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Are bacteria more like plants or animals? Growth rate and resource dependence of bacterial C : N : P stoichiometry

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Summary

1. We examined the relative importance of resource composition (carbon : phosphorus molar ratios which varied between 9 and 933) and growth rate (0·5–1·5 h^{-1}) to biomass carbon : nitrogen : phosphorus stoichiometry and nucleic acid content in *Escherichia coli* grown in chemostats, and in other heterotrophic prokaryotes using published literature.
2. *Escherichia coli* RNA content and the contribution of RNA-P to total cellular P increased with increasing growth rate at all supply C : P ratios. Growth rate had a much stronger effect on biomass C : P than did supply C : P, and increased RNA content resulted in low biomass C : P and N : P ratios.
3. However, we observed only twofold variations in biomass C : P and N : P ratios in the experiments, despite a difference of two orders of magnitude in C : P and N : P supply. The response of biomass C : P and N : P ratios to alteration of the supply C : P and N : P ratios revealed that *E. coli* was strongly homeostatic in its elemental composition.
4. This result, and a literature survey, suggest that each heterotrophic bacterial strain regulates its elemental composition homeostatically within a relatively narrow range of characteristic biomass C : P and N : P ratios.
5. Thus shifts in the dominance of different bacterial strains in the environment are probably responsible for the large variation in bacterial biomass C : P, as has been suggested for crustacean zooplankton. These findings indicate that bacteria are more like animals than plants in terms of biomass C : P and N : P homeostasis.

Key-words: C : N : P ratios, growth rate hypothesis, homeostasis, nucleic acid, phosphorus

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Introduction

There can be an imbalance in the ratio of carbon (C) to other elements, especially phosphorus (P), among different trophic levels or functional groups (Sterner *et al*. 1998; Sterner & Elser 2002). At the base of the food web, phytoplankton have a wide range of biomass C : P mole ratios (C : P hereafter), from 100 up to ≈4000. This large range probably reflects interspecific and intraspecific variations among marine (Goldman, McCarthy & Peavey 1979) and freshwater species (Rhee 1978; Gächter & Bloesch 1985; Sterner *et al*. 1993). The C : P ratios of phytoplankton consumers such as crustacean zooplankton are typically much smaller and less variable; the total range of body C : P in taxa studied to date varies from 50 to 200, showing limited intraspecific variability (strong physiological homeostasis) with most variation associated with differences among species (Elser *et al*. 2000b). Such imbalances of biomass C : P between predator and prey have important ecological consequences, such as impacts on population dynamics and nutrient recycling (Elser & Urabe 1999; Sterner & Elser 2002).

One proposed cause of variable C : P or nitrogen (N) : P ratios in different organisms is interspecific differences in RNA content, reflecting differences in their life history (Elser *et al*. 1996; Elser *et al*. 2000a). For example, fast-growing cladocerans (especially *Daphnia*) contain as much as 1·5% P and 10% RNA dry weight, while slow-growing copepods contain only 0·6% P and 2% RNA. Thus differences in P content between *Daphnia* and adult copepods can be explained largely by the difference in RNA content (assuming RNA is ≈9% P; Sterner 1995; Elser *et al*. 1996). This mechanism relating C : N : P stoichiometry, RNA allocation and the life history of organisms is called the growth rate hypothesis (GRH, Elser *et al*. 1996; Elser *et al*. 2000a). While the GRH originally emerged from interspecific comparisons among biomass C : P, N : P and maximum growth rate for different species under

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ideal growth conditions (Elser *et al*. 1996; Main, Dobberfuhl & Elser 1997), it may also be relevant to intraspecific variations such as ontogenetic differences in biomass C : P and N : P ratios (Hessen 1990; Hessen & Andersen 1990; Villar-Argaiz, Medina-Sánchez & Carrillo 2002) because growth rates and RNA contents generally decline with age (Gorokhova & Kyle 2002).

Although many questions relating to the GRH have been examined in crustacean zooplankton, the dominant decomposers in lakes and oceans are heterotrophic bacteria (Cotner & Biddanda 2002), and therefore these organisms are likely to have large impacts on the stoichiometric composition of these waters. In heterotrophic bacteria collected from lakes and coastal waters and grown in the laboratory, biomass C : P varied from 8 to ≈500 (Tezuka 1990; Kirchman 2000), suggesting that the biomass C : P of these organisms is more variable than that of multicellular consumers such as zooplankton, and is perhaps comparable with that of phytoplankton (see Figure 1 of Sterner *et al.* 1998). However, little attention has been paid to factors causing large variations in bacterial C : P. Tezuka (1990) found positive relationships between the C : nutrient ratio of culture media and that of lake bacteria, implying that biomass elemental composition could closely reflect the elemental composition of supplied resource (weak homeostasis), as in phytoplankton (Rhee 1978; Sterner *et al*. 1993). Given that both phytoplankton and bacteria are osmotrophic, incorporating nutrients as individual rather than 'prepackaged' units, as herbivores and predators do, it is probable that bacteria would have a biomass stoichiometry that varied similarly to that of phytoplankton, although this question has not yet been examined.

On the other hand, many studies of enterobacteria such as *Escherichia coli* have shown that RNA (more specifically ribosomal RNA) content increases with growth rate (Maaloe & Kjeldgaard 1966; Neidhardt, Ingraham & Schaechter 1990; Hanegraaf & Muller 2001). There are also considerable variations in RNA content or RNA : DNA ratios in natural bacteria, with implications for growth and productivity (Kemp, Lee & LaRoche 1993; Lee & Kemp 1994; Jeffrey *et al*. 1996). Among these studies, Kemp *et al*. (1993) documented positive correlations between RNA content and growth rate for four marine bacterial isolates. Thus there is ample evidence that increased RNA could reduce bacterial biomass C : P as suggested by the GRH. Indeed, phytoplankton biomass C : P decreases from ≈1000 to 100 with increasing growth rate in continuous culture experiments (Goldman *et al*. 1979; Gächter & Bloesch 1985). However, simultaneous measurements of biomass C, N, P and nucleic acid content are still required for a direct evaluation of these correlations.

We now have alternatives (resource dependency and growth rate dependency) for biomass C : P and N : P stoichiometry of various organisms in the basal part of planktonic food webs. Although a contrast between phytoplankton (weak homeostasis) and zooplankton (strong homeostasis) is reasonably well established, information on the relative importance of and/or the interactions between these alternatives for microbes is incomplete. To improve understanding of stoichiometric homeostasis and its determinants in heterotrophic bacteria, we measured biomass C, N, P and nucleic acid content simultaneously in *E. coli* K-12 under a wide range of substrate C : P and growth rates. We also surveyed the literature on the homeostasis of biomass C : P and N : P for cultured and natural freshwater lake bacteria, and compared these with the results of our experiments. We propose a possible mechanism for highly variable biomass C : N : P stoichiometry in bacteria in nature.

Materials and methods

EXPERIMENTAL PROCEDURE

We chose to culture *E. coli*, admittedly not a very representative species for aquatic studies. But because RNA production is an essential outcome of growth in all organisms, we presumed that physiological responses are similar in bacteria growing in many different habitats. Our literature survey, which included several aquatic bacteria (see below), supported this view.

All culture media were defined as shown in Table 1 (Neidhardt, Bloch & Smith 1974; Wanner, Kodaira & Neidhardt 1977). We used glucose and ammonium chloride for C and N sources, respectively, and held C and N concentrations constant. The concentration of potassium phosphate was manipulated to create a gradient in supply C : P mole ratio from 9·3 to 934 and in supply N : P from 7·2 to 721. The pH of media after mixing all compounds was 7·3–7·4.

At the beginning of the experiment, three 33 ml chemostats were inoculated with *E. coli* grown in batch culture in a culture medium with a particular target C : P, and the same medium was fed to the chemostats at dilution rates of 0·5, 1·0 and 1·5 h^{-1} (except for the first run at a supply C : P of 9·3, in which dilution rates were 0·5, 1·1 and 1·7 h^{-1}). The chemostats were mixed continuously with filtered, hydrated air, and maintained at 37 °C in the dark. We began collecting samples when the chemostat reached steady-state (≈36 h after inoculation). Outflowing medium containing *E. coli* was collected and filtered onto precombusted glass-fibre filters (Whatman GF/F) or polycarbonate filters (Osmotics, 0·2 μm pore size). The former were used to determine bacterial C and N contents; the latter were processed for bacterial P and nucleic acids. C and N contents were determined using a CHN analyser (model 2400, Perkin-Elmer, MA, USA), and P contents were measured by acid-persulfate digestion and subsequent soluble reactive phosphorus analysis (APHA 1992) using an Alpkem Flow Solution 3000 Analyser (ANTEC GmbH, Pinneberg, Germany). The pH of outflowing medium was 7·2–7·3.

Table 1. The composition of defined media used in the present study

Compound	Chemicals	Concentrations in medium (mM)
Buffer	Tris–HCl, pH 7·6	80
Chelate	Tricine, pH 7·6	4
Macronutrients	NaCl	50
	$MgCl_2$	0·523
	K_2SO_4	0·276
	$FeSO_4$	0·010
	$CaCl_2$	5×10^{-4}
Micronutrients	$(NH_4)_6(MoO_7)_{24}$	3×10^{-6}
	H_3BO_3	4×10^{-4}
	$CoCl_2$	3×10^{-5}
	$CuSO_4$	1×10^{-5}
	$MnCl_2$	8×10^{-5}
	$ZnSO_4$	1×10^{-5}
Vitamins	*p*-Aminobenzoic acid	0·01
	p-Dihydroxybenzoic acid	0·01
	p-Hydroxybenzoic acid	0·01
	Panthothenate, hemicalcium salt	0·01
	Thiamine-HCl	0·01
C source	Glucose	1·984
N source	NH_4Cl	9·52
P source	KH_2PO_4 (supply C : P & N : P = 9·3 & 7·2)	1·32
	KH_2PO_4 (supply C : P & N : P = 93 & 72)	0·132
	KH_2PO_4 (supply C : P & N : P = 233 & 180)	0·0528
	KH_2PO_4 (supply C : P & N : P = 467 & 361)	0·0264
	KH_2PO_4 (supply C : P & N : P = 933 & 721)	0·0132

Nucleic acids were determined by extracting the cellular contents via sonication followed by staining with the fluorochrome RiboGreen (Molecular Probes, OR, USA), which reacts with DNA and RNA (Jones *et al*. 1998; Gorokhova & Kyle 2002). A polycarbonate filter containing *E. coli* and 5 ml TE buffer (1×, Molecular Probes) with 0·167% w/v *N*-laurosarcosyl (Sigma, MO, USA) were transferred to a 50 ml snap-cap vial. Samples were sonicated on ice for 2 min using a 5 mm tip on a Microson Ultrasonic Cell Disruptor XL set at 10% maximum output, then incubated for 1–2 h on a shaker at room temperature. From each tube, three replicate 75 µl subsamples were pipetted into wells of a black microplate, and 75 µl RiboGreen working solution (750 nM in 1 × TE buffer) were added to each well. Negative control samples (containing all reagents but no *E. coli*) and standard DNA (calf thymus, Sigma) and RNA (Type III from baker's yeast, Sigma) solutions, diluted in the TE buffer with sarcosyl, were also distributed into the microplates and processed in the same way. The microplate was incubated in the dark at room temperature for 5 min. The samples were then measured in an FL600 Microplate Fluorescence Reader using KC4 software (Bio-Tek, VT, USA) with peak excitation at 480 nm and peak emission at 520 nm. After an initial scan, 7·5 µl RNase A (Promega, WI, USA), 10 µg ml^{-1} in 1 × TE buffer, were added and the plate was rescanned after 20–40 min. Differences in the sample scans relative to differences observed in RNA and DNA standards were used to determine RNA and DNA concentrations in the extracts.

DATA ANALYSIS

Biomass N, P, RNA and DNA contents were converted to relative values (percentage of dry weight) by assuming that C content was 50% of dry weight (Neidhardt *et al*. 1990). The relative amounts of RNA-P and DNA-P to total biomass P were calculated by assuming that P occupies 9% of the mass of nucleic acids (calculated from the elemental composition of five nucleotides). The effects of dilution rate (= growth rate) and supply C : P on *E. coli* C : N : P stoichiometry and nucleic acid contents (Y) were examined by stepwise multiple regression analysis using the following model:

$$Y = a_1(\text{growth rate}) + a_2(\text{supply C : P}) + \text{intercept},$$

where a is the partial regression coefficient.

To compare the nutrient ratios of bacteria and culture media, we modelled stoichiometric homeostasis (Sterner & Elser 2002) by describing a linear relationship between the relative changes in these two parameters with the following equation:

$$\frac{dy}{y} = \frac{1}{H}\frac{dx}{x} \qquad \text{eqn1}$$

where x is a supply nutrient ratio in the culture media, y is the same ratio in bacteria (measured with identical units), and $H$is a regulatory coefficient measuring the degree of homeostasis. Equation 1 can be integrated to give:

$$y = cx^{\frac{1}{H}} \qquad \text{eqn 2}$$

where c is a constant of integration. Equation 2 can be expressed in linear form using logarithms:

$$\log(y) = \log(c) + \frac{\log(x)}{H} \qquad \text{eqn 3}$$

Equation 3 indicates that if one plots the logarithm of a nutrient ratio in a consumer (bacteria) *vs* the logarithm of the ratio of the same nutrients in its resources, the slope ($1/H$) relates directly to the strength of homeostatic maintenance of nutrient ratio within the consumer biomass. H will vary between 1·0 (no homeostasis) when $\log(y) = \log(c) + \log(x)$ and ∞ (no variation in consumer nutrient ratios, or strict homeostasis) when $\log(y) \approx \log(c)$. Sterner & Elser (2002) present several examples of the use of this analysis to quantify different degrees of homeostasis in diverse consumer–resource pairs.

Results and discussion

ESCHERICHIA COLI BIOMASS IN CHEMOSTATS

Adding more P to the culture media increased *E. coli* biomass in chemostats at low growth rates (Fig. 1a). When the growth rate was 0·5 h^{-1}, biomass was ≈80 µg C ml^{-1} for supply C : P ratios of 9·3 and 93, but only 7 µg C ml^{-1} at a supply C : P of 933. However the

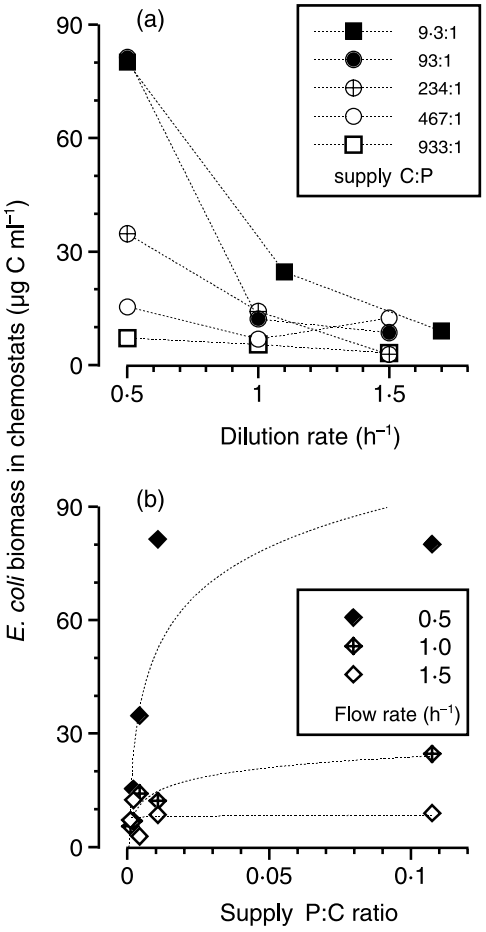

Fig. 1. (a) Effects of growth (dilution) rate and supply C : P molar ratio on the biomass of *Escherichia coli* grown in chemostats; (b) the response of *E. coli* biomass to the P content of media for each growth rate.

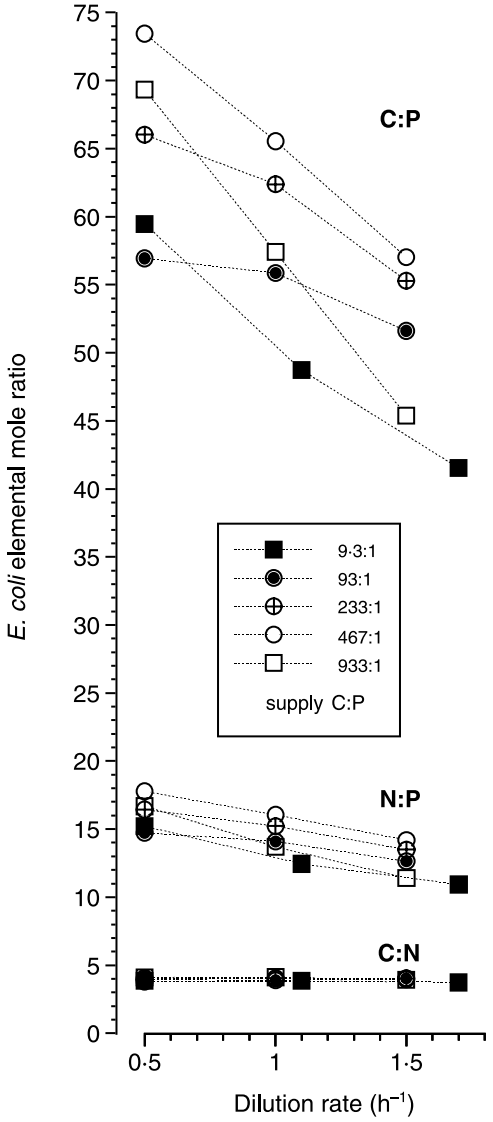

Fig. 2. As for Fig. 1, but for the biomass C : N : P stoichiometry of *Escherichia coli* grown in chemostats. Ratios are on a molar basis.

differences in biomass diminished when growth rates increased. Theoretically, the biomass of organisms in a chemostat should increase with increasing concentration of a limiting resource at a constant growth rate (Fiechter, Käppeli & Meussdoerffer 1987; Neidhardt *et al*. 1990). With our various media P : C ratios, there was some evidence that *E. coli* biomass was P-limited at low growth rates (Fig. 1b). At the highest growth rate, however, there was little effect of increased P concentration (higher P : C ratio) in the medium.

BIOMASS CNP STOICHIOMETRY, NUCLEIC ACID CONTENT AND GROWTH – EVALUATION OF THE GROWTH RATE HYPOTHESIS

Consistent with the GRH, the biomass C : P and N : P ratios of *E. coli* decreased with increasing growth rate in all media, while biomass C : N changed little (Fig. 2; Table 2). The 200% increase in growth rate from 0·5 to 1·5 h^{-1} led to a 20% reduction in biomass C : P from 65·1 to 52·4 (the average of the treatments), and a 21% reduction in biomass N : P from 16·5 to 13·0. These responses reflect the increase in P content (from 2 to 2·8%), with growth rate while the N content did not increase. N content (14–15%) and subsequently biomass C : N were affected by the supply C : P. However, supply C : P was not statistically significant in stepwise multiple regressions for biomass C : P and N : P ratios (Table 2), suggesting that variations in biomass C : P and N : P were independent of supply C : P.

Bacterial RNA content is strongly associated with growth rate (see Introduction). Consistent with this and with the GRH, we also observed that the RNA content of *E. coli* increased considerably with increasing growth rate, whereas DNA content was stable around 2·5% of *E. coli* dry weight (Fig. 3). Thus RNA : DNA ratios rose from 3·5–5·5 to 7–9 as growth rate increased. This increased RNA was probably ribosomal RNA, as it generally dominates the cellular RNA pool (65–81% cellular RNA; Neidhardt *et al*. 1990; Lehninger, Nelson & Cox 1993) and increases in proportion to other types of RNA with increasing growth rate (Neidhardt *et al*. 1990). Similar trends of growth-dependent

Table 2. Results of stepwise regression analysis for *E. coli* C : N : P stoichiometry and biochemical composition

Category	ANOVA R^2	ANOVA F	ANOVA P	Variable	Partial regression coefficient	Standard regression coefficient	t	P
N content	0·39	8·3	0·013	*S*	$7{\cdot}7 \times 10^{-4}$	−0·62	−2·88	0·013
				I	14·8			
P content	0·58	17·8	0·001	*D*	0·6	0·76	4·2	0·001
				I	1·7			
Biomass C : P	0·58	17·7	0·001	*D*	−15·1	−0·76	−4·2	0·001
				I	73·1			
Biomass N : P	0·67	25·9	<0·001	*D*	−3·6	−0·82	−5·1	<0·001
				I	18·1			
Biomass C : N	0·40	8·7	0·011	*S*	$2{\cdot}1 \times 10^{-4}$	0·63	2·9	0·011
				I	3·9			
DNA content	0·22	3·7	0·075	*D*	0·3	0·47	1·9	0·075
				I	2·2			
RNA content	0·77	20·0	<0·001	*D*	9·9	0·85	6·1	<0·001
				S	$3{\cdot}7 \times 10^{-3}$	0·25	1·8	0·097
				I	4·4			
RNA : DNA	0·69	29·3	<0·001	*D*	3·3	0·83	5·4	<0·001
				I	3·0			
Proportion of DNA-P to total P	0·45	4·9	0·029	*D*	−1·2	−0·49	−2·3	0·042
				S	$1{\cdot}4 \times 10^{-3}$	0·43	2·0	0·066
				I	10·2			
Proportion of RNA-P to total P	0·52	6·6	0·011	*D*	22·9	0·65	3·3	0·007
				S	$1{\cdot}6 \times 10^{-2}$	0·35	1·7	0·106
				I	29·9			

R^2 is the coefficient of multiple determination; F is the variance ratio of multiple regression; t is the t-statistic. *D*, *S* and *I* denote dilution rate, supply C : P ratio and *Y* intercept of the regression lines, respectively.

variation in RNA content have also been observed in cyanobacteria (Binder & Liu 1998; Lepp & Schmidt 1998); yeast (Brown & Rose 1969; Aiking & Tempest 1976); and freshwater eukaryotic algae (Rhee 1978). Within the range of growth rates that we applied, the RNA and DNA contents in our experiments were similar to those of *E. coli* recorded by Jacobsen (1974; cited by Neidhardt *et al.* 1990) and the *E. coli* RNA : DNA ratios were almost identical to those reported by Skjold, Juarez & Hedgcoth (1973) and of freshwater and enterobacteria summarized by Dortch *et al*. (1983).

Were increased RNA amounts sufficient to affect the cellular P pool? The contributions of RNA-P to total cellular P were at least 40–50% at the lowest growth rate of 0·5 h^{-1} and 70–80% at 1·5 h^{-1} (Fig. 3). DNA-P, on the other hand, was ≈10% of total cellular P and decreased with increasing growth rate. Thus RNA was the dominant pool of biomass P, especially at high growth rate; this result is also consistent with the GRH. Although similar observations are limited, Vadstein (1998) also found that about 60% of cellular P was bound in DNA + RNA + lipid fraction at the early stationary phase in two bacterial strains isolated from a eutrophic lake. The lipids of those isolated strains could have been entirely phospholipids, as in *E. coli* (Neidhardt *et al.* 1990). However, cellular lipid content (9·1% dry weight for *E. coli*; Neidhardt *et al.* 1990) as well as phospholipid P content (≈4%, Elser *et al.* 1996) are generally less than those of RNA (see above). Furthermore, as growth rates increase, cell size also increases (Cotner, Ogdahl & Biddanda 2001), decreasing the surface-to-volume ratio and probably decreasing the percentage of cellular P that would be in phospholipids. These results indicate that RNA-P is the internal pool having the largest effect on cellular-P in bacteria, at least under balanced growth conditions. The P content of *E. coli* in our experiments (P_{ec}) can be expressed as a linear function of RNA content (RNA_{ec}):

$$P_{ec} = 4{\cdot}17 \times 10^{-2}\, RNA_{ec} + 1{\cdot}64$$
$$(r^2 = 0{\cdot}34,\ F_{1,13} = 6{\cdot}6,\ P = 0{\cdot}02).$$

Stepwise multiple regression revealed that although supply C : P was marginally associated with three out of five variables in Fig. 3, the effects of dilution rate on nucleic acid components (especially RNA content) were much stronger (Table 2). We conclude that variations in *E. coli* RNA content were regulated mainly by changes in the growth rate. To explore specifically whether RNA content could be responsible for changes in *E. coli* stoichiometry, we plotted *E. coli* biomass elemental ratios against RNA content (Fig. 4). Increased RNA content with growth rate corresponded with low biomass C : P and N : P, strongly supporting the GRH within this organism.

There are substantial data to support the relationship between bacterial nucleic acid content and growth, that indirectly support the GRH. Herbert, Phipps & Strange (1971) pointed out '... and in the case of bacteria,

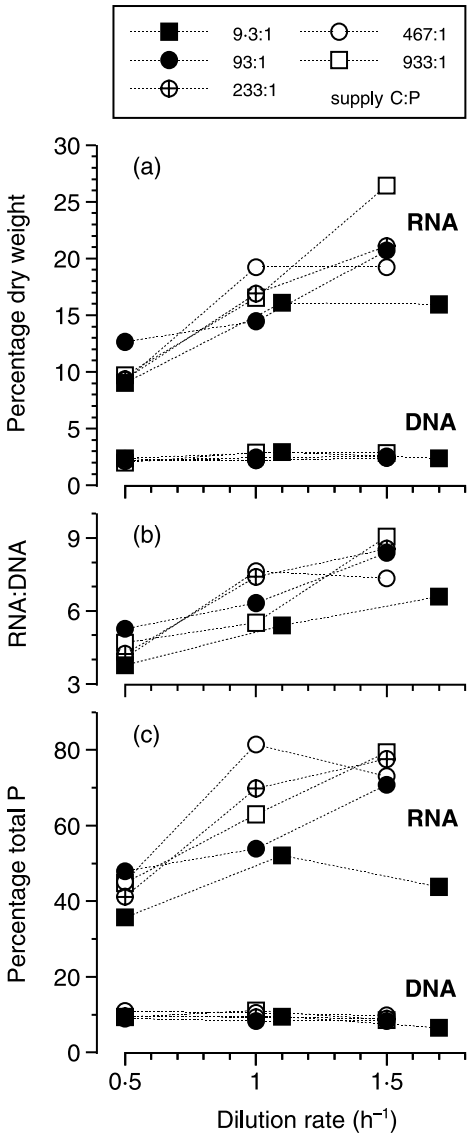

Fig. 3. As for Fig. 1, but for (a) DNA and RNA contents; (b) RNA : DNA ratio; (c) contributions of DNA-P and RNA-P to total P pools for *Escherichia coli* grown in chemostats. RNA : DNA ratios are expressed as mass ratios.

(P) may account for up to 3% of the bacterial dry weight. For example, nucleic acids contain roughly 10% phosphorus and fast-growing bacteria may contain 25% of their dry weight as RNA and DNA.' The importance of RNA for cellular C : P is also given by the literature survey of Kirchman (2000) who compared the amount of RNA, DNA and phospholipid among fast- and slow-growing bacteria. However, few studies have directly examined the importance of the increased amount of P due to growth (= RNA) in terms of biomass C : N : P stoichiometry. We examined this issue and found strong support for the GRH. It appears that the GRH is valid for intraspecific variations in biomass C : N : P stoichiometry for many microbes because the response of increased RNA content with increased growth rate is apparently ubiquitous, as discussed above.

IMPLICATIONS OF VARIABLE C : N : P STOICHIOMETRY IN NATURAL BACTERIA

Our data show that variations in *E. coli* C : P and N : P are strongly determined by growth rate. However, the observed variation, ≈20% reduction in the biomass C : P and N : P ratios caused by a 200% increase in growth rate, was small compared with the large variations observed in lake bacteria; biomass C : P ratios vary between 8 and 500 (Tezuka 1990). So we are left with the question of whether growth rate variation is sufficient to explain this large variation in bacterial biomass stoichiometry in nature.

One might suspect that our experiments did not sufficiently represent the natural variance in growth rate, and that we therefore observed low stoichiometric variability in the chemostat relative to nature. Growth rates (= dilution rates) of *E. coli* (0·5–1·7 h^{-1} in our experiments, corresponding to the specific growth rates of 8–28 day^{-1}) were high compared with those of bacteria in natural waters [up to 7·7 day^{-1}, but typically less in terms of specific growth rate calculated from Table 1 of Ducklow & Carlson (1992)]. However, our multiple regression analysis predicted that *E. coli* biomass C : P would be around 73 (SE = 4·0; Table 2) at zero growth, and Jacobsen (1974; cited by Neidhardt *et al.* 1990) demonstrated that *E. coli* RNA content was nearly constant at growth (= dilution) rates <0·5 h^{-1}, supporting the view that our range of growth rates adequately represented stoichiometric variation in *E. coli*. In Jacobsen's study, RNA content was still ≈10% of dry weight (and that of DNA increased to 5% dry weight) even at growth (= dilution) rates of 0·03–0·05 h^{-1}, corresponding to specific growth rates of 0·50–0·83 day^{-1} (within the range of *in situ* rates). Thus *E. coli* biomass C : P may never reach 500, even at very low growth rates. In addition, Chrzanowski & Kyle (1996) cultured *Pseudomonas fluorescens* at a growth (= dilution) rate of 0·03 h^{-1} and found that biomass C : P was 77–210, even though they used much higher supply C : P ratios (1200–2500) than ours (see below). From these observations we argue that the intraspecific growth-dependent variation in biomass C : N : P stoichiometry is not sufficient completely to explain the wide variation in bacterial biomass C : N : P stoichiometry.

Therefore we return to the prediction of a strong relationship between bacterial biomass C : N : P stoichiometry and supply C : N : P stoichiometry as shown by Tezuka (1990). In contrast to this prediction, *E. coli* biomass C : P and N : P ratios in the present study were quite stable, despite the growth-dependent variations, across a broad range of supply C : P and N : P ratios. We applied the model of Sterner & Elser (2002) to identify the degree of stoichiometric homeostasis (Fig. 5), and found that there was no significant correlation between log(biomass C : P or N : P) and log(supply C : P or N : P): H in equation 3 was effectively ∞ in both cases (Table 3). These findings indicate that *E. coli* was strongly homeostatic in its C : P and

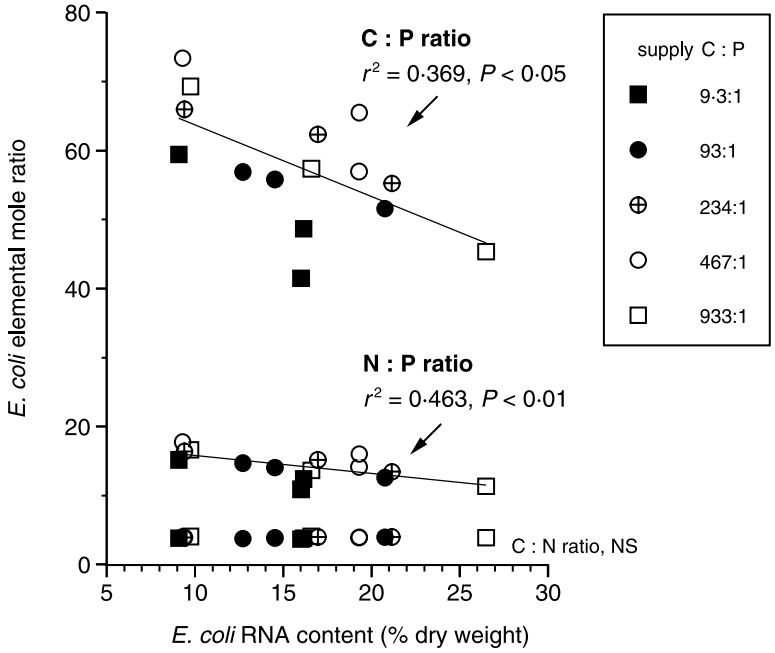

Fig. 4. Relationship between biomass C : N : P stoichiometry and relative RNA content of chemostat-grown *Escherichia coli*. Solid lines, regression lines.

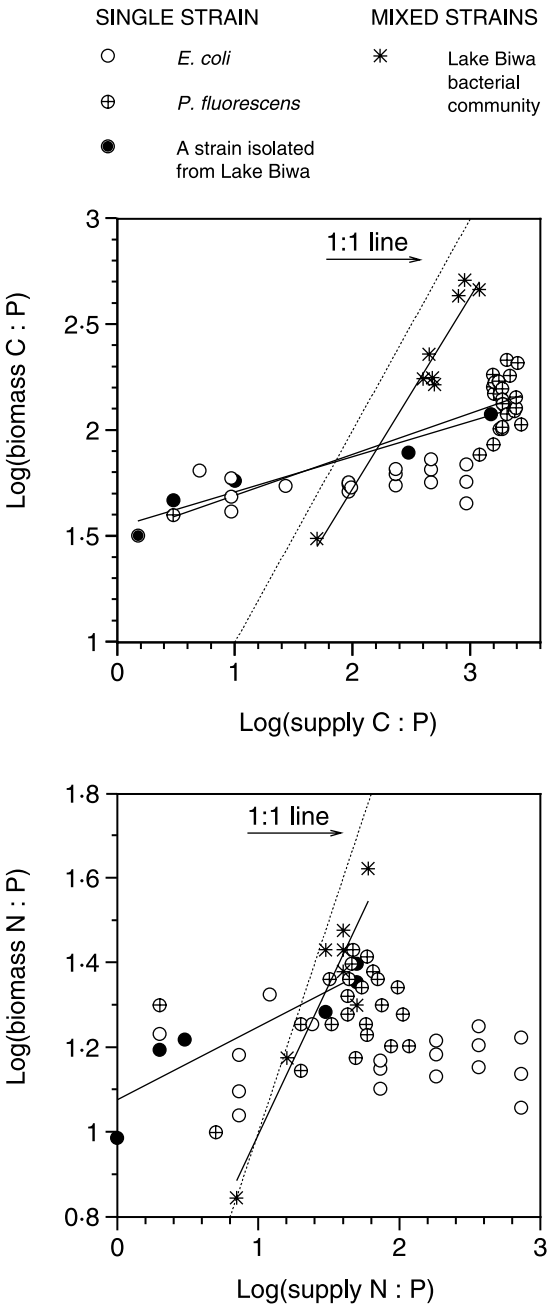

Fig. 5. Relationship between log(biomass elemental ratio) and log(supply elemental ratio) for three bacterial strains [*Escherichia coli*, present study; Nakano (1994); *Pseudomonas fluorescens*, Nakano (1994); Chrzanowski & Kyle (1996); an unidentified bacterium isolated from Lake Biwa, Nakano (1994)] and one group of mixed strains collected from Lake Biwa (Tezuka 1990). Regression lines (solid lines) are also shown if they are statistically significant.

N : P ratios. This conclusion was also supported by stepwise multiple regression, which did not indicate significant independent effects of supply C : P on biomass C : P and N : P ratios, respectively (Table 2). Thus, resource dependency is also insufficient completely to explain the wide variation in biomass C : N : P stoichiometry in natural bacteria.

An important difference in the study of Tezuka (1990) relative to ours was that he worked with lake water (<1 μm fraction). That inoculum would have contained many bacterial strains, each with different characteristic C, N and P requirements. The slopes of a regression line for his data in Fig. 5 were close to 1 (0·91 and 0·71 for C : P and N : P, respectively; Table 3), indicating that this community exhibited almost no homeostasis within a range of supply C : P similar to ours (50–1200), and even within the smaller supply N : P of 7–60. A possible explanation as to why Tezuka (1990) observed that lake bacteria C : P and N : P ratios varied directly with the supply C : P and N : P ratios is that he may have selected different strains with characteristic biomass C : P and N : P values that best matched the C : P and N : P ratios he supplied. Variations in biomass C : P (and N : P) of *in situ* bacteria could have been generated by shifts in the dominance of different strains in the environment, as has been observed for crustacean zooplankton (Gulati, Siewertsen & Liere 1991; Hessen, Andersen & Faafeng 1992).

The study of Nakano (1994) supports this suggestion. He isolated a bacterium from Lake Biwa, where the work of Tezuka (1990) was performed. That single strain showed strong homeostasis, as indicated by the modest slopes of the log–log plots in Fig. 5 (cf. Table 3) even though supply C : P was 1·5–2000 (1·0–50 for supply N : P; Nakano 1994). At least one strain of bacteria in Lake Biwa shares the lack of responsiveness of biomass C : P and N : P ratios that we observed in *E. coli*. Furthermore, we found that *P. fluorescens* studied by Nakano (1994) and Chrzanowski & Kyle (1996) was also strongly homeostatic (Fig. 5; Table 3). Together with the previously discussed observations from zooplankton, these results suggest that two of the main groups of heterotrophs in lakes, zooplankton

Table 3. Summary of the regression analyses for data shown in Fig. 5 of log(biomass elemental ratio) *vs* log(supply elemental ratio)

Strains in Fig. 5	Category	Regression analysis				
		R^2	F	P	Slope	H*
E. coli (N = 18) [1]	C : P	0·106	1·9	0·187	0·027	∞
	N : P	0·007	0·1	0·745	−0·007	∞
P. fluorescens (N = 23) [2]	C : P	0·500	21·0	<0·001	0·194	5·17
	N : P	0·118	2·8	0·109	0·084	∞
Strain from Lake Biwa (N = 6) [3]	C : P	0·951	78·1	<0·001	0·167	5·99
	N : P	0·819	18·1	0·013	0·172	5·80
Mixed strains from Lake Biwa (N = 8) [4]	C : P	0·947	107·0	<0·001	0·906	1·10
	N : P	0·848	33·6	0·001	0·708	1·41

*H values in equation 3 (= 1/slope), treated as infinity in insignificant relationships (P > 0·05).
Culture conditions for each data set: [1] continuous culture (dilution rate, 0·5–1·5 h^{-1}, present study) and batch culture (early stationary phase, Nakano 1994); [2] continuous culture (dilution rate, 0·03–0·09 h^{-1}, Chrzanowski & Kyle 1996) and batch culture (early stationary phase, Nakano 1994) [3] batch culture (early stationary phase, Nakano 1994), and [4] batch culture (stationary phase, Tezuka 1990).

and bacteria, and not just phagotrophic heterotrophs (metazoans such as crustacean zooplankton), are likely to be more strongly homeostatic than autotrophs. Therefore heterotrophic bacteria are more like animals in terms of C : N : P homeostasis.

FUTURE DIRECTIONS

Finally, we propose one possible explanation of how the biomass C : N : P of *in situ* bacteria varies through shifts in the dominance of different strains. Generally, *in situ* bacteria grow slowly (Ducklow & Carlson 1992) and may have RNA : DNA ratios as low as 0·3–1 (by mass), as observed by Lee & Kemp (1994) for marine bacteria. We might speculate that such bacteria have biomass C : P as high as 500, simply because of their low RNA content. However, DNA content in terms of percentage dry weight increases at low growth rate. Kirchman (2000) points out that the DNA content of slow-growing bacteria in the ocean can reach up to 10% dry weight (also see Simon & Azam 1989; Posch *et al*. 2001). We calculated the biomass C : P of such bacteria, assuming that C is 50% dry weight, nucleic acid is 9% P, and RNA : DNA is 0·3 or 1 (Fig. 6). In contrast to speculation, the calculated biomass C : P is 110, nearly the Redfield ratio of 106 (Redfield 1958), and 72 when RNA : DNA is 0·3 and 1, respectively, and when DNA is 10% dry weight. Our calculation also suggests that bacteria would have a biomass C : P as high as 500 if they contained little DNA relative to their biomass (less than 2% dry weight). This might be possible, mechanistically at least, because the DNA content of bacteria in natural waters is variable. For example, cellular DNA content varied from 1% up to 25%, with the mode around 10% dry weight, in an Austrian lake bacterial community (Figure 2 of Posch *et al*. 2001; also see Button & Robertson 2001).

There are many bacterial strains in natural waters that grow slowly (low RNA : DNA ratio), and those with low DNA content (e.g. a few per cent) may have higher biomass C : P than their conspecifics. Biomass C : P at a community level may increase if bacteria with such low DNA contents dominate, and this may be caused by increases in supply C : P, as observed in the experiment of Tezuka (1990). This high biomass C : P would not be observed in *E. coli* as its DNA content would still be ≈5% at low growth rate (Jacobsen 1974; cited by Neidhardt *et al*. 1990). Rather, the growth of bacteria with intrinsically low DNA content close to that of eukaryotes such as yeasts (0·1–0·6%

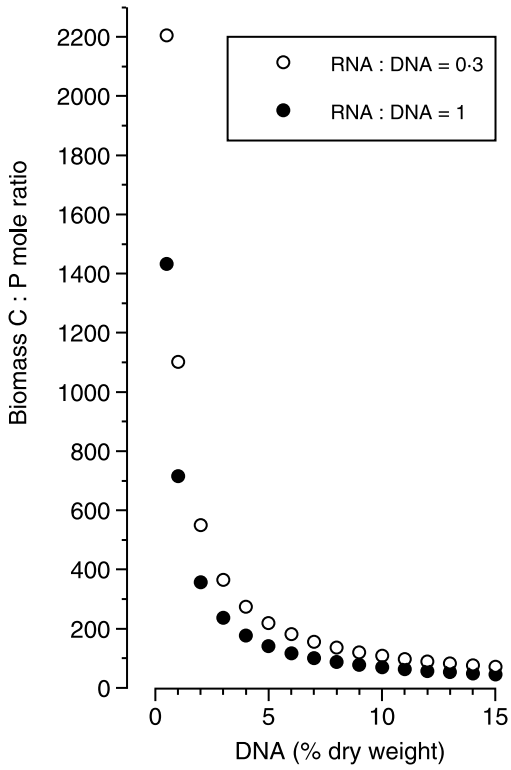

Fig. 6. Theoretical biomass C : P of slow-growing bacteria as estimated from their nucleic acid content. Bacterial RNA : DNA ratio is assumed to be either 0·3 (○) or 1 (●); and C : dry weight is set at 50%.

dry weight; Brown & Rose 1969; Fiechter *et al*. 1987) and phytoplankton (0·4–0·8% dry weight; Holm-Hansen, Sutcliffe & Sharp 1968; Dortch *et al*. 1983) may be responsible for high biomass C : P at a community level.

Addressing these issues will require more comprehensive studies of natural microbial organisms, to determine whether they behave homeostatically in response to changes in resource stoichiometry. Our work suggests that this should be the case, but these studies need to be performed on more strains isolated from natural waters and soils. If they are not homeostatic, and instead bacterial community C : N : P ratios routinely track supply C : N : P ratios, this would suggest little effect of heterotrophic bacteria on the relative availability of nutrients at any external supply ratio–nutrients would be regenerated in about the same ratio as they are supplied (Elser & Urabe 1999). However, the existence of certain supply ranges where bacterial communities behave homeostatically could have quantitative effects on the relative availability of different nutrients, as has been shown for crustacean zooplankton (Elser & Urabe 1999; Sterner & Elser 2002).

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References

Aiking, H. & Tempest, D.W. (1976) Growth and physiology of *Candida utilis* NCYC 321 in potassium-limited chemostat culture. *Archives of Microbiology* **108**, 117–124.

APHA (1992) *Standard Methods for the Examination of Water and Wastewater*, 18th edn. American Public Health Association, Washington, DC.

Binder, B.J. & Liu, Y.C. (1998) Growth rate regulation of rRNA content of a marine *Synechococcus* (cyanobacterium) strain. *Applied and Environmental Microbiology* **64**, 3346–3351.

Brown, C.M. & Rose, A.H. (1969) Effects of temperature on composition and cell volume of *Candida utilis*. *Journal of Bacteriology* **97**, 261–272.

Button, D.K. & Robertson, B.R. (2001) Determination of DNA content of aquatic bacteria by flow cytometry. *Applied and Environmental Microbiology* **67**, 1636–1645.

Chrzanowski, T.H. & Kyle, M. (1996) Ratios of carbon, nitrogen and phosphorus in *Pseudomonas fluorescens* as a model for bacterial element ratios and nutrient regeneration. *Aquatic Microbial Ecology* **10**, 115–122.

Cotner, J.B. & Biddanda, B.A. (2002) Small players, large role: microbial influence on biogeographical processes in pelagic aquatic ecosystems. *Ecosystems* **5**, 105–121.

Cotner, J.B., Ogdahl, M.L. & Biddanda, B.A. (2001) DsDNA measurement in lakes with the fluorescent stain, PicoGreen, and application to bacterial bioassays. *Aquatic Microbial Ecology* **25**, 65–74.

Dortch, Q., Roberts, T.L., Clayton, J.J.R. & Ahmed, S.I. (1983) RNA/DNA ratios and DNA concentrations as indicators of growth rate and biomass in planktonic marine organisms. *Marine Ecology Progress Series* **13**, 61–71.

Ducklow, H.W. & Carlson, C.A. (1992) Oceanic bacterial production. *Advances in Microbial Ecology* **12**, 113–181.

Elser, J.J. & Urabe, J. (1999) The stoichiometry of consumer-driven nutrient recycling: theory, observations, and consequences. *Ecology* **80**, 735–751.

Elser, J.J., Dobberfuhl, D.R., MacKay, N.A. & Schampel, J.H. (1996) Organism size, life history, and N : P stoichiometry. *Bioscience* **46**, 674–684.

Elser, J.J., Sterner, R.W., Gorokhova, E. *et al.* (2000a) Biological stoichiometry from genes to ecosystems. *Ecology Letters* **3**, 540–550.

Elser, J.J., Fagan, W.F., Denno, R.F. *et al.* (2000b) Nutritional constraints in terrestrial and freshwater food webs. *Nature* **408**, 578–580.

Fiechter, A., Käppeli, O. & Meussdoerffer, F. (1987) Batch and continuous culture. *The Yeasts*, *Vol. 2*, 2nd edn (eds A.H. Rose & J.S. Harrison), pp. 99–129. Academic Press, London.

Gächter, R. & Bloesch, J. (1985) Seasonal and vertical variation in the C : P ratio of suspended and settling seston of lakes. *Hydrobiologia* **128**, 193–200.

Goldman, J.C., McCarthy, J.J. & Peavey, D.G. (1979) Growth rate influence on the chemical composition of phytoplankton in oceanic waters. *Nature* **279**, 210–215.

Gorokhova, E. & Kyle, M. (2002) Analysis of nucleic acids in *Daphnia*: development of methods and ontogenetic variations in RNA–DNA content. *Journal of Plankton Research* **24**, 511–522.

Gulati, R.D., Siewertsen, K. & Liere, L.V. (1991) Carbon and phosphorus relationships of zooplankton and its seston food in Loosdrecht lakes. *Memorie Dell'istituto Italiano di Idrobiologia* **48**, 279–298.

Hanegraaf, P.P.F. & Muller, E.B. (2001) The dynamics of the macromolecular composition of biomass. *Journal of Theoretical Biology* **212**, 237–251.

Herbert, D., Phipps, P.J. & Strange, R.E. (1971) Chemical analysis of microbial cells. *Methods in Microbiology* **5B**, 209–344.

Hessen, D.O. (1990) Carbon, nitrogen and phosphorus status in *Daphnia* at varying food conditions. *Journal of Plankton Research* **12**, 1239–1249.

Hessen, D.O. & Andersen, T. (1990) Bacteria as a source of phosphorus for zooplankton. *Hydrobiologia* **206**, 217–223.

Hessen, D.O., Andersen, T. & Faafeng, B. (1992) Zooplankton contribution to particulate phosphorus and nitrogen in lakes. *Journal of Plankton Research* **14**, 937–947.

Holm-Hansen, O., Sutcliffe, W.H. Jr & Sharp, J. (1968) Measurement of deoxyribonucleic acid in the ocean and its ecological significance. *Limnology and Oceanography* **13**, 507–514.

Jeffrey, W.H., Von Haven, R., Hoch, M.P. & Coffin, R.B. (1996) Bacterioplankton RNA, DNA, protein content and relationships to rates of thymidine and leucine incorporation. *Aquatic Microbial Ecology* **10**, 87–95.

Jones, L.J., Yue, S.T., Cheung, C.-Y. & Singer, V.L. (1998) RNA quantitation by fluorescence-based solution assay: RiboGreen reagent characterization. *Analytical Biochemistry* **265**, 368–374.

Kemp, P.F., Lee, S. & LaRoche, J. (1993) Estimating the growth rate of slowly growing marine bacteria from RNA content. *Applied and Environmental Microbiology* **59**, 2594–2601.

Kirchman, D.L. (2000) Uptake and regeneration of inorganic nutrients by marine heterotrophic bacteria. *Microbial Ecology of the Oceans* (ed. K.L. Kirchman), pp. 261–288. Wiley–Liss, New York.

Lee, S. & Kemp, P.F. (1994) Single-cell RNA content of natural marine planktonic bacteria measured by hybridization with multiple 16S rRNA-targeted fluorescent probes. *Limnology and Oceanography* **39**, 869–879.

Lehninger, A.L., Nelson, D.L. & Cox, M.M. (1993) *Principles of Biochemistry*. Worth, New York.

Lepp, P.W. & Schmidt, T.M. (1998) Nucleic acid content of *Synechococcus* spp. during growth in continuous light and light/dark cycles. *Archives of Microbiology* **170**, 201–207.

Maaloe, O. & Kjeldgaard, N.O. (1966) *Control of Macromolecular Synthesis: A Study of DNA, RNA and Protein Synthesis in Bacteria*. W.A. Benjamin, New York.

Main, T.M., Dobberfuhl, D.R. & Elser, J.J. (1997) N : P stoichiometry and ontogeny of crustacean zooplankton: a test of the growth rate hypothesis. *Limnology and Oceanography* **42**, 1474–1478.

Nakano, S. (1994) Carbon : nitrogen : phosphorus ratios and nutrient regeneration of a heterotrophic flagellate fed on bacteria with different elemental ratios. *Archiv für Hydrobiologie* **129**, 257–271.

Neidhardt, F.C., Bloch, P.L. & Smith, D.F. (1974) Culture medium for Enterobacteria. *Journal of Bacteriology* **119**, 736–747.

Neidhardt, F.C., Ingraham, J.L. & Schaechter, M. (1990) *Physiology of the Bacterial Cell: A Molecular Approach*. Sinauer Associates, Sunderland. MA.

Posch, T., Loferer-Krößbacher, M., Gao, G., Alfreider, A., Pernthaler, J. & Psenner, R. (2001) Precision of bacterioplankton biomass determination: a comparison of two fluorescent dyes, and of allometric and linear volume-to-carbon conversion factors. *Aquatic Microbial Ecology* **25**, 55–63.

Redfield, A.C. (1958) The biological control of chemical factors in the environment. *American Scientists* **46**, 205–221.

Rhee, G.-Y. (1978) Effects of N : P atomic ratios and nitrate limitation on algal growth, cell composition, and nitrate uptake. *Limnology and Oceanography* **23**, 10–25.

Simon, M. & Azam, F. (1989) Protein content and protein synthesis rates of planktonic marine bacteria. *Marine Ecology Progress Series* **51**, 201–213.

Skjold, A.C., Juarez, H. & Hedgcoth, C. (1973) Relationships among deoxyribonucleic acid, ribonucleic acid, and specific transfer ribonucleic acids in *Escherichia coli* 15T- at various growth rates. *Journal of Bacteriology* **115**, 177–187.

Sterner, R.W. (1995) Elemental stoichiometry of species in ecosystems. *Linking Species and Ecosystems* (eds C.G. Jones & J.H. Lowton), pp. 240–252. Chapman & Hall, New York.

Sterner, R.W. & Elser, J.J. (2002) *Ecological Stoichiometry. The Biology of Elements from Molecules to the Biosphere*. Princeton University Press, Princeton, NJ.

Sterner, R.W., Hagemeier, D.D., Smith, W.L. & Smith, R.F. (1993) Phytoplankton nutrient limitation and food quality for *Daphnia*. *Limnology and Oceanography* **38**, 857–871.

Sterner, R.W., Clasen, J., Lampert, W. & Weisse, T. (1998) Carbon: phosphorus stoichiometry and food chain production. *Ecology Letters* **1**, 146–150.

Tezuka, Y. (1990) Bacterial regeneration of ammonium and phosphate as affected by the carbon : nitrogen : phosphorus ratio of organic substrates. *Microbial Ecology* **19**, 227–238.

Vadstein, O. (1998) Evaluation of competitive ability of two heterotrophic planktonic bacteria under phosphorus limitation. *Aquatic Microbial Ecology* **14**, 119–127.

Villar-Argaiz, M., Medina-Sánchez, J.M. & Carrillo, P. (2002) Linking life history strategies and ontogeny in crustacean zooplankton: implications for homeostasis. *Ecology* **83**, 1899–1914.

Wanner, B.L., Kodaira, R. & Neidhardt, F.C. (1977) Physiological regulation of a decontrolled *lac* operon. *Journal of Bacteriology* **130**, 212–222.

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