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# Variation in elemental stoichiometry and RNA:DNA in four phyla of benthic organisms from coral reefs

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## Summary

**1.** The elemental composition of organisms has been linked to traits such as growth rates, through investment of phosphorus (P) in RNA (called the Growth Rate Hypothesis) and through ecological processes such as susceptibility to predation and herbivory.

**2.** To test the predictions of the Growth Rate Hypothesis, we assessed the elemental composition and RNA of four phyla of common, co-occurring benthic coral reef organisms, corals, and green, brown and red macroalgae, whose patterns of dominance are predicted to be strongly influenced by differences in growth and herbivory and thus elemental stoichiometry. We assessed the importance of phylogeny and functional form, two important attributes for predicting the composition of macroalgal communities, on the variation in elemental composition.

**3.** Over these widely divergent taxonomic groups of organisms, we found support for links between elemental composition and RNA in tissues. The RNA:DNA in corals was positively correlated with % nitrogen (N) and negatively correlated with carbon (C):N ratios of tissues while RNA:DNA and RNA concentrations in macroalgae were positively correlated with %N, N:P ratios and negatively correlated with C:N ratios. Corals had higher concentrations of P and lower C:P ratios than macroalgae. Among the macroalgal lineages, C:P was higher in the brown algae than in red and green algae. Overall, the variation in elemental composition of macroalgae was relatively low compared with the variation reported for terrestrial plants and similar to that in corals.

**4.** Analysis of phylogenetic sources of variation (family and genus) in elemental composition and RNA found that genus accounted for a significant proportion of variation in both corals and macroalgae (15–47%), but the largest source of variation (50–97%) was unexplained by our statistical model and thus likely attributable to species and environmental factors. Although elemental composition of macroalgae did not vary in a way that was consistent with functional form models, genera with larger thalli had higher tissue N than smaller forms and more lineages with greater complexity of form had lower C:P ratios than less complex brown algal lineages.

**5.** Our data indicate that the despite relatively low levels of variation in elemental composition compared with terrestrial organisms, elemental stoichiometry varied significantly among coral reef taxa and with the structure of organisms and thus may have potential for predicting variation in growth rates and patterns of consumption on coral reefs.

**Key-words:** Chlorophyta, coral, coral reefs, ecological stoichiometry, Great Barrier Reef, Lizard Island, macroalgae, Phaeophyta, Rhodophyta

## Introduction

Variation in the elemental composition of plants and animals is proposed to be important in determining growth rates and abundance of organisms (Sterner & Elser 2002). The concentrations and proportions of elements such as phosphorus (P), nitrogen (N), silicon (Si), iron (Fe), carbon (C) and others have been linked with metabolic processes (e.g. photosynthesis, respiration, N fixation and calcification), organism performance (growth and reproduction), interactions with other organisms (predation, competition, facilitation) and ecosystem function (nutrient cycling and exchange with the atmosphere and oceans). The Growth Rate Hypothesis (Elser *et al.* 2000) proposes

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that P concentrations in organisms vary predictably with their investment in RNA, which in turn gives rise to variation in growth rates and other ecological processes. Here, we use a comparative approach to investigate the variation in elemental composition and RNA of corals and macroalgae on coral reefs.

In the tropics, coral reefs are important sites of primary and secondary production, supporting extremely high levels of biodiversity (Hughes 1989). Benthic primary production on coral reefs is performed by a range of different photoautotrophs and by hermatypic corals, which have a symbiosis with photosynthetic dinoflagellates contained within their tissues. Corals and macroalgae compete for space on reefs (McCook, Jompa & Diaz-Pulido 2001). The dominance of corals vs. macroalgae on coral reefs is attributed to differences in the growth rates of macroalgae and corals (McCook, Jompa & Diaz-Pulido 2001), the ability of corals to acquire nutrients via predation (Houlbrèque *et al.* 2004; Rahav *et al.* 1989) and also grazing of algae (Hughes *et al.* 1994), which is influenced by both herbivore pressure and algal structure and composition (Hay 2009). Thus, elemental composition may influence many of the processes proposed to be important in determining the community composition of coral reefs. Here, we assess variation in the elemental composition and nucleic acid concentrations in corals and macroalgae from Lizard Island on the Great Barrier Reef.

Elemental composition varies with phylogeny, functional traits, life-history traits and environment (e.g. Duarte 1992; Sterner & Elser 2002; Broadley *et al.* 2004; Kerkhoff *et al.* 2006; Lovelock *et al.* 2007). The four lineages of coral reef benthic organisms we studied come from highly divergent phylogenetic groups, which may give rise to fundamental differences in their elemental composition (Sterner & Elser 2002; Quigg *et al.* 2003; Watanabe *et al.* 2007). Hermatypic corals (Scleractinia, Cnidaria and Kingdom Animalia) can acquire carbon from photosynthesis via their symbiotic dinoflagellate partners but also acquire nutrients and carbon from feeding on suspended particulate matter (e.g. Porter 1976; Sebens & Johnson 1991). We anticipated that because Scleractinia are animals with low investment in cell wall materials, compared with plants and macroalgae they should have higher concentrations of N, P and RNA in their tissues and lower C:P, C:N and N:P ratios than macroalgae (excluding their skeleton). Corals are also expected to have higher levels of homeostasis in elemental composition (less variation) compared with macroalgal lineages, as has been observed in contrasts of terrestrial animals and plants, partially because of their comparatively smaller vacuoles for storage (Sterner & Elser 2002).

Coral reef macroalgae are comprised of members of all three major macroalgal lineages: the green algae (Chlorophyta, Kingdom Viridiplantae), red algae (Rhodophyta, Kingdom Viridiplantae) and brown algae (Phaeophyta, Kingdom Stramenopilae). The brown algae are the least differentiated algal group with a smaller range of tissue types than other lineages (Bell & Mooers 1997) and contain high concentrations of algin and phlorotannin (Amsler & Fairhead 2006). Low levels of cellular differentiation and large investment in polysaccharides may give rise to relatively carbon rich thalli in brown algae. In the green algae, thalli are more complex than in the brown algae (Bell & Mooers 1997). Green algae structure ranges from single cells to corticated macrophytes that can attain large sizes through lateral spread (e.g. *Caulerpa*). In the red algae, species are typically small (<1 m). Thalli of the Rhodophyta do not have true paraenchyma, but instead growth occurs from apical or intercalary cell division and the fusion of cells (a thallus is essentially a group of fused filaments). Previous studies of terrestrial and marine plants have found that diverse lineages of plants follow similar rules of scaling among elements and with plant size (e.g. Duarte 1992; Kerkhoff & Enquist 2006; Kerkhoff *et al.* 2006), but Bell & Mooers (1997) found differences in the relationships describing thallus structure among plants/green algae and brown algal lineages. Thus, because of the differences in thallus structure among algal phyla, our hypothesis was that a large proportion of the variation in elemental composition and RNA in macroalgae may be explained at higher taxonomic levels and that brown algae would have higher C:P, C:N and N:P ratios and less RNA than would green and red lineages.

The study of the ecology of macroalgal communities has been strongly influenced by functional form models developed by Littler & Littler (1980) and Steneck & Dethier (1994). Functional form classifications are useful because of their simplicity and the limited data on specific functional traits for tropical marine macroalgal species. The functional forms of macroalgae include filaments, sheets, corticated macrophytes, leathery macrophytes and calcified forms. These classifications have been linked to rates of primary productivity, thallus longevity, susceptibility to herbivores and damage by waves (Littler & Littler 1980; Steneck & Dethier 1994), all of which may be linked to the stoichiometry of thalli. Here, we assessed whether variation in elemental composition and RNA was consistent with the macroalgal functional form groupings.

In summary, we surveyed the elemental composition and RNA from corals and macroalgae at Lizard Island in the northern Great Barrier Reef lagoon to test the following hypotheses. We tested whether variation in elemental composition (P, C:P, N:P) of benthic organisms was correlated with RNA of tissues. We assessed whether corals (Kingdom Animalia) had higher and less variable elemental composition than macroalgae (Kingdom Chlorophyta, Rhodophyta and Stramenopila). We assessed whether macroalgal lineages differ in their elemental composition, testing the hypothesis that the (Phaeophyta) had higher C:P ratios and more variable elemental composition than Chlorophyta and Rhodophyta. Finally, for macroalgal lineages, we assessed whether variation in functional form of macroalgae was linked to variation in elemental composition of thalli.

## Materials and methods

### SITE DESCRIPTION AND BENTHIC COMMUNITY SAMPLING

Algae and corals were collected from within lagoonal habitats at Lizard Island, Queensland, which is situated in the northern section of the Great Barrier Reef (14·67°S, 145·46°E). Corals and macroalgae were collected by hand from habitats that ranged from 0 to 15 m water depth during three sampling campaigns in June 2008, March 2009 and October 2009. The lagoon is shallow, and thus, sampling was more intense at shallow depths where macroalgae were abundant (Supporting information Fig. S1). Samples were placed in plastic bags containing seawater from where they were collected and processed in the laboratory within 1 h of collection to limit degradation of RNA. Samples were identified to the lowest possible taxonomic level (after Littler & Littler 2003; Cribb 1983; Veron & Stafford-Smith 2000). From each sample, a number of subsamples of tissue were removed from the algae or scraped from the coral into a 2-mL cryo-tube using a scalpel and immediately frozen in liquid $N_2$. The remaining sample was frozen at −20 °C for storage and transport to the laboratory at The University of Queensland in Brisbane. Samples were freeze-dried for analysis of total carbon (C), nitrogen (N) and phosphorus (P). Coral tissue was airbrushed from the coral skeleton prior to freeze-drying, and skeletal fragments and symbiotic algae were removed from the tissue slurry by centrifugation at 5500 g for 5 min. Maximum thallus size of macroalgae genera was obtained from taxonomic guides (Cribb 1983; Littler & Littler 2000, 2003). Macroalgae thalli form was classified as corticated macrophytes, calcifying corticated macrophytes, spheres, sheets or filaments after Steneck & Dethier (1994). As spherical forms were only present in green algal lineages, these were removed from the analysis.

### TISSUE C, N AND P AND NATURAL STABLE ISOTOPES

Nitrogen and C concentrations (presented as % dry mass) of tissues were determined using a PDZ Europa ANCA-GSL elemental analyzer (Sercon Ltd., Cheshire, UK) at the UC Davis Stable Isotope Facility. The P concentration in finely ground tissue was determined using an acidified persulphate autoclave digestion of the organic compounds followed by quantification of the released orthophosphate in a colourimetric assay with ammonium molybdate and malachite green (Van Veldhoven & Mannaerts 1987).

### DNA AND RNA DETERMINATION

The relative quantities of RNA and DNA were determined by a fluorescence assay using 2,7-diamino-10-ethyl-9-phenylphenanthridinium bromide (ethidium bromide, EtBr) as described in Reef *et al.* (2010). Briefly, EtBr binds specifically to double-stranded polynucleotides and while doing so emits a yellow fluorescence when irradiated with UVR. Hydrolysis of the nucleic acid strands results in loss of fluorescence. We used the sequential enzymatic method (Bentle, Dutta & Metcoff 1981) to quantify the contribution of the RNA and DNA fractions to the EtBr-polynucleotide complex by the sequential addition of RNase and DNase in excess to specifically hydrolyse first RNA and then DNA.

Approximately 500 mg of tissue were ground in liquid $N_2$ and homogenized (Tissue-Tearor; BioSpec Products Inc., Bartlesville, OK, USA) in 1 mL of homogenizing buffer (1 M NaCl, 2% Sarkosyl) and left on ice for 30 min. Homogenized samples were then spun at 5000 g, and 100 µL of supernatant was transferred to a tube with 1 mL of extraction buffer: 20 mM Tris-acetate (pH 7·5), 1 mM $MgCl_2$, 0·8 mM $CaCl_2$, 6·5 µM EtBr, 0·01% Proteinase-K. All reagents were molecular grade and certified DNase and RNase free. Extraction was then carried out at 37 °C for 90 min in the dark, mixing occasionally. Following incubation, 100 µL of each sample was aliquoted to three triplicate wells in a 96-well plate (µClear black; Greiner Bio-One GmbH, Frickenhausen, Germany). Fluorescence was measured using a plate reader (SpectraMax M2; Molecular Devices, Sunnyvale, CA USA) with excitation set at 365 nm and emission measured at 592 nm. This measurement was designated F1. Samples were then incubated with 0·01 mg DNase free RNase A (Fermentas Inc., Burlington, ON, Canada) for 45 min at 37 °C. Fluorescence was measured again (designated F2), after which samples were incubated with 2 U of RNase free DNase 1 (Fermentas Inc.) for 45 min at 37 °C. A final fluorescence measurement was then made (F3). The contribution of RNA to the total fluorescence was measured as F1–F2 and that of DNA as F2–F3. Remaining fluorescence at F3 was attributed to background fluorescence of the sample. Alongside the samples, each plate included standards consisting of blank aliquots (homogenizing buffer 1:10 v/v with no tissue) as well as blanks spiked with 5, 10 and 20 µg of pure DNA or RNA (DNA from calf thymus and RNA from *Torula* yeast, Sigma-Aldrich Corp., St Louis, MO, USA) to control for interplate variability and to suppress slight changes in background fluorescence due to weak fluorescence by the nucleases. In all plates, both RNA and DNA standards exceeded the amount of DNA and RNA in the samples and were reduced to zero following incubation with respective nuclease. To test our analytical accuracy and to determine the fluorescence ratio between RNA and DNA, we spiked a series of samples with known quantities of nucleic acids and measured the fluorescence contributed by the spikes. The DNA-EtBr complex has a higher fluorescence yield than the RNA-EtBr complex, and fluorescence data were normalized to account for this difference. As the extraction efficiency of the nucleic acids was not known, we conservatively present the data as RNA:DNA ratio, assuming that if cells were broken then both RNA and DNA were released into the extraction buffer. For the samples collected in 2009, we also assessed the concentration of RNA and DNA in the algal tissue, expressing this as µg $g^{-1}$ wet tissue. The RNA:DNA ratio and concentration of RNA in tissues were significantly correlated (Fig. 1). A linear regression of the

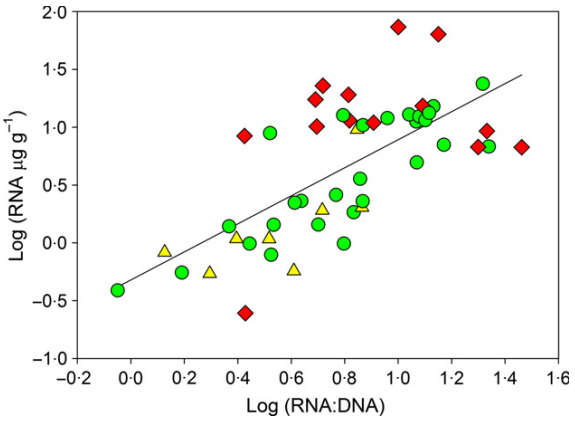


**Fig. 1.** Relationship between RNA expressed as µg $g^{-1}$ and the ratio of RNA:DNA in macroalgal tissue collected from the Lizard Island lagoon, Queensland, Australia. The linear regression is the form: Log(RNA, µg $g^{-1}$) = −0·321 + 1·213 × Log (RNA:DNA), $R^2 = 0{\cdot}48$, $P < 0{\cdot}0001$. Points are individual samples of the Rhodophyta (red diamonds), Chlorophyta (green circles) and Phaeophyta (yellow triangles).

form Log(RNA, μg $g^{-1}$) = −0·321 + 1·213 × Log (Ratio RNA:DNA) was fitted to the relationship ($R^2$ = 0·48, $P < 0·0001$). This relationship had a similar slope to that observed in six studies of higher plants (Fig. S2). Thus, we consider the ratio of RNA:DNA to be indicative of the concentration of RNA in tissues, although the extraction efficiency is not known and is likely to vary among taxa.

### DATA ANALYSIS

Analyses of the variation in RNA:DNA and concentrations of RNA and elemental composition were performed on values for individual specimens using regression analyses. To assess differences among the phyla (corals and macroalgae) and functional form of macroalgae, we used mean values for all genera of coral or macroalgae. The number of samples per genera varied from 1 to 24 (Table S1, Supporting information). For analysis of variation in elemental composition and RNA among lineages of corals and macroalgae, we used nested ANOVA's following the approach of Broadley *et al.* (2004) and McGill (2008), where genus was nested within family which was nested within Phyla (for macroalgae) and where variation due to species, environment and time of sampling are assigned to the error term. Only genera with three or more samples were used in this analysis (which excluded 15 genera, see Table S1). Phyla (for macroalgae), family and genus were considered random factors in the models. The proportion of variance explained at each level in the hierarchy was estimated using the 'varcomp' function in the R package 'ape' following McGill (2008). The relationships between elemental composition, RNA:DNA ratio and maximum thalli size of the genera were analysed using generalized linear models with Phylum as a fixed effect in the model and maximum thalli size as a random continuous variable. We verified the assumptions of normality and homoscedasticity of the models by visually inspecting residual plots. Data were log-transformed, if necessary, to reduce heteroscedasticity.

## Results

For corals, RNA:DNA increased significantly with %N and was positively correlated with N:P ratios and decreased with C:N of tissues, although relationships were weak and highly variable (Table 1, Fig. 2). In macroalgae, the RNA:DNA ratio was significantly correlated with %N and negatively correlated with C:N ratio while the concentration of RNA in tissues was positively correlated with % N and N:P ratio and negatively correlated with the C:N ratio (Fig. 3). There were no significant relationships between the RNA:DNA ratio or RNA concentration and %P in either corals or macroalgae.

**Table 1.** Spearman Rank correlations among elemental composition and the RNA:DNA ratio or RNA concentration in corals and macroalgae.

| | Corals RNA:DNA ($N$ = 64) | Macroalgae RNA:DNA ($N$ = 92) | Macroalgae RNA (μg $g^{-1}$) ($N$ = 36) |
|---|---|---|---|
| %P | 0·065 (0·274) | 0·125 (0·751) | 0·268 (0·213) |
| %N | **0·262 (0·031)** | **0·280 (0·010)** | **0·668 (< 0·0001)** |
| C:P | 0·156 (0·303) | −0·043 (0·861) | 0·189 (0·118) |
| C:N | **−0·272 (0·034)** | **−0·330 (0·0008)** | **−0·610 (< 0·0001)** |
| N:P | 0·256 (0·155) | 0·172 (0·179) | **0·474 (0·0004)** |

All values were log-transformed prior to analysis. Significant linear relationships are in bold with $P$ value in parentheses

The mean ratio of RNA:DNA was similar among coral and red and green macroalgal lineages (Fig. 4a; $P > 0·05$). The Phaeophyta (brown algae) tended to have lower values of RNA:DNA, but this was not significant. However, the concentration of RNA in macroalgal tissues was significantly different among macroalgal lineages with the Phaeophyta and Chlorophyta having lower RNA concentrations in their tissues than Rhodophyta (Fig. 4b, $F_{2,45}$ = 11·075; $P$ = 0·0001).

The %P of tissues differed significantly among the different groups of benthic coral reef organisms (Fig. 5, $F_{3,47}$ = 27·33, $P < 0·0001$). Corals have double the P concentrations of macroalgal tissues. In macroalgae, using RNA per wet weight of tissues (Fig. 1), we estimate that RNA-P is 0·0058% of dry weight (assuming 80% water content of algae and that RNA is 0·087% P, Sterner & Elser 2002), which is 0·38% of the total P pool. C:P ratios of corals were significantly lower than macroalgal lineages ($F_{3,36}$ = 44·9, $P < 0·0001$). %P of the three macroalgal lineages was similar, but the Phaeophyta had a significantly higher C:P compared with Rhodophyta and Chlorophyta (post hoc least significant difference tests of differences between means are $P$ = 0·008 and 0·009, respectively). %N was not significantly different among lineages ($P > 0·05$). C:N ratios followed a similar pattern among lineages as C:P ratios (data not shown). The ratio of N:P was higher than the Redfield Ratio in all lineages and tended to be higher in macroalgae compared with corals ($F_{3,36}$ = 2·80, $P$ = 0·054), but was not significantly different among macroalgal lineages. The coefficient of variation of elemental ratios did not vary significantly among the different lineages (mean coefficient of variation was 0·41 ± 0·05), but corals had the highest levels of variation in C:N, N:P and C:P ratios (coefficient of variation 0·54 ± 0·18) and the Rhodophyta the lowest variation (0·30 ± 0·03).

For both corals and macroalgae, most of the variation in elemental composition and RNA:DNA was contained within the error term (53–97%, Table 2) associated with species or factors associated with environment (e.g. site, depth or time of sampling). Genus explained a significant proportion of variation in %N, C:N, C:P, N:P ratios and RNA:DNA (16–47%) and was particularly high for %N in both corals and macroalgae (~40%). Higher taxonomic classification (phyla, family) did not explain a significant proportion of the variation except for %P where family explained a significant, but low proportion of the variance in the data (corals 10·8% and macroalgae 2·7%, Table 2). The proportion of variance unexplained by the model, that is, within the error term and thus associated with species and environmental factors, was highest for %P (89% and 97% for corals and macroalgae, respectively). To assess how much of this unexplained variation may be explained by environmental factors, we tested for a relationship

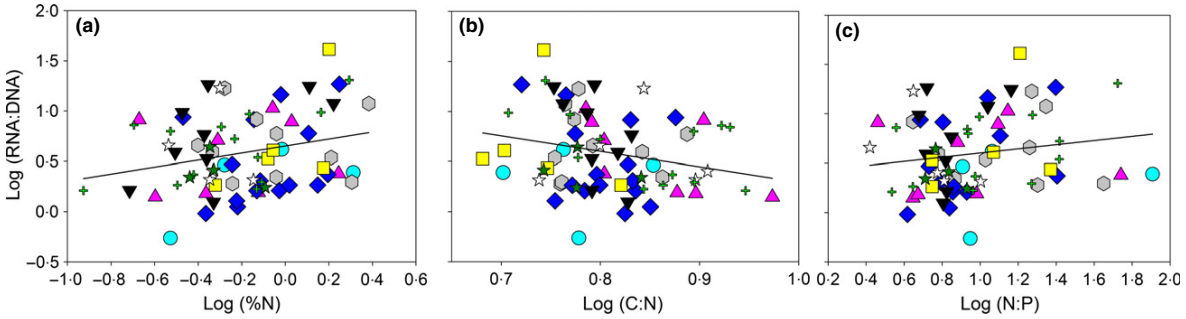


**Fig. 2.** Variation in log(RNA:DNA ratio) of corals with (a) log (%Nitrogen); (b) log (Carbon:Nitrogen) and (c) log (Nitrogen: Phosphorus). Points are individual samples with coral families indicated in colour: Acroporidae – pink upward triangles; Poritidae – light green cross; Merulinidae – blue diamonds; Diploastreidae – aqua circles; Mussidae – grey hexagons; Lobophylidae – yellow squares; Agaricidae – white stars; Pocilloporidae – black downward triangle; Dendrophyllidae – dark green stars.

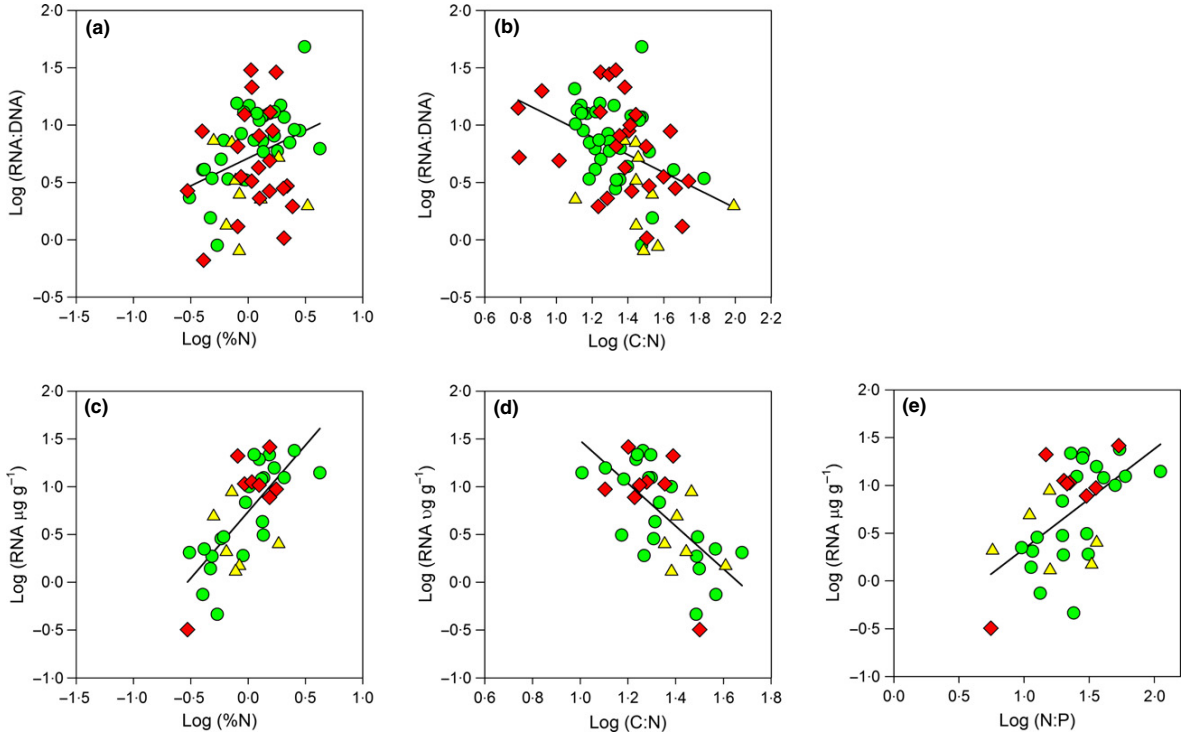


**Fig. 3.** Variation in log(RNA:DNA) of macroalgae with (a) log (%Nitrogen); and (b) log (Carbon:Nitrogen) ratio. Variation in the log (RNA concentration in µg g$^{-1}$ wet weight) with (c) log (%Nitrogen); (d) log (Carbon: Nitrogen); and (e) log (Nitrogen: Phosphorus). Points are individual samples that are coloured for macroalgal phyla: Chlorophyta – green circles; Phaeophyta – yellow triangles; Rhodophyta – red diamonds.

between %P and sampling depth. We did not find a significant relationship between depth and %P; however, our sampling was skewed towards shallow depths, which reduced our power to detect depth effects.

Tests of whether variation in elemental ratios and RNA:DNA was associated with variation in functional form of macroalgae thalli indicated that although means among functional forms were variable, functional form did not explain a significant proportion of variance for any parameter. However, calcifying corticated macroalgae tended to have lower C:P, lower N:P and higher RNA:DNA and concentrations of RNA than other forms of thalli even though these differences were not significant (Table 3). Additionally, we found that %N increased significantly with maximum size of thalli of genera (Fig. 6, $P = 0{\cdot}0123$). There were no other significant relationships among maximum thalli size and elemental composition, although in the green macroalgae, there was a tendency for RNA:DNA to also increase with maximum size (data not shown).

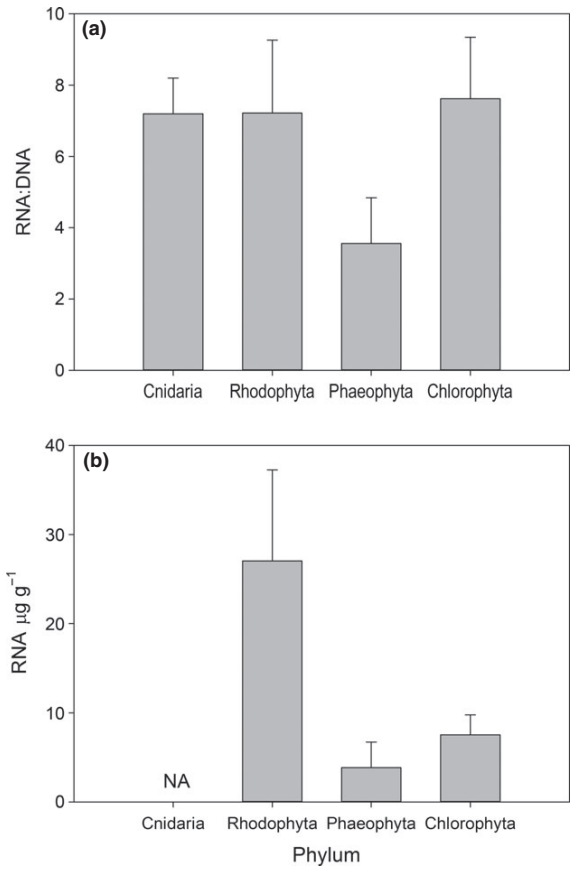


**Fig. 4.** (a) Variation in RNA:DNA in four phyla of benthic coral reef organisms. (b) Variation in the concentration of RNA in macroalgal lineages. Values are means and standard errors for 4–9 genera in panel (a) and 3–5 genera in Panel (b). NA indicates data not available.

## Discussion

The Growth Rate Hypothesis links %P to the amount of RNA and the growth rates of a range of animals and bacteria (Elser *et al*. 2003). Although a strong relationship between P, RNA and growth rates has been observed over many taxa, we found positive but highly variable (non-significant) relationships between %P and C:P and RNA:DNA, but stronger links to %N (and C:N and N:P), similar to that observed in experimental studies (Reef, Pandolfi & Lovelock 2012). A study of phytoplankton also found little evidence to support the Growth Rate Hypothesis (Flynn *et al*. 2010), although in mangroves, RNA:DNA was correlated with C:P ratio of tissues and growth rates of tree species (Reef *et al*. 2010). High variability in RNA:DNA in corals has been observed (fivefold differences, Buckley & Szmant 2004). The high variation in the %P-RNA:DNA relationship may indicate high levels of variation among genera (and species) in the allocation of P to RNA vs. lipids (Gates & Edmunds 1999). Lipids can reach 40% of coral tissue biomass (Stimson 1987) and approximately half of the lipid present can be as phospholipids (Imbs *et al*. 2010). The variation in lipid concentrations among coral taxa is high and is a function of environmental conditions and reproductive stage (Leuzinger, Anthony & Willis 2003; Buckley & Szmant 2004; Imbs *et al*. 2010), which could also contribute to the weak relationship between RNA:DNA and C:P. An assessment of the relationship between the values of lipids in eight corals from the Great Barrier Reef (Leuzinger, Anthony & Willis 2003) and C:P ratios of the same genera from our data set found a significant linear relationship, with high concentrations of lipids at low C:P ratios (Fig. S3), consistent with high allocation of P to lipids in corals as P concentrations increase.

In macroalgae, the storage of P in vacuoles (or 'luxury' uptake), which is an adaptation to low and variable nutrient supply (Fitzgerald & Nelson 1975; Raven 2013), may obscure any %P (or C:P) – RNA relationship for macroalgae (Reef, Pandolfi & Lovelock 2012). The capacity for 'luxury' uptake of P may be particularly well developed in coral reef species because nutrient concentrations in the water column are often very low, particularly in carbonate systems (Lapointe, Littler & Littler 1992), leading to the evolution of highly developed P uptake and storage mechanisms. In macroalgae, we calculated the proportion of P associated with RNA (RNA-P). P in RNA accounted for *c*. 0·4% of total P in samples, which is a very low proportion compared with animals, phytoplankton, bacteria (Elser *et al*. 2003) and higher plants (Veneklaas *et al*. 2012). This is possibly due to low RNA concentrations in macroalgae (mean of 13 μg g$^{-1}$ fresh weight, Fig. 1), which, while similar to other studies of macroalgae (e.g. Su & Gibor 1988; Kim *et al*. 1997; Chan *et al*. 2004), is low compared with other taxonomic groups that can have up to 25% RNA per dry weight (Elser *et al*. 2003). The extraction efficiency of RNA in our study is not known as establishing extraction efficiency was not our priority given the wide range of taxa used in the study, but is known to be low particularly in the Rhodophyta (Kim *et al*. 1997). Links between P and RNA and growth in macroalgae may exist, but the quantitative assessment of RNA and different pools of P (e.g. vacuolar, lipid, water soluble P esters) are needed to assess this hypothesis (Agren 2004; Elser *et al*. 2010).

Among the groups of benthic coral reef organisms we studied, we found support for fundamental differences in the elemental composition of animals (corals) and photoautotrophs (macroalgae) of the reef benthos (Fig. 5). Corals clearly had higher %P (and lower C:P) than macroalgae, a trend similar to other published comparisons of elemental stoichiometry between animals and plants (Sterner & Elser 2002). But variation in elemental ratios of corals was equivalent to that of macroalgae. This high level of variation in elemental composition of corals (similar to co-occurring autotrophs) may reflect differences in levels of heterotrophy among groups of corals (Houlbrèque *et al*. 2004; Hoogenboom, Connolly & Anthony

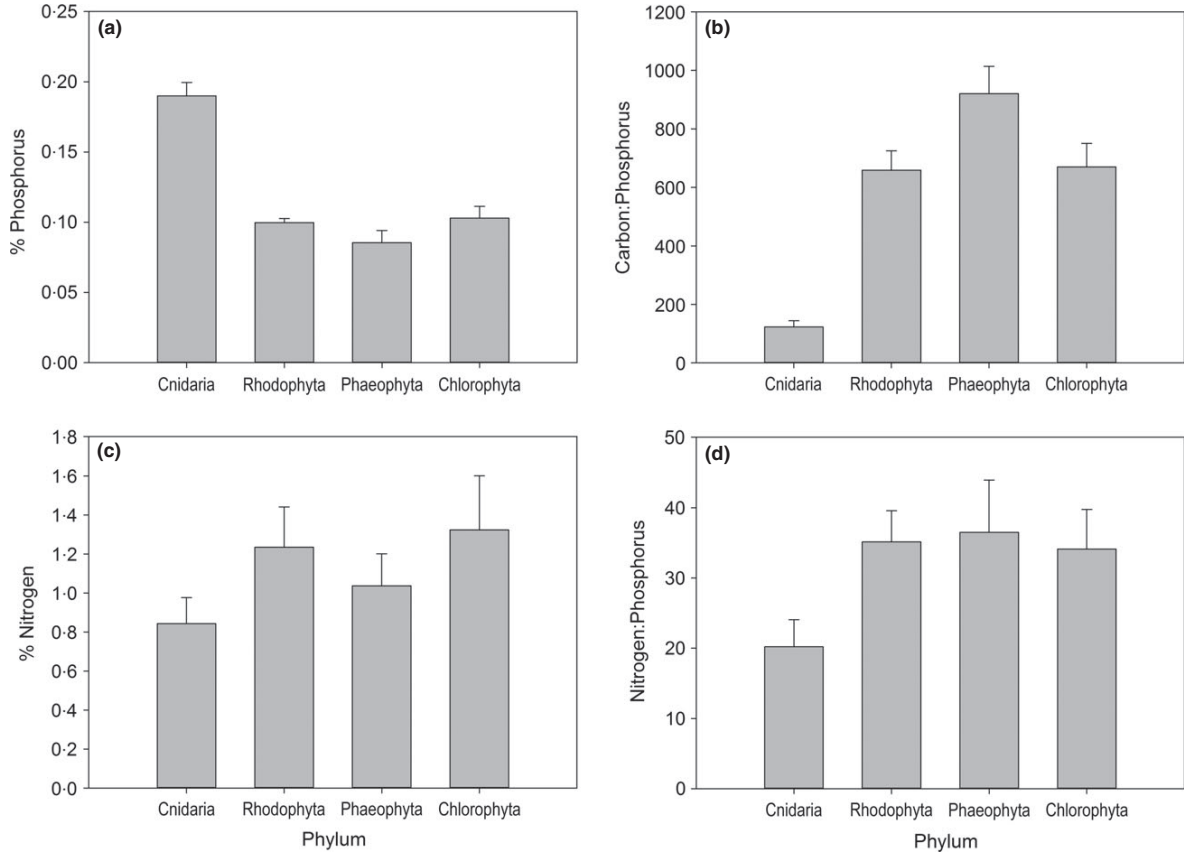


**Fig. 5.** Variation in elemental composition of tissues of coral reef benthic organisms from four phyla. (a) Phosphorus (% dry mass); (b) carbon to phosphorus ratios (C:P); (c) nitrogen (% dry mass); and (d) nitrogen to phosphorus ratios (N:P). Values are means and standard errors for 3–5 genera.

**Table 2.** Proportion of variance (%) (±SD) in elemental composition and RNA:DNA explained by phylogenetic groupings in corals and macroalgae from Lizard Island

| | Corals | | | Macroalgae | | | |
|---|---|---|---|---|---|---|---|
| | Family | Genus | Error | Phyla | Family | Genus | Error |
| %N | 1·7 (41·1) | **37·4 (195·3)** | 60·9 (249·9) | <0·01 (0·02) | <0·01 (<0·01) | **47·0 (230)** | 53·0 (245) |
| %P | **10·8 (473)** | 0·22 (74·5) | 88·9 (136) | <0·01 (0·08) | **2·7 (4·7)** | <0·01 (0·01) | 97·3 (282·4) |
| C:N | <0·01 | **32·6 (93·2)** | 67·4 (133·0) | 21·2 (223·8) | 5·2 (109·5) | **20·6 (221·3)** | 53·0 (354·6) |
| C:P | 11·2 (95) | **14·8 (146)** | 73·9 (284) | 10·9 (147·4) | 12·0 (153·3) | **18·8 (192)** | 58·0 (329·8) |
| N:P | 3·3 (55) | **24·4 (148)** | 72·3 (257) | <0·01 (0·01) | <0·01 (<0·01) | **41·6 (223)** | 58·3 (225·9) |
| RNA:DNA | <0·01 (<0·01) | **26·3 (6·65)** | 73·7 (11·3) | 25·6 (116) | <0·01 (<0·01) | 14 (88) | 60·4 (183·2) |

Variance components were scaled to sum to 100%. Significant contributions of phyla, family or genus to variance ($P < 0·05$) determined by hierarchical nested ANOVA are indicated in bold. The error term includes variation due to species and environmental conditions including those associated with sampling site, date and depth.

2008), but also could reflect other differences, for example differences in types of symbionts (Knowlton & Rohwer 2003). Irrespective of the underlying causes, the high level of variation in elemental composition among coral genera may influence coral growth rates and susceptibility to predation (e.g. Rotjan & Lewis 2005; Hoogenboom, Rodolfo-Metalpa & Ferrier-Pagès 2010).

The variation we found in the C:P ratio of macroalgal lineages was low compared with published variation in higher plants where, for example, C:P values can exceed 3000 (Elser *et al.* 2000; Sterner & Elser 2002; Reich & Oleksyn 2004; Lovelock *et al.* 2007). Our data indicate there is a much greater level of homeogeneity of elemental composition among macroalgae, even across widely

**Table 3.** Variation in elemental composition, RNA (μg $g^{-1}$) and RNA:DNA among differing macroalgal growth forms

| | Filament | Sheet | Corticated macrophyte (CM) | CM-calcified |
|---|---|---|---|---|
| %P | 0·12 ± 0·02 (4) | 0·094 ± 0·007 (4) | 0·095 ± 0·003 (10) | 0·098 ± 0·004 (6) |
| %N | 1·37 0·47 (6) | 1·00 ± 0·21 (4) | 1·43 ± 0·16 (10) | 0·87 ± 0·05 (5) |
| C:P | 669 ± 146 (5) | 819 ± 127 (3) | 860 ± 71 (10) | 562 ± 35 (5) |
| C:N | 22·8 ± 3·1 (6) | 35·6 ± 6·8 (4) | 24·2 ± 2·9 (10) | 23·8 ± 1·9 (5) |
| N:P | 38·4 ± 10·2 (5) | 32·2 ± 6·8 (3) | 40·6 ± 4·9 (10) | 26·1 ± 1·6 (5) |
| RNA (μg $g^{-1}$) | 7·7 ± 6·7 (2) | NA | 7·5 ± 1·8 (5) | 28·9 ± 13·6 (4) |
| RNA:DNA | 4·4 ± 1·1 (3) | 3·7 ± 0·3 (2) | 6·5 ± 1·9 (8) | 9·0 ± 2·4 (5) |

Values are means ± standard errors. Number of genera represented is in parenthesis.

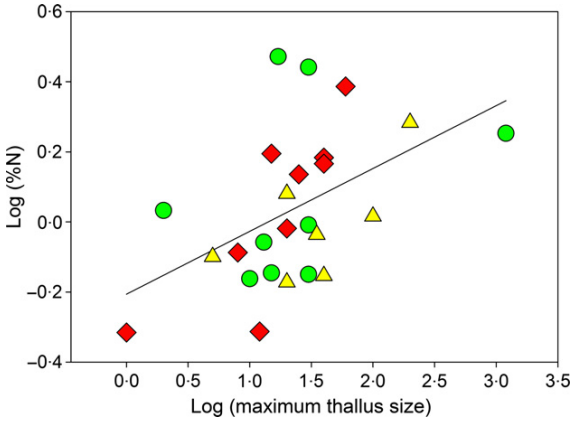


**Fig. 6.** The relationship between %N of tissues (log-transformed) and maximum size of macroalgal genera (log-transformed). Each point is one genus from the Chlorophyta (green circles), Phaeophyta (yellow triangles) and Rhodophyta (red diamonds). The line is the regression of the form $Y = -0{\cdot}206 + 0{\cdot}179 \times X$, $R^2 = 0{\cdot}24$, $P = 0{\cdot}0123$.

divergent lineages (two kingdoms, three phyla), compared with variation in higher plants (within a phyla). Studies reviewed by Elser *et al*. (2010) found that both freshwater and marine macroalgae tended to have higher levels of homeostasis than terrestrial plants. In our study region, the elemental composition of tropical *Sargassum* grown over a gradient in nutrient availability was also relatively homeostatic (Shaffelke & Klumpp 1998). Higher levels of homeogeneity in elemental composition in aquatic compared with terrestrial autotrophs may influence a range of ecosystem processes, including the ecology of grazers. For example, reduced tolerances of extremely high or extremely low C:P ratios in marine grazers (Elser *et al*. 2006), reduced specialization among grazers (more generalists), overall higher levels of grazing (Cry & Pace 1993; Shurin, Gruner & Hillebrand 2006) and high sensitivity to pollution (Lapointe 1997) may be some of the ecological consequences of the relatively low and tightly constrained C:P of corals and macroalgae.

We found support for our hypothesis that the less differentiated Phaeophyta (Bell & Mooers 1997) gives rise to higher mean C:P ratios (and lower RNA) compared with the more differentiated tissues of the Rhodophyta and Chlorophyta. The differences observed among the major algal lineages are congruent with earlier observations of C:N:P ratios (Atkinson & Smith 1983) and energetic value of tissues of the different lineages (Paine & Vadas 1969). A high level of investment in defensive compounds in the Phaeophyta, for example phlorotannins and diterpenoids (Amsler & Fairhead 2006), are also likely to contribute to high C:P ratios and to the reduced palatability often observed for this group compared with green and red species (e.g. Paul & Hay 1986; Boyer *et al*. 2004; Mantyka & Bellwood 2007). Instead, specialized grazers may focus on younger tissue in the Phaeophyta, which may also reduce the impacts of grazing on the whole thallus (Hay, Poore & Lovelock 2011). In red and green algal lineages, highly toxic compounds and calcified tissues may interact with elemental composition to deter grazers (Cruz-Rivera & Hay 2003).

The proportion of variation in the data that was explained by higher taxonomic groupings was low, with a higher proportion of variation explained at the genus level or contained within the error term of our models, which encompasses species and environmental factors (Table 3). Studies of marine (Duarte 1992) and terrestrial (Broadley *et al*. 2004; Kerkhoff *et al*. 2006; Watanabe *et al*. 2007) plants also indicate a relatively low proportion of the variation in elemental composition was attributed to higher taxonomic grouping (order, family). Variation in %P was significantly associated with family in our analysis, although at a low level (2·7 and 10·8%). In plants, genome size varies significantly among families, as well as among species, and has been linked to functional traits (Bainard *et al*. 2012). Variation in %P in macroalgae could reflect variation in genome size among families (and genera and species), although this remains to be assessed.

Our analysis of differences in elemental stoichiometry or RNA among functional forms did not indicate that the commonly used functional form classifications of macroalgae (Steneck & Delthier 1994) were linked to elemental composition. In contrast, Gusewell (2004) found differences in elemental composition between grasses and forbs and between stress tolerant and ruderal species. However, comparison among functional forms found a tendency for higher levels of C:P in the structurally complex corticated

macrophytes compared with filamentous forms. This is consistent with predictions of increasing C:P in structurally complex higher plants (Sterner & Elser 2002; Elser *et al.* 2010). We also observed a trend towards high levels of RNA:DNA (and concentrations of RNA) in calcifying macroalgae compared with other growth forms, although this was not significant (Table 3). Calcification provides physical defence against herbivory (e.g. Paul & Hay 1986) although potentially at some cost, as growth rates of calcifying forms are lower than that of fleshy algae (Delgado & Lapointe 1994). High levels of RNA and low levels of C:P (Table 3) in this group may be associated with the metabolic processes required for calcification and nutrient uptake. Cylindrical thalli forms are highly efficient in resource acquisition, maintaining high surface area to volume ratios compared with other forms (Niklas 2000). Our observations, which %N tends to increase with maximum thalli size, may be due to the predominance of cylindrical forms in coral reef taxa with large thalli sizes (e.g. *Caulerpa*). Modification of functional form models of macroalgae to include thalli size or surface area to volume ratios, or other functional traits may increase their utility (Padilla & Allen 2000).

A possible limitation to our study is the use of RNA:DNA ratio to compare taxa because RNA:DNA may covary with genome size (Hessen, Ventura & Elser 2008), such that taxa with larger genomes have lower RNA:DNA, and thus, variation in RNA:DNA ratio may reflect variation in genome size rather than allocation to RNA. We assessed the RNA:DNA ratio because of the variation in tissue composition among taxa and the resulting variation in nucleic acid extraction efficiency, and because of its widespread use in the literature (e.g. Buckley & Szmant 2004; Hessen, Ventura & Elser 2008). While there are few estimates of genome size of coral species, there are genome size data for macroalgae (Plant DNA C-values Database, release 6.0, December 2012, M.D. Bennett & I.J. Leitch, http://data.kew.org/cvalues/). An assessment of genome size and RNA:DNA ratio over 23 macroalgal genera in our study did not reveal any significant relationship (Fig. S4), suggesting RNA:DNA may be independent of variation in genome size in our study. However, species level variation in genome size may be a factor that should be considered further in both macroalgae and corals.

## Conclusions

The positive relationship between C:P and RNA proposed by the Growth Rate Hypothesis was not strongly supported for corals or macroalgae. This may arise due to the adaptations to low nutrient environments of coral reefs. The quantification of pools of P within tissues may give further insights into the utility of the Growth Rate Hypothesis framework for predicting the ecology of benthic organisms on coral reefs. Elemental composition of organisms of the coral reef benthos varied with differences in phylogeny and macroalgal structure. However, variation in elemental composition was surprisingly low compared with terrestrial plants given the widely divergent lineages examined. Some of the consequences of greater homogeneity in the elemental composition of tissues in marine vs. terrestrial systems may give rise to some of the fundamental differences observed between terrestrial and marine ecosystems, including enhanced grazing and high sensitivity to pollution in marine ecosystems.

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## Supporting Information

Additional Supporting information may be found in the online version of this article:

**Table S1.** Number of individual samples for each genus used in the study.
**Fig. S1.** Frequency of sampling effort over depth for corals (panel A) and macroalgae (panel B).
**Fig. S2.** Relationship between RNA and RNA:DNA ratio in higher plant species.
**Fig. S3.** The relationship between lipids in coral genera (mg $cm^{-2}$) and C:P ratio.
**Fig. S4.** A. Variation in RNA:DNA ratio of macroalgal genera with genome size of species within the same genera (1C values).