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Element content of *Pseudomonas fluorescens* varies with growth rate and temperature: A replicated chemostat study addressing ecological stoichiometry

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Abstract

Ecological stoichiometry is emerging as a central organizing framework upon which our perceptions of aquatic trophic dynamics are being reshaped. The microbial component of aquatic systems is crucial to overall nutrient dynamics, yet little data are available addressing the ecological stoichiometry of microorganisms. *Pseudomonas fluorescens*, a commonly encountered bacterium, was used as a model organism to investigate the relationships among temperature, growth rate, and element stoichiometry. *P. fluorescens* was grown in chemostats at low dilution rates (ranging between 0.03 and 0.13 h^{-1}) and realistic environmental temperatures (ranging between 14°C and 28°C). Cells accumulated elements as an interactive function of temperature and growth rate. The highest element concentrations corresponded to cells growing slowly under low temperatures and to cells growing rapidly under warm conditions. Additionally, small cells had higher concentrations of elements than did large cells. Element ratios (C : N, C : P, and N : P) varied more as a function of growth rate than of temperature. The same dissolved resource pool could conceivably yield bacteria of differing element content simply as an interactive function of growth rate and temperature.

Only within the past decade or so has a considerable body of information on competition theory, growth dynamics, and matter and energy flow been integrated into a formal theory of ecosystem function that advances the view of aquatic trophic dynamics set in place by Lindeman (1942) and Odum (1956, 1957). This construct, known as ecological stoichiometry (Sterner and Elser 2002), is emerging as a central organizing framework upon which our perceptions of aquatic trophic dynamics are being reshaped (Grover 1997; Sterner et al. 1997).

One of the facets of ecological stoichiometry deals with the movement of multiple nutrients from the dissolved state into autotrophs and the subsequent incorporation and regeneration of these nutrients by metazoan consumers. When coupled with growth dynamics, a mass balance approach focusing on the comparison of nutrient ratios in the dissolved pool to the ratio of the same elements in major consumers allows for predictions of element regeneration rates and eventual shifts in trophic structure. Less characterized, but perhaps equally important to the development of ecological stoichiometry, is the flow of dissolved nutrients to bacteria and, subsequently, to their consumers. Apart from the autotrophs, the heterotrophic bacteria represent the only other group of organisms capable of moving significant amounts of dissolved nutrients into particulate matter. Clearly, the influence of microbial processes on biogeochemical processes cannot be underestimated (Cotner and Biddanda 2002).

It is not surprising, given the importance of bacterial metabolism to ecosystem function, that a few have begun the process of integrating microbial (bacterial) physiology into the framework of ecological stoichiometry using both experimental (Chrzanowski et al. 1996; Makino et al. 2003; Cotner et al. 2006) and theoretical approaches (Grover 2003, 2004). Much of this work focuses upon the cellular C : P ratio but fails to recognize that several aspects of bacterial growth physiology affect this ratio and must be considered simultaneously to explain the variation in cellular element content. What is known of these aspects of bacterial physiology stems largely from cultures grown in rich laboratory media under conditions rarely, if ever, encountered in natural systems. Even using culture studies, there are very few data on the element content of cells and even less on the relationship between element content and growth rate. Perhaps the most extensive data characterizing the element content of cells is that of Porter (1946, summarized in Reiners 1986), whereas the relationship between element content and growth rate was derived by Schaechter et al. (1958) and reviewed by Bremer and Dennis (1987). Previously, Makino et al. (2003) confirmed some of these data. Briefly, an increase in growth rate requires an increase in ribosome content (the growth rate hypothesis, Elser et al. 2000). Consequently, changes in growth rate should be reflected in the cellular C : P or N : P ratio since ribosomes are rich in P compared with C and N: the P content of bacteria and other organisms should rise with growth rate.

Temperature also has profound effects on cellular function and this is particularly true for osmotrophs. Temperature affects diffusion of nutrients, cytoplasm viscosity, enzymatic reactions, and cell size (Koch 1996). RNA content of cells also appears to be affected by temperature: increasing as temperature decreases. Thus, changes in element stoichiometry of cells as a function of temperature are difficult to predict a priori.

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Table 1. Concentration of *Pseudomonas fluorescens* (10^8 cells mL^{-1}) growing in chemostats at different temperatures and growth rates (mean±SE, $n = 3$ chemostats).

Growth rate (h^{-1})	Temperature (°C)		
	14	20	28
0.03	1.97±0.31	2.56±0.23	4.14±0.59
0.06	2.86±0.30	3.49±0.19	3.93±0.27
0.10	3.43±0.11	3.17±0.24	4.09±0.21
0.13	3.22±0.26	2.68±0.06	2.29±0.14

The difficulty faced when applying stoichiometric concepts to bacteria lies with knowing if the basic physiology characterizing growth in culture, particularly batch culture, can be applied to cells growing under ecologically relevant conditions where growth under single- or multiple-nutrient limitation is common, where temperatures vary over wide ranges, and where *rapid* growth may be considered a doubling per day. In this work we investigated the element stoichiometry of a bacterium with cosmopolitan distribution under realistic environmental temperatures and growth rates. We used a replicated chemostat approach manipulating both temperature and growth rate to characterize the element content of cells and their macromolecular composition. We used a low-P medium because there is extensive evidence for P limitation of bacterial growth in aquatic habitats (Morris and Lewis 1992; Chrzanowski and Grover 2001).

Materials and methods

Pseudomonas fluorescens (ATCC 3214) stock cultures were maintained on standard mineral base (SMB) (White and Hegeman 1998) supplemented with 10 mmol L^{-1} glucose. For experimental purposes, *P. fluorescens* was grown in SMB in continually stirred and aerated 800-mL chemostats (Applikon) at four dilution rates ($D = 0.03$, 0.06, 0.10, 0.13 h^{-1}) and three temperatures (14°C, 20°C, 28°C). Triplicate chemostats were prepared for each dilution rate–temperature combination. Medium delivered to chemostat reactors was prepared to yield element concentrations (C, N, and P) of 600 : 30 : 1 (molar) where P (0.1 mmol L^{-1}) was the growth-limiting nutrient. Reactors were assumed to be in steady state after three complete turnovers at a given temperature and dilution rate. Outflow was aseptically captured into presterilized 1-liter bottles (Nalgene). When growing in SMB, optimal growth of this

Table 2. Volume of *Pseudomonas fluorescens* (μm^3 $cell^{-1}$) when growing at different temperatures and growth rates in chemostats (mean±SE, $n = 3$ chemostats).

Growth rate (h^{-1})	Temperature (°C)		
	14	20	28
0.03	0.1179±0.018	0.1455±0.018	0.1195±0.038
0.06	0.1437±0.011	0.0980±0.015	0.1495±0.006
0.10	0.1945±0.009	0.1667±0.009	0.1597±0.016
0.13	0.2119±0.010	0.1599±0.009	0.1682±0.016

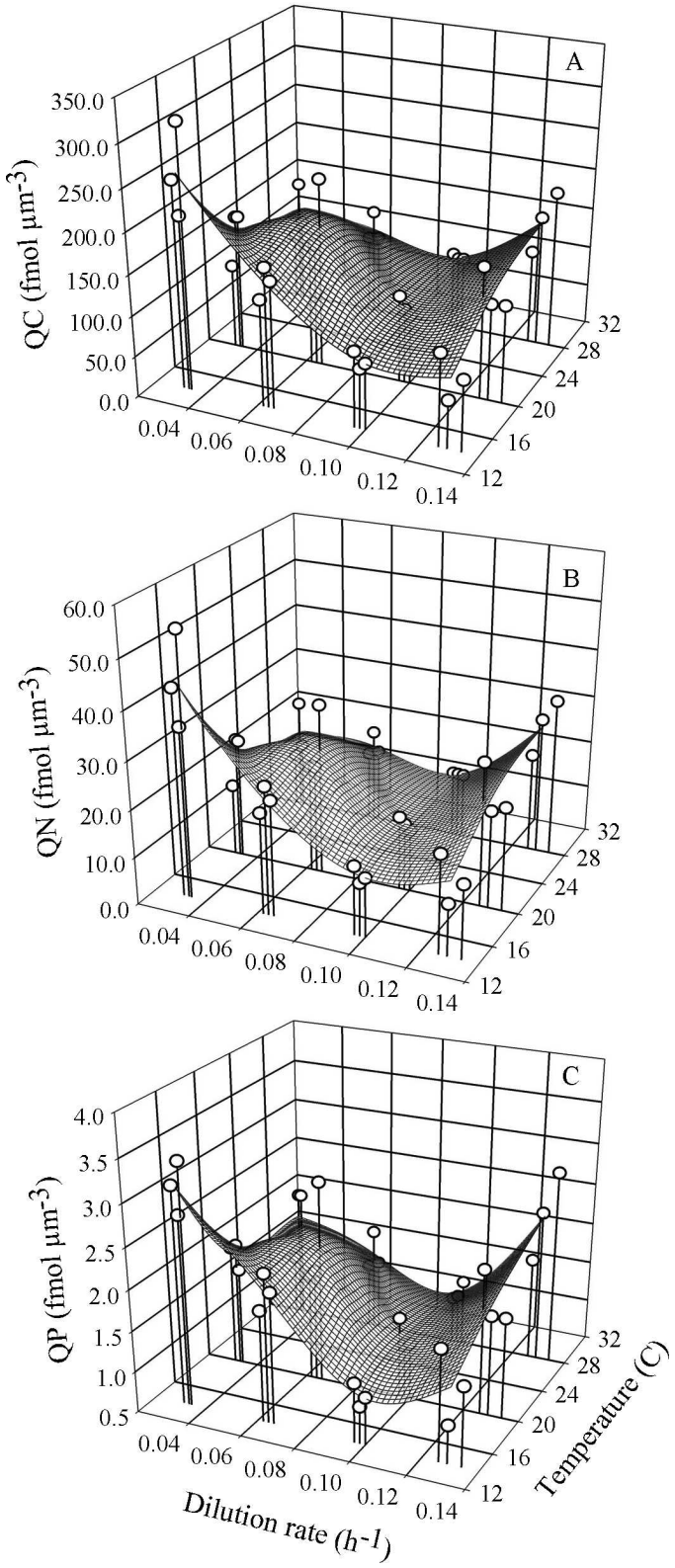

Fig. 1. (A) Cell quota of carbon (QC), (B) nitrogen (QN), and (C) phosphorus (QP) for *Pseudomonas fluorescens* grown in chemostats at different growth rates and temperatures. Data from individual chemostats are shown as a solid line and open circle. The contour mesh fits the mean for the replicate chemostats along the growth rate–temperature matrix. Mean values and standard errors are given in Table 3.

Table 3. Carbon, nitrogen, and phosphorus content of *Pseudomonas fluorescens* grown at different temperatures and growth rates in chemostats (mean±SE, $n = 3$ chemostats).

Growth rate (h^{-1})	Temperature (°C)	Element (fmol μm^{-3})		
		C	N	P
0.03	14	260.9±31.7	44.5±5.5	3.11±0.18
	20	145.6±21.1	21.8±3.4	1.88±0.10
	28	111.4±20.6	16.0±3.7	1.78±0.28
0.06	14	152.3±11.5	24.16±1.6	2.1±0.13
	20	176.1±27.2	26.9±4.4	2.2±0.37
	28	109.1±11.21	16.7±1.5	1.5±0.14
0.10	14	82.6±5.6	12.7±0.9	1.1±0.08
	20	95.2±6.2	14.3±0.7	1.3±0.10
	28	92.7±1.5	13.8±0.4	1.2±0.07
0.13	14	86.5±15.6	15.8±2.8	1.4±0.25
	20	133.7±14.1	24.2±3.0	1.9±0.18
	28	157.4±22.6	27.6±3.6	2.3±0.32

strain occurred between 28°C and 30°C at a maximal growth rate (μ_{max}) of 0.49 h^{-1}. Chemostat dilution rates (hereafter growth rate) ranged from 6% to 27% of μ_{max} at 28°C, from 13% to 56% of μ_{max} at 20°C, and from 21% to 92% of μ_{max} at 14°C.

Bacterial abundance was determined by direct epifluorescent microscopic enumeration (×1,250) of formaldehyde-preserved (2% final concentration) samples using 4,6-diamidino-2-phenylindole as the fluorochrome (Porter and Feig 1980). Cell volume (V) of bacteria was determined from length and width of at least 100 cells according to the formula for a cylinder capped by two hemispheres:

$$V = \left(\pi\left[\left(0.5W^2\right)(L-W)\right] + \left[(4/3)\pi\right]\left[0.5W^3\right]\right) \quad (1)$$

where W is the maximum cell width and L is the maximum cell length in micrometers. Length and width of individual cells were determined from digital images (Olympus DP70 camera) and imaging software (Simple PCI, Compix.). The element content was determined from cells collected on precombusted glass-fiber filters (Whatman GF/F). The C and N content of cells was determined using a CHN analyzer (Perkin-Elmer series 2200 CHN Analyzer). The P content of cells was determined from persulfate digests and subsequent analyses of soluble reactive P (Strickland and Parsons 1972). Ratios of elements are reported as mol : mol. Bulk DNA and RNA were extracted according to the methods of Gorokhova and Kyle (2002) with minor modifications and were quantified using the fluorochrome RiboGreen© (Invitrogen) in combination with a Fugi FLA-3000G imager.

Graphical and statistical analyses were performed using Sigma Plot (v10) and SigmaStat (v3.5).

Results

Chemostat conditions—Growth medium fed to chemostats was prepared as 10-liter batches 18 times. There was little variability in each of the critical element nutrients among the various batches of medium: C (from glucose), N (from NH_4Cl), and P (from KH_2PO_4) averaged (± SE) 63.95 ± 3.18, 2.48 ± 0.06, and 0.11 ± 0.002 mmol L^{-1}, respectively. The average C : N : P ratio of the media was 592 : 23 : 1 and was very close to the target ratio of 600 : 30 : 1. Chemostats were operated at ecologically relevant growth rates and temperatures. Generation times ranged between 5.3 and 23.1 h (growth rates of 0.13 and 0.03 h^{-1}) at each of three temperatures (14°C, 20°C, and 28°C).

Abundance and cell volumes—The concentration of bacteria within reactors only varied about twofold ($1.97 \pm 0.31 \times 10^8$ to $4.14 \pm 0.31 \times 10^8$ cells mL^{-1} [mean ± SE]) across the range of growth conditions (Table 1). Cell abundance was influenced by temperature (analysis of variance [ANOVA], $p < 0.01$), growth rate ($p < 0.01$), and the interaction of temperature and growth rate ($p < 0.01$). Cells tended to be more abundant when reactors were held at warm temperatures than when held at cooler temperatures.

Cell size was strongly regulated by growth rate (Table 2, ANOVA, $p < 0.001$), with the largest cells associated with the highest growth rates. Cell size (as volume) was not influenced by temperature (Table 2, ANOVA $p > 0.12$, *see* Discussion) or by the interaction between temperature and growth rate (Table 2, ANOVA, $p > 0.15$).

Cell quotas of C, N, and P—Figure 1 presents the element content of cells normalized to cell volume for each replicate chemostat. The mean concentration of each element (cell quota: QC, carbon; QN, nitrogen, and QP, phosphorus) for each group of replicate chemostats is presented as a contoured mesh fitted to the data. When presented in three-dimensional space, the distribution of each element appears as a saddle, with highest concentration of each element found at the extremes of the growth rate–temperature matrix. The highest element concentrations were found to correspond to cells growing slowly under low temperatures and to cells growing rapidly under warm conditions. These data are further summarized in Table 3. The highest element content was found for cells grown at 0.03 h^{-1} and 14°C where QC, QN, and QP averaged 260.9 ± 31.7, 44.5 ± 5.5, and 3.11 ± 0.18 fmol μm^{-3}, respectively. The lowest element content

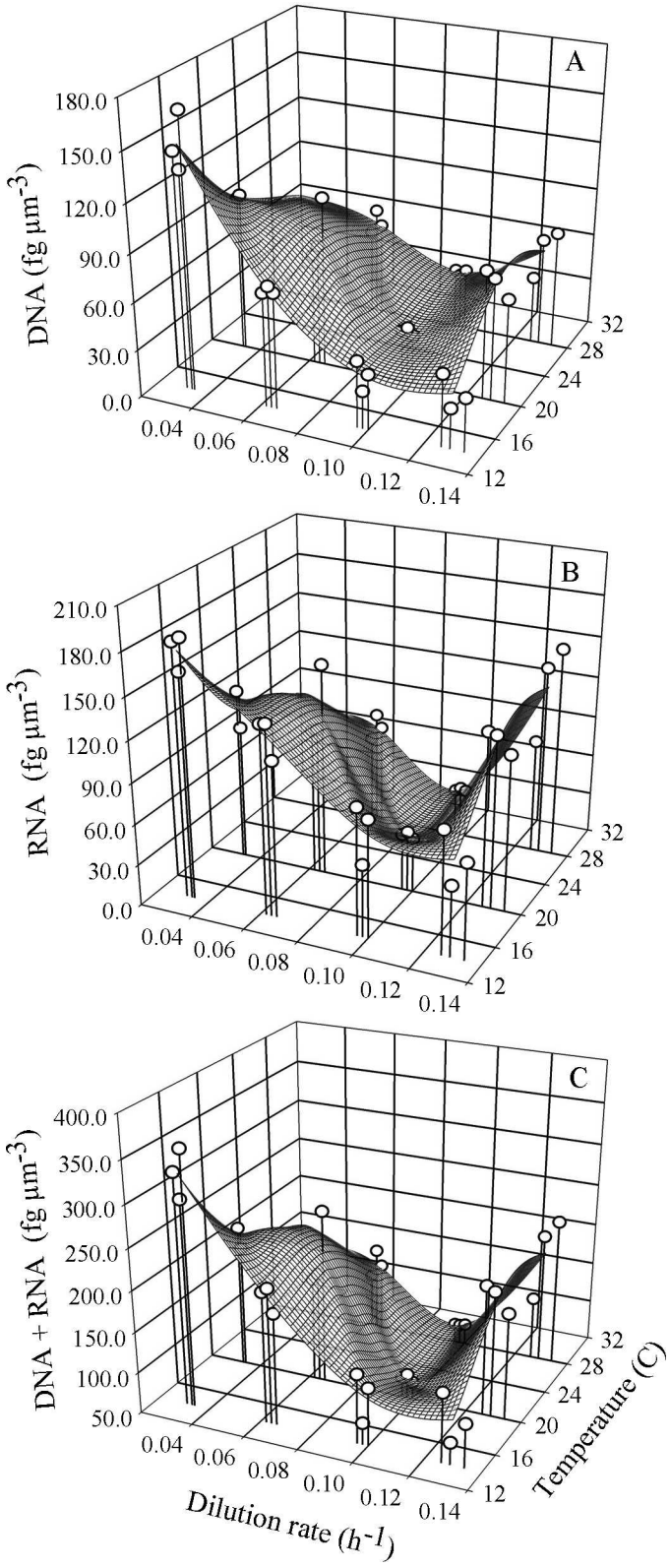

Fig. 2. (A) Cell quota of DNA, (B) RNA, and (C) total nucleic acids for *Pseudomonas fluorescens* grown in chemostats at different growth rates and temperatures. Data from individual chemostats are shown as a solid line and open circle. The contour mesh fits the mean for the replicate chemostats along the growth rate–temperature matrix. Mean values and standard errors are given in Table 4.

was found for cells grown at the two highest growth rates (0.10 and 0.13 h^{-1}) but at the lowest temperature (14°C, Table 3).

Examination of the data presented in Fig. 1 suggests that *P. fluorescens* accumulates elements as an interactive function of growth rate and temperature and this is supported by statistical analyses (ANOVA; temperature × growth rate, $p < 0.001$ for each element). Consider Fig. 1A, B, and C: follow the increasing concentration of elements along the temperature gradient for cells grown at 0.14 h^{-1} as well as the decreasing concentration of elements along the growth-rate gradient for cells held at 14°C. However, statistical analyses generally failed to identify temperature alone as a significant factor driving the element content of cells (ANOVA: QC, $p > 0.09$; QN, $p < 0.05$; QP, $p > 0.25$). Growth rate, however, explained a significant amount of the variation in cell element content for each element (ANOVA, $p < 0.001$).

Cell quotas of nucleic acids—The nucleic acid concentrations of cells growing within each replicate chemostat and the contours of mean nucleic acid concentration are presented in Fig. 2. When portrayed in three-dimensional space the distribution of nucleic acids assumed a saddle shape (Fig. 2C), with the greatest concentration of total nucleic acids (DNA + RNA) found when cells were grown slowly at low temperature or rapidly at warm temperature. Visual evaluation of the contour plots suggests that the nucleic acid content of cells varied as a function of growth rate and temperature similar to the variation observed for cell quotas of the various elements. Missing data (0.03 h^{-1} at 28°C, Table 4) precludes a statistical assessment of the interaction of growth rate and temperature as combined factors regulating the variability of nucleic acids. However, temperature alone was not sufficient to explain the variability in nucleic acid concentration (ANOVA, $p > 0.35$). Growth rate, however, did explain a significant proportion of the variation in nucleic acid concentration (ANOVA, $p < 0.001$, for DNA and RNA).

The distribution of nucleic acids appears slightly different when DNA and RNA are considered separately. The highest concentration of DNA (150.9 fg μm^{-3}) occurred when cells were grown slowly at low temperatures (Table 4). Under these conditions the DNA concentration was more than double the DNA concentration of cells grown rapidly at warm temperatures (61.8 fg μm^{-3}, Table 4). The distribution of RNA concentration across the growth–temperature matrix was similar to that of DNA except at high growth rates. At high growth rates, RNA concentration increased to a greater extent than did DNA. This resulted in a shift in the relative contribution of RNA to the total nucleic acid pool. At low dilution rates, RNA was approximately 50% of the total nucleic acid pool, but at high growth rate, RNA was approximately 65% of the total nucleic acid pool.

Element ratios—Element ratios (C : N, C : P, and N : P) for each dilution rate–temperature combination for each replicate chemostat are plotted in Fig. 3. Means are plotted as contours in Fig. 3 and fully characterized in Table 5.

Table 4. Nucleic acid content of *Pseudomonas fluorescens* grown at different temperatures and growth rates in chemostats (mean±SE, $n = 3$ chemostats).

Growth rate (h^{-1})	Temperature (°C)	Nucleic acid (fg μm^{-3})		
		DNA	RNA	Total
0.03	14	150.9±10.2	177.1±7.2	327.9±16.5
	20*	99.7–97.5	98.2–124.7	210.1
	28			
0.06	14	73.6±1.4	129.6±8.2	203.2±8.7
	20	84.5±11.4	122.5±15.8	218.5±27.1
	28	60.5±4.4	73.6±5.5	134.1±9.9
0.10	14	33.6±5.3	77.1±12.3	110.7±17.5
	20	33.2±1.2	42.2±1.2	75.7±2.5
	28	37.8±1.0	36.3±0.7	74.1±0.6
0.13	14	34.9±5.8	70.5±10.9	105.4±31.1
	20	74.5±4.5	122.5±4.2	191.4±8.7
	28	61.8±9.7	125.9±21.4	187.7±16.7

* Data for two chemostats: Values are for each chemostat. Total is the sum of the means.

The C : N ratio varied from 5.5 to 7.0 (Table 5, Fig. 3A). Even though the numerical change in the ratio was slight, the variation was significantly influenced by the interaction of temperature and growth rate (ANOVA, $p < 0.01$). The highest C : N ratio was found in cells growing slowly at high temperature (C : N ~ 7) and the lowest in cells growing rapidly at low temperatures (C : N ~ 5.5). Within a given growth rate, C : N tended to decrease with temperature.

C : P ratios were variable and not influenced by temperature (ANOVA, $p > 0.45$). There was a significant effect of growth rate on the C : P ratio (ANOVA, $p < 0.05$). Lower C : P ratios were associated with rapidly growing cells and probably reflect the increased P content associated with these cells (compare Fig. 1C with Fig. 3B).

The average N : P ratios varied only slightly over the growth conditions and typically ranged between 11 and 12. The distribution of N : P across the temperature–growth rate matrix was influenced by the interaction of temperature and growth rate (ANOVA $p < 0.02$); however, the effects of temperature and growth rate could not be individually teased apart. In fact, the interaction effect appears to be driven by the N : P ratio of cells grown at slow growth rate. Under slow growth conditions, N : P decreased from ~14 at 14°C to ~9 at 28°C (Table 5, Fig. 3C).

Discussion

Cell size and element content—It is well established that cell size increases as growth rate increases (Henrici 1928; Schaechter et al. 1958; Neidhardt et al. 1990). It would seem logical to expect that C content would be a simple function of cell size (volume); however, there is considerable uncertainty associated with this metric. Data from both natural systems and cultures have suggested that small bacteria contain less water than large cells and are therefore disproportionately rich in C when compared with large cells (Lee and Fuhrman 1987; Simon and Azam 1989; Kroer 1994), whereas others have found the reverse (Fagerbakke et al. 1996). Much of these data stem from batch cultures and some of the variability associated with C : volume relationships may reflect the inherent variability of batch culture (Wanner and Egli 1990) as well as the variability induced by the limiting nutrient at the onset of stationary phase (Vrede et al. 2002). P-limited chemostat cultures of *Pseudomonas* (previous studies) tend to confirm that slow-growing cells are small compared with more rapidly growing cells (Chrzanowski et al. 1988; Chrzanowski and Kyle 1996) and the data presented in Table 2 also support this conclusion. In Fig. 4, we plot both average cell volume and C content in the three-dimensional space created by the growth rate–temperature matrix. *Pseudomonas* growing at low growth rate and cold temperatures were small but rich in C. Similarly, *Pseudomonas* growing rapidly near the optimal growth temperature were densely packed with C (Fig. 4). In general, larger cells tended to have lower C content.

Considerably less is known about the relationship between cell size and other essential elements (such as N and P). Evaluation of Fig. 4B,C suggests that the relationship between cell size and C content was also true for N and P content. The data clearly demonstrate that small cells are richer in elements than are large cells and that the relationship is not easily predicted from growth rate alone or temperature alone.

Cell size and temperature—Temperature exerts an effect upon cell size that confounds simple interpretation of C : volume relationships and of volume : growth rate relationships. In natural systems, the distribution of cell size varies as a function of environmental temperature. Small cells are more common in warm waters than in cold (Chrzanowski et al. 1988; Wiebe et al. 1992). This finding appears to be opposite of what might be expected from growth dynamics. Growth rates of bacterial assemblages are generally thought to be greater under warm (summer) conditions than under cold (winter) conditions, so the distribution of cell size should be expected to shift to larger cells during warm periods. The dominance of small cells has been attributed, in part, to grazing pressure on large

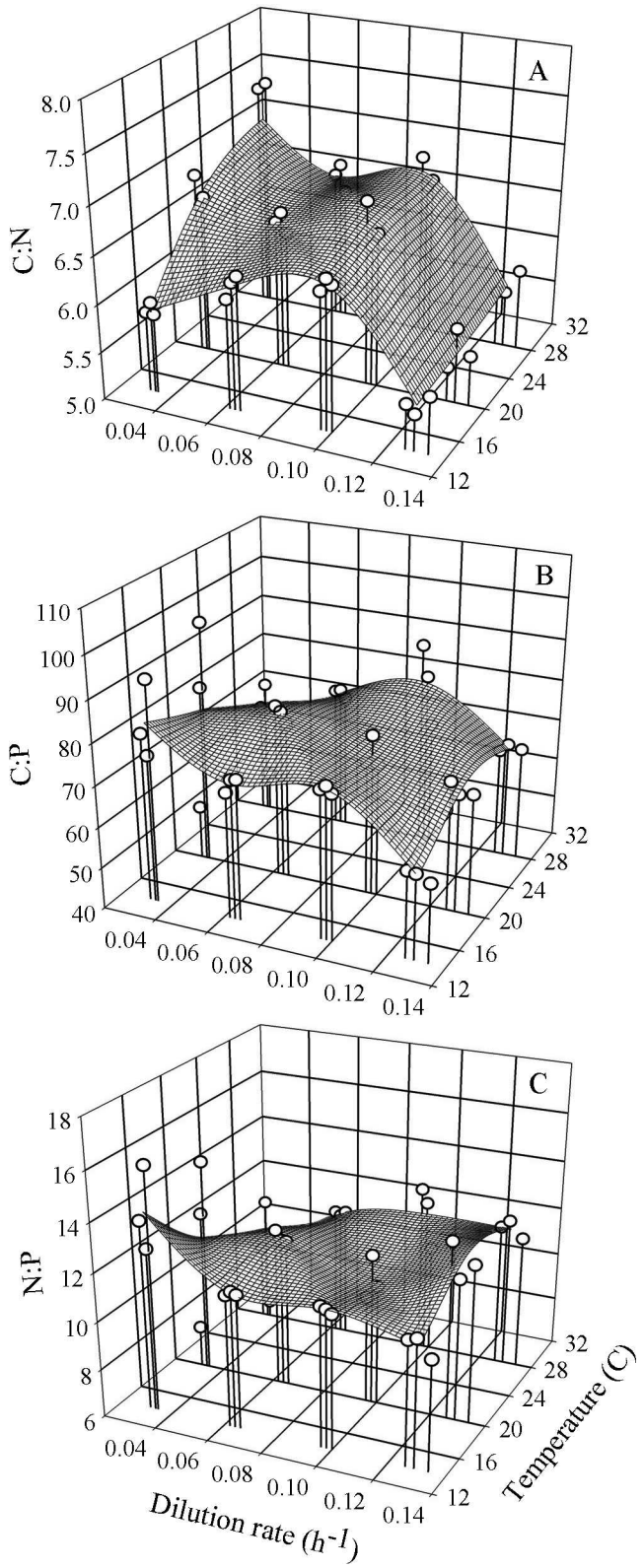

Fig. 3. Element ratios of *Pseudomonas fluorescens* grown in chemostats at different growth rates and temperatures. Data from individual chemostats are shown as a solid line and open circle. The contour mesh fits the mean for the replicate chemostats along the growth rate–temperature matrix. Mean values and standard errors are given in Table 5. (A) C : N, (B) C : P, and (C) N : P.

cells as well as to shifts in community structure. However, chemostat studies, where growth rate was held constant, revealed an inverse relationship between cell size and temperature (Hagström and Larsson 1984; Chrzanowski et al. 1988): cells growing at a constant rate are *larger* when the environmental temperatures are colder. This finding is particularly important to application of the principles of ecological stoichiometry to the microbial food web since the change in cell size is likely associated with changes in the composition of cells (and consequently their element ratios) as they respond to changing temperature. We were unable to detect a statistically significant relationship between cell size and temperature alone even though cells growing at 14°C were larger than cells growing at other temperatures. It is likely that a wider range of temperatures, coupled with a wider range of growth rates will be required to further tease apart the temperature–size relationship.

Macromolecular composition of cells and cellular P content—The macromolecular composition of bacteria shifts as an exponential function of growth rate. RNA, protein, and to a lesser extent DNA all increase as growth rate increases; however, each component increases at a different rate. When normalized to cell mass (as dry weight), protein content decreases slightly as growth rate increases, DNA remains essentially constant, and cell mass and RNA content increase (Ingraham et al. 1983). The decrease in protein content and corresponding increase in cell mass with increasing growth rate is consistent with findings that large, rapidly growing cells have higher water content than smaller slow-growing cells.

The increase in RNA concentrations with increasing growth rate is of particular interest. It has been well established that there is a close correlation between RNA content and growth rate, and that the variation in RNA content is due almost exclusively to changes in ribosome content (Tempest and Hunter 1965). RNA is approximately 9% P (by weight, Sterner and Elser 2002). Therefore rapidly growing cells should have an increased demand for P and cellular C : P ratios should fall as growth rate increases (*see* Fig. 3B, Chrzanowski et al. 1996; Makino et al. 2003). Additionally, if changes in cell size and C content are considered, the C : P may be expected to decrease disproportionately to increasing growth rate (especially if small slow-growing cells are indeed C rich compared with rapidly growing cells; again, *see* Fig. 3B).

There is an apparent contradiction between some of the patterns exhibited in Figs. 1C and 2C and the principle that rapid growth requires high contents of RNA and hence P. At 14°C, amounts of P and RNA (normalized to cell volume) decline as growth rate increases (this is also true for amounts of P and RNA per cell, data not shown). These surprising patterns appear to result from complicating changes in cell mass and volume. At low growth rates, bacterial cells might undergo periods of temporary quiescence during which protein synthesis ceases and nutrients accumulate in reserves (Koch 1996). Combined with a tendency to expel water, slow-growing bacteria might thus undergo a change in element composition. If so,

Table 5. Element ratios in *Pseudomonas fluorescens* grown at different temperatures and growth rates in chemostats (mean±SE, n = 3 chemostats).

Growth rate (h^{-1})	Temperature (°C)	Ratio (mol : mol)		
		C : N	C : P	N : P
0.03	14	5.87±0.04	83.29±5.26	14.18±0.97
	20	6.67±0.09	77.67±13.42	11.65±2.11
	28	7.04±0.30	63.27±7.10	8.99±1.33
0.06	14	6.30±0.08	72.80±1.14	11.56±0.05
	20	6.55±0.06	80.32±1.08	12.27±0.13
	28	6.55±0.08	74.17±0.59	11.33±0.05
0.10	14	6.49±0.04	75.89±0.45	11.69±0.07
	20	6.67±0.13	74.39±2.22	11.15±0.52
	28	6.73±0.09	80.23±5.65	11.92±0.75
0.13	14	5.49±0.06	60.35±0.57	11.00±0.22
	20	5.55±0.12	69.96±0.82	12.61±0.42
	28	5.67±0.09	67.44±0.56	11.89±0.19

it might be instructive to adopt a stoichiometric perspective and examine P and RNA contents as a function of cell C in relation to growth rate (Fig. 5). Excluding two apparent outliers, P content normalized to C content increases with growth rate ($r = 0.61$, $p < 0.01$). Because P is the limiting nutrient for these cultures, cellular content that increases with growth rate is expected theoretically (Droop 1974), following the equation

$$QP = \frac{\mu'_{\max} Q_{\min}}{\mu'_{\max} - D} \quad (2)$$

where $\mu'_{\max}$ is the maximal growth rate and $Q_{\min}$ is the minimal P content at which growth vanishes. For P content normalized to cell or to volume, this relationship is not observed because of changes in cell mass and volume that make the normalized P content decrease with growth rate at some temperatures. However, Droop's relationship fits the data on cellular P normalized to C, provided the highly variable data at the lowest dilution rate are excluded. Moreover, a model with temperature-dependent growth parameters (Table 6, Fig. 5) fits better than one with common parameters for all temperatures (nonlinear regression, partial $F_{4,21} = 2.25$, $p < 0.10$).

RNA normalized to C is more variable than P normalized to C, but also tends to increase with growth rate ($r = 0.20$, not significant). Some of this variability in RNA content is likely due to measurement error. But also, when growth rate is less than about 25% of maximal, as was the case for much of our data, the RNA content of bacteria becomes independent of growth rate as cells maintain a minimum complement of ribosomes and a large pool of uncharged transfer RNA (Ingraham et al. 1983).

Macromolecular composition of cells and temperature—In their classic paper, Schaechter et al. (1958) reported that, for "a given medium, cell size and composition are almost independent of growth temperature. The characteristics of the cells would therefore seem to be determined primarily by the pattern of biochemical activities imposed by the medium." This position argues that cellular stoichiometry is purely a function of the growth rate. The cellular stoichiometry of an organism would be the same when grown in any medium (i.e., resource pool) that allows the organism to attain a specific growth rate (*see* for example Ingraham et al. 1983, p. 276). However, this notion was modified by Tempest and Hunter (1965) who, working in chemostats, varied temperature independently of growth rate and found changes in macromolecular composition. For a given growth rate, RNA content increases as temperature decreases and the pattern is similar for two types of limitation, carbon and mineral element. Our data tend to confirm this result for slow-growing cells (*see* Fig. 2B, 0.03 h^{-1}), but also indicate that the relationship between RNA content and temperature may shift as cells approach optimal growth conditions (*see* above). *P. fluorescens* appears to reverse the relationship between RNA content and temperature at more rapid growth rates (*see* Fig. 2B, 0.13 h^{-1}). The physiology behind this finding appears straightforward; the rate at which a ribosome processes information is essentially constant; therefore, to maintain a given growth rate in the face of decreasing temperature, ribosome content must increase (Yun et al. 1996). Similarly, under more favorable growth conditions, ribosome content must increase to keep pace with metabolic demand.

The imbalance of it all—Stoichiometric theory holds that there are two potential outcomes if there is an imbalance between the ratio of elements in prey and the ratio of elements in predators; if the element ratio of the prey is within the range of that of the predator, the predator will excrete the element consumed in excess (nutrient regeneration); if the element ratio of the prey is not within the range of that of the predator, the predator will not be able to maintain a significant presence in the trophic structure and will be displaced by one that can take better advantage of the resource pool. Much of these arguments are based on predator–prey systems in which the prey are slow growing and predator(s) have narrow ranges in their element ratios, i.e., they are largely homeostatic.

How does this apply to bacteria and their predators? It is clear that the element composition (most clearly the C : N and C : P ratios) of bacteria varies as an interactive function

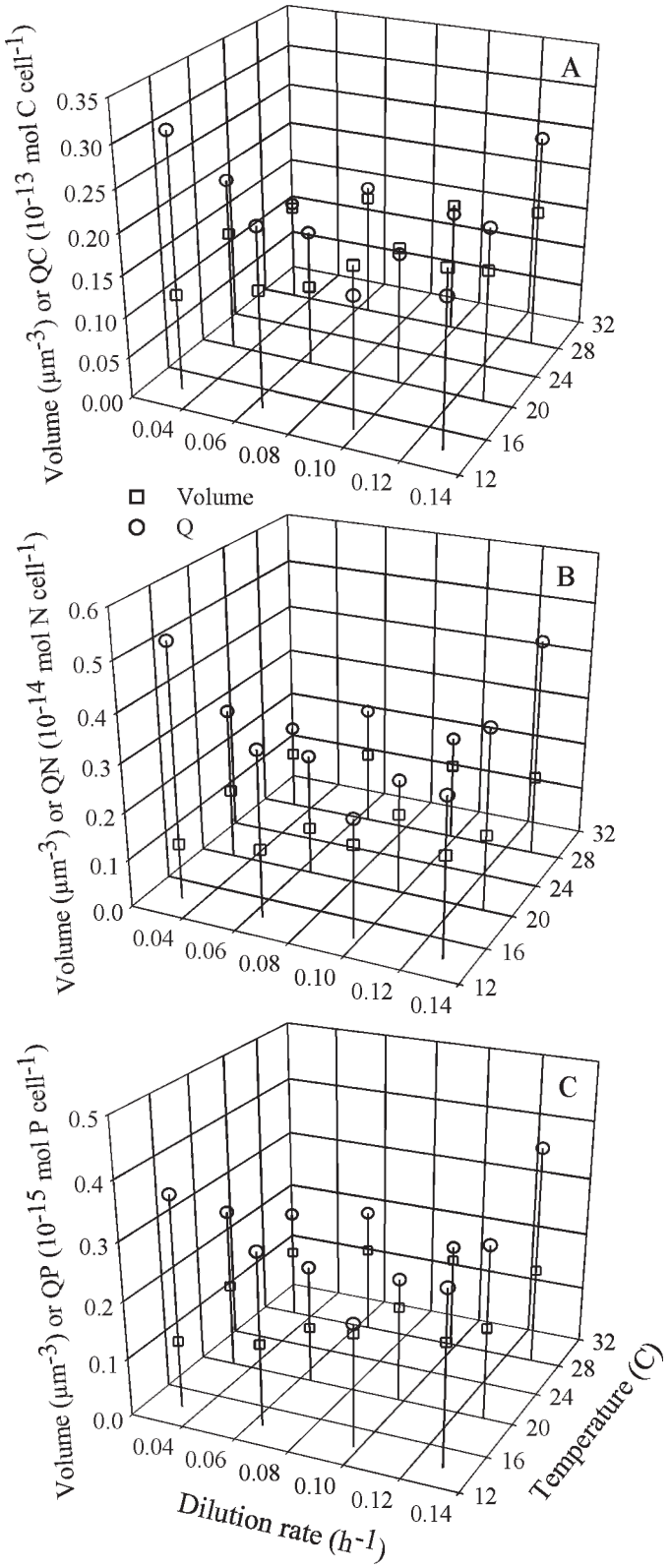

Fig. 4. The relationship between cell size and element content of *Pseudomonas fluorescens* grown in chemostats at different growth rates and temperatures. Data points represent the mean value generated from triplicate chemostats. (A) Carbon, (B) nitrogen, and (C) phosphorus.

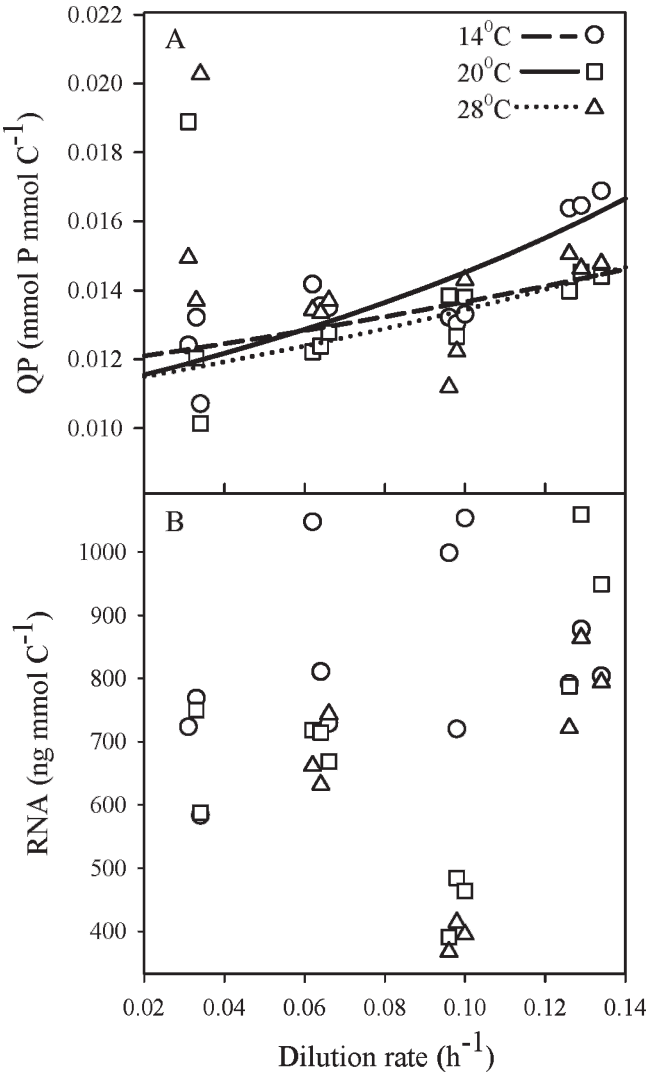

Fig. 5. Cell contents of (A) P and (B) RNA normalized to cell C plotted in relation to growth rate. Lines (panel A) fit the data to nonlinear regression models of the Droop equation (*see* Discussion).

of growth rate and temperature. Predators ingest bacterial cells as single packages of defined mass and volume. Predicting the size, element content, and macromolecular content of these packages is complicated by these interactions. The standard theory coupling growth and nutrient content in microbes (Droop 1974) and its extensions (e.g., Thingstad 1987; Grover 2003) appears inadequate to model these relationships unless attention focuses only on the stoichiometric ratio of the limiting nutrient to C. The decrease of limiting element content normalized to bacterial cell or to cell volume seen at some temperatures is especially unanticipated. New theory appears necessary to predict sizes and masses of cells in addition to compositional ratios. Despite this challenge, stoichiometric theory affords some insights into microbial predator–prey interactions.

The efficiency by which elements are transferred from the dissolved state through the microbial loop by predators

Table 6. Parameters of the Droop equation fitted to cell content of P normalized to cell carbon content (data from the lowest growth rate has been excluded).

Temperature (°C)	$\mu'_{max} \pm SE$	$Q_{min} \pm SE$
14	0.41±0.078	0.011±0.00075
20	0.57±0.20	0.011±0.00086
28	0.72±0.33	0.012±0.00092

of bacteria may change depending upon the environment in which the prey bacterium grows (both the physical condition, i.e., temperature, and the resource conditions). The same dissolved resource pool could conceivably yield prey of differing "quality" (i.e., C : N or C : P) simply as an interactive function of growth rate and temperature. Simply stated, the same prey may be a high-quality food at some times and a poor-quality food at others even though the resource environment does not change. Much depends upon whether an aquatic system is more "chemostat-like" with a single limiting nutrient and a quasi-steady supply to the dissolved state regulated by a myriad of physical and biological interactions, or if an aquatic system is more "batch culture-like" where growth continues until some critical resource become unavailable. We have recently shown (Shannon et al. 2007) that the ubiquitous protozoan *Ochromonas* ingests bacteria with low C : N (i.e., high QN) or low C : P (i.e., high QP) ratios at significantly higher rates than it ingests other bacteria. Thus it seems likely that the trophic flow of nutrients sequestered in bacteria will depend largely upon the growth state of the bacteria themselves.

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