation, and P content (Elser et al. 1996, 2003), thus causing variability in

the stoichiometric composition of organisms (lower C:P and N:P ratios). During this experiment, P contents in the formulated diet were ca. 4.6 times higher than in the seagrass diet and caused negative associations between growth rates and N: P ratios in sea urchin ST, which support the GRH. In contrast, N contents in the formulated diet were only 1.4 times higher than in the seagrass diet, which could be slightly limiting at high growth rates and cause dilution of protein N to body components. For instance, in trees, a rapid decrease in N concentration has been reported to occur during foliage expansion and bud break (Bauer et al. 1997), and analogous patterns may also occur in animals. Overall, our results indicate that growth rates can determine not only C:P and N:P ratios (Elser et al. 1996; Sterner and Elser 2002), but also C:N ratio, possibly through changes in the allocation of proteins versus body components. In
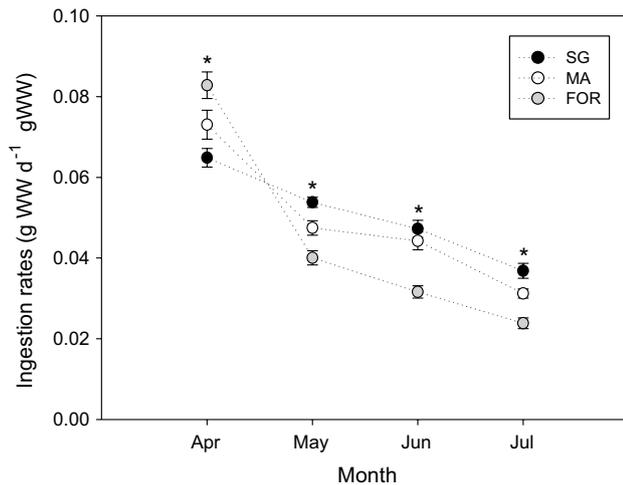
Fig. 5 Sea urchin ingestion rates for each type of diet during the experiment expressed in terms of g WW of diet per g WW of sea urchin per day. Diet labels as in Fig. 1. *Error bars* are SE. In SNK, the presence of a significant Di × Mo interaction is indicated with *asterisks*

addition, our results suggest that the sign of the relationship can be dual, depending on nutrient demands at high increases in body size.

Among investigated tissues, gut attained the highest values of variability in nutrient ratios across diets (ca. 7.2–22 %) and displayed closer patterns to those of combined soft tissues, particularly for C:P and N:P ratios, whereas muscle and gonad consistently displayed variations below 10 % (ca. 3.2–8.5 % and 3–9.3 %, respectively, for muscle and gonad). For individuals fed seagrass, more distinctive differences in nutrient ratios compared to the other two diets may be due to the higher contribution of guts to the overall weight of soft tissues. In contrast, gonad was, by large, the tissue with the highest contribution to the total weight of ST in both macroalgae and formulated diets, which explains higher similarities but also suggests the importance of less abundant tissues in understanding patterns across the whole individual. The reasons behind nutrient patterns within each tissue remain, however, unclear, although it is likely that they are related to distinctive physiological functions, as well as to patterns of tissue growth and turnover rates induced by the availability of specific nutrients in the diet (Heflin et al. 2012). For instance, gonad tissue displayed the largest increase from initial values (by 78–97 %), particularly in June–July, whereas gut displayed slightly lower rates (ca. 81–90 % increase) and a more gradual growth, such as that of muscle (ca. 61–63 % increase from initial weight). Also, compared to muscle (only 5.2 % fat), gonads and guts have larger lipid contents (23 and 14.1 %, respectively; see Prado et al. 2012), and carbohydrate storage (Moss and Lawrence 1972), which
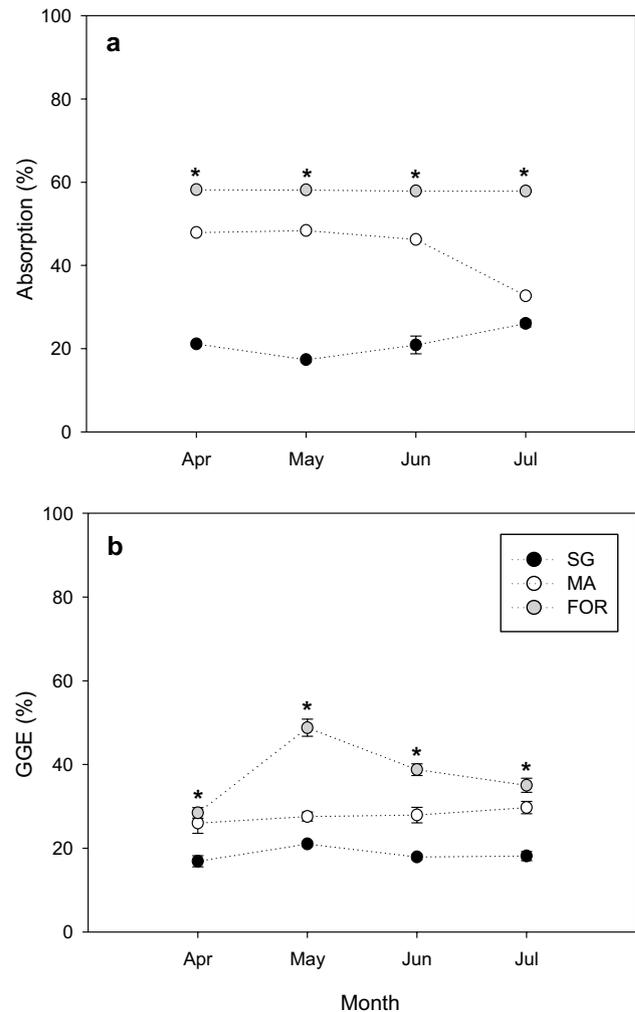

Fig. 6 a Sea urchin absorption (% caloric decline from food to feces) and **b** growth efficiencies (% growth per dietary ingestion) obtained during the experiment. Diet labels as in Fig. 1. *Error bars* are SE. In SNK, significant differences among diets during each experimental month are indicated with *asterisks*. In SNK, the presence of a significant Di × Mo interaction is indicated with *asterisks*

may have distinctive rates of incorporation and turn over within each tissue. Hence, the absence of any significant trends in gonads may be due to rapid growth rates and turnover patterns of storage components, although allocation strategies may vary across species and cause shifts in nutrient ratios at the onset of reproduction (Færøvig and Hessen 2003). For muscle and gut, a steady decline in C:P and N:P ratios from juvenile and adult sizes may reveal an increase in the nutrient storage capacity in older individuals with lower nutritional demands (Cazcarra and Petit 2010). However, these patterns contrast with previous studies in zooplankton showing an increase in nutrient ratios through developmental stages (e.g., Laspoumaderes et al. 2010; Villar-Argaiz et al. 2002) in accordance with patterns predicted for the GRH at higher growth rates.

Table 5 Summary of correlation analyses

Correlations	*df*	*R*	*F*	*P*
(a) C:N:P in diets–C:N:P in ST				
C:N	11	−0.74	12.45	**0.0054**
C:P	11	0.23	0.608	0.4535
N:P	11	0.60	5.818	**0.0365**
(b) C:N:P in diets–growth (g WW month^{-1})				
C:N	11	0.81	20.37	**0.00111**
C:P	11	0.87	32.36	**<0.001**
N:P	11	0.81	20.14	**<0.001**
(c) Growth (g WW month^{-1})–C:N:P in ST				
C:N	11	0.93	69.39	**<0.001**
C:P	11	−0.38	1.71	0.2196
N:P	11	−0.63	6.77	**0.0263**
(d) C:N:P in diets–ingestion (g WW g WW^{-1})				
C:N	11	0.032	0.010	0.9216
C:P	11	0.062	0.032	0.8481
N:P	11	0.195	0.399	0.5415
(e) C:N:P in diets–absorption (% cal. decline)				
C:N	11	−0.78	16.22	**0.0024**
C:P	11	−0.84	25.10	**<0.001**
N:P	11	−0.80	18.65	**0.0015**
(f) C:N:P in diets-growth eff. (growth·ingestion^{-1})				
C:N	11	−0.72	11.02	**0.0072**
C:P	11	−0.82	21.82	**<0.001**
N:P	11	−0.81	20.13	**0.0011**

(a) monthly nutrient ratios in diets and in sea urchin soft tissues (ST) (C:N, C:P, and N:P); (b) monthly nutrient ratios in diets and sea urchin growth rates (g WW month^{-1}); (c) sea urchin growth rates and monthly nutrient ratios in sea urchin ST (C:N, C:P, and N:P); (d) nutrient ratios in diets and monthly ingestion rates for each diet (g DW d^{-1}); (e) nutrient ratios in diets and monthly estimates of food absorption (% caloric decline); (f) nutrient ratios in diets and growth efficiency (body growth ingestion^{-1} × 100)

Boldfaced *P* values are statistically significant at the *P* < 0.05 level

Ingestion rates expressed in terms of food consumed per unit weight of sea urchin (g WW diet g WW of sea urchin^{-1} d^{-1}), were higher on the formulated diet during the first month of the experiment, and then showed increased values on vegetal diets, particularly seagrass. Hence, our results support the theoretical preference for more nutritious food (e.g., Cebrian and Lartigue 2004; Goecker et al. 2005; Meunier et al. 2012), but further suggest that as time progressed some degree of compensation for lower nutritional quality does also occur to fulfill consumer requirements (e.g., Cruz-Rivera and Hay 2000; Valentine and Heck 2001), with some exceptions for organisms with stoichiometric plasticity (Meunier et al. 2012). This shift in consumption explains the missing association between ingestion per unit weight and dietary C:N:P ratios, in spite of significantly higher average rates for seagrass

and macroalgal diets. Overall, these results suggest that the effectiveness of marine herbivores in alleviating reduced nutrient availability in primary producers through increased consumption per unit weight (see review by Heck and Valentine 2007) is possibly a short-term response.

Although several studies have shown compensatory feeding responses by invertebrate herbivores in terrestrial, freshwater, and marine ecosystems (e.g., Rueda et al. 1991; Cruz-Rivera and Hay 2000; Valentine and Heck 2001; Fink and Von Elert 2006), less is known about whether stoichiometric homeostasis is maintained as a result of increasing feeding rates. Fink and Von Elert (2006) found a pronounced compensatory feeding response to low nutrient food in the gastropod *Radix ovata* that reduced food quality effects on snail growth and preserved soft-body nutrient stoichiometry. Our results suggest that increased feeding rates could helped to balance nutritional deficiencies and allow for moderate variability in C:N:P ratios across diet treatments, although they could not compensate for growth effects (ca. 4 times lower in the seagrass diet). Also importantly, ingestion rates per unit weight showed a continuous decrease throughout the experiment for all diets, suggesting that larger animals have lower nutrient requirements and feed less (weight specific) compared to smaller ones. Given that there was considerable growth throughout the experiment, the interpretation of this result is difficult, but it could be the result of metabolic processes regulating the incorporation and release of C, N, and P and that were not directly investigated in this study, such as respiration and nutrient storage among others (see review in Frost et al. 2005).

Acquisition mechanisms such as absorption efficiencies were higher for the nutrient-rich formulated diet and red macroalgae than for seagrass. In fact, seagrass absorption efficiency by consumers is usually low (e.g., 19 and 23 % in *L. variegatus*, Lowe and Lawrence 1976; this study; 40 % in Trochoid gastropods, Peduzzi 1987; and 18–38 % in fish, Klumpp and Nichols 1983; Velimirov 1984), possibly because structural compounds such as lignin and cellulose are largely indigestible and may constrain the acquisition of labile nutrients despite the presence of symbiotic microbes in the consumers' guts (Lawrence and Klinger 2001). Fernandez and Boudouresque (2000) also conducted an experiment to evaluate absorption rates in sea urchins fed three types of artificial food—vegetal, mixed, and animal based—and reported higher values for the latter diet, which also featured higher levels of protein content. Accordingly, the growth efficiency (g WW gained per g WW of diet) was ca. 2 times higher for individuals fed the formulated diet and ca. 1.5 times higher for those fed macroalgae diets compared to seagrass. Low absorption rates of seagrass by sea urchins may also help explain why higher consumption of seagrass per unit of sea urchin weight may not be an efficient compensatory mechanism, although it might

have helped to maintain growth efficiency through periods of higher growth. Other specialized herbivores such as zooplankton (Frost et al. 2004) and insects (Zanotto et al. 1993) may achieve differential post-ingestion acquisition of elements, whereas omnivorous consumers may partly accomplish compensatory regulation through selective feeding on other more nutritious local resources (Frost et al. 2005).

The sea urchin, *L. variegatus*, is a generalist omnivore that commonly occurs in seagrass meadows of the northern Gulf of Mexico and the Caribbean (Valentine and Heck 2001). Although sea urchins could partly compensate for decreased nutrient concentrations in primary producers by shifting to animal prey, submerged vegetation is consistently reported as the dominant component of their diet (see review in Watts et al. 2007). Seagrass habitats and macroalgal beds are particularly susceptible to nutrient enrichment due to their general proximity to land. Given the significant associations in C:N and N:P ratios observed between sea urchins and in their diet, variability in the stoichiometric composition is expected for individuals living in contrasting nutrient regions (e.g., Prado et al. 2010; Baggett et al. 2013). In addition, our results suggest that more intense herbivory patterns in oligotrophic tropical regions (Cronin et al. 1997) may be partly due to increased ingestion rates to compensate for nutritional imbalances (see also Valentine and Heck 2001), at least until certain thresholds of organisms' performance (Boersma and Elser 2006).

To conclude, our results show that ranges of stoichiometric homeostasis similar to other studies (e.g., Persson et al. 2010), with a clear influence of growth rates, which in turn, were tightly controlled by the nutrient availability of food (Heflin et al. 2012). Different species may, however, have variable capacities for preserving their stoichiometric composition and/or implementing compensatory mechanisms (Villar-Argaiz et al. 2002; Valentine and Heck 2001; Malzahn and Boersma 2012). For sea urchins, nutrient-limited individuals showed moderate preservation of internal nutrients, but reduced growth and body size, characteristics indicative of chronic nutritional stress (Heflin et al. 2012). This suggests that nutrient-limitation signals can travel up the food chain (Malzahn et al. 2007) and potentially impact the functioning of coastal ecosystems such as seagrass meadows and macroalgal beds.

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