Vol. 71: 257–269, 2014
doi: 10.3354/ame01680

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Published online February 14

Prey element stoichiometry controls ecological fitness of the flagellate *Ochromonas danica*

Thomas H. Chrzanowski*, Briony L. L. Foster

Department of Biology, PO Box 19498, The University of Texas Arlington, Arlington, Texas 76019, USA

ABSTRACT: In aquatic systems, the protist–bacteria consumer–resource interaction is one of the few interactions that link and regulate the flow of dissolved nutrients to higher trophic levels. While this interaction is well characterized from the perspective of top-down control of bacteria, less understood is how the interaction may regulate consumer success. Here, we consider the protist–bacteria consumer–resource interaction and explore relationships among food quality (measured as ratios of C:N, C:P, or N:P), prey type (measured by prey-size and taxonomic affiliation), and consumer success (measured by growth rate). We use a model consumer, the flagellate *Ochromonas danica*, and a variety of bacterial species as resources. Rates at which prey were ingested corresponded to prey quality for metrics of food quality based on C (C:N and C:P). Prey quality also strongly influenced consumer fitness. A large imbalance between the element ratio of the consumer and that of its prey had a negative impact on consumer fitness, particularly when excess C or excess N was consumed. Prey type, as a phylogenetic group of bacteria, also influenced consumer success. *Betaproteobacteria* were readily consumed but failed to support consumer growth, mainly as a result of a nutrient imbalance between consumer and resource.

KEY WORDS: Bacterivory · Food quality · Ecological stoichiometry · Prey selection

INTRODUCTION

A growing body of theory focuses on consumer–resource interactions and addresses how such things as prey edibility, prey quality, and predator diet-breath influence the types and quantities of nutrients ingested and regenerated by consumers (Grover 2004, Jiang & Morin 2005, Cebrian et al. 2009). For the most part, nutrients consumed in excess of that required for maintenance and growth will be regenerated (Grover 2004, Hessen & Anderson 2008). Competition among potential users for these recycled nutrients, and the efficiency at which nutrients are utilized, may bring about shifts in community structure thereby linking biodiversity to ecosystem function (Cardinale et al. 2009, Hillebrand et al. 2009). Additionally, increasing discrepancies between nutrients contained in resources and those required by a consumer may place a major constraint on consumer fitness and subsequent energy transfer through ecosystems (Dickman et al. 2008, Sinsabaugh et al. 2009). In both terrestrial and aquatic systems, this constraint may be expressed by changes in grazing rates; nutrient imbalance between resource and consumer leads to increased grazing rates by consumers, lower consumer growth rates, and lower trophic efficiency (Mitra et al. 2007, Hessen & Anderson 2008, Hillebrand et al. 2009).

Apart from situations where a resource (prey) supplies a consumer with an essential biochemical (Jónasdóttir 1994, Müller-Navarra et al. 2004), simple variations in prey quality have gone far in explaining not only the consequences of consuming excess nutrients in terms of consumer behavior and success, but also the consequences in terms of ecosystem dynamics (Anderson et al. 2005, Hessen & Anderson

*Corresponding author: chrz@uta.edu

2008, Wang et al. 2009). For instance, work focused upon metazoan zooplankton consumers of phytoplankton revealed that a consumer's behavior and fitness hinges upon a balance between food quantity and food quality, where quantity was measured as carbon (C) availability and quality was measured by the ratio of nutrient elements (C, nitrogen [N], and phosphorus [P]) within prey (Hessen et al. 2002). Imbalances in nutrient elements between zooplankton and their resources leads to excess C consumption (relative to P consumption) that reduces fitness and makes dissolved organic C available to osmotrophs, potentially modulating C flow through the food-web (Hessen & Anderson 2008).

Functioning in a trophic level lower, yet parallel to the metazoan zooplankton–phytoplankton consumer–resource interaction, is the nanoflagellate–bacteria consumer–resource interaction. This interaction has been well-studied from the perspective of how predation upon bacteria by flagellates regulates both bacterial standing crop and the transfer of C to higher trophic levels (Gebre-Mariam & Taylor 1989, Chrzanowski & Šimek 1993, Okamura et al. 2012, Šimek et al. 2013). Similar to the metazoan zooplankton–phytoplankton consumer–resource interaction, imbalances between the nutrient requirements of flagellates and their prey are thought to result in excretion of nutrients (N and P; excess N or P consumed relative to C) that permit continued growth of bacteria and enhanced C decomposition (Wang et al. 2009). Equally important to such ecosystem dynamics, yet less studied, is how predation upon bacteria translates to consumer fitness and the potential for flagellates to transfer nutrients to higher trophic levels.

When the flagellate–bacteria consumer–resource interaction is considered from the broad perspective of trophic dynamics, it is practical to consider the bacteria as a single group whose members have an equivalent nutritional value to a flagellate consumer; as exemplified by the use of single-value conversion factors to convert bacterial size to biomass (Watson et al. 1977, Bratbak 1985). However, the nutritional value of a bacterium as prey, if considered as element content (quota, element content cell^{-1}) or stoichiometric ratios of elements (Sterner & Elser 2002), varies within a species as a function of growth (Kemp et al. 1993, Eccleston-Parry & Leadbeater 1995, Chrzanowski & Grover 2008, Løvdal et al. 2008, Grover & Chrzanowski 2009) and among species (phylogenetic diversity) (Nakano 1994, Salcher et al. 2007, Barlett & Leff 2010).

Given that different species of bacteria have different demands for nutrients and that demand varies with growth, then resources supporting bacteria in an aquatic environment will not meet the demands of all members of the bacterial community all of the time. Changing resource pools bring about dramatic changes in the characteristics of bacteria as different members of the bacterial community make the transition from a non-growing to a growing state. Rates and concentrations of nucleic acid and protein synthesis increase, ribosome content increases, cells increase in length, and there are striking changes in molecules expressed at the cell surface (White 2007). As a consequence of growth, cells accumulate mineral nutrients, quotas of various elements increase, and ratios of C:P and N:P change. Growing members of a bacterial community become richer in critical elements (N and P), and, theoretically, are of higher food-quality to a consumer than non-growing cells (Shannon et al. 2007).

Here, we considered the flagellate–bacteria consumer–resource interaction in a model system and explored the relationships among food quality, prey type, and consumer fitness. We used as a consumer, the flagellate bacterivore *Ochromonas danica*, and as a resource, growing and non-growing bacteria from a variety of phylogenetic groups. We asked whether consumer–resource interactions at the lowest trophic level in aquatic systems function similarly to the well-studied zooplankton–phytoplankton consumer–resource interactions.

MATERIALS AND METHODS

In these experiments, the flagellate consumer *Ochromonas danica* was fed bacteria harvested from mid-exponential (growing bacteria) and from stationary (non-growing bacteria) phases of growth in R2A broth (Teknova). The rate at which *O. danica* ingested prey and its subsequent growth rate were determined. Element analysis of nutrients contained in the consumer and prey (as C, N, and P) permitted assessment of how the ingestion rate and consumer growth rate varied as functions of prey size and prey food quality (as molar ratios of C:N, C:P, and N:P).

Ochromonas is the most species-rich genus in the Chrysophyceae and is distributed throughout freshwaters (Edgar & Andersen 2003). *O. danica* contains plastids, but will not grow autotrophically at ambient CO_2 levels (Aaronson & Baker 1959). Robust phagotrophic growth occurs when it preys upon bacteria, while osmotrophic growth only appears possible on rich organic media (Foster & Chrzanowski 2012a).

O. danica (UTEX 1298) was maintained bacteria-free in *Ochromonas* medium (OM; 40 ml liver extract, plus 1 g 960 ml^{-1} each of glucose, tryptone, and yeast extract; Starr 1978) at room temperature (RT, ~23°C). Bacteria were maintained at RT on R2A agar (Difco). The bacteria used as prey (see Table 1) were existing laboratory strains, obtained from the American Type Culture Collection (ATCC) (www.atcc.org/) or were isolated from freshwater (Foster & Chrzanowski 2012b). The identity of all strains was verified by 16S rRNA gene sequencing. Briefly, genomic DNA was extracted (QIAmp DNA Mini kit, Qiagen), the DNA encoding the 16S rRNA gene was amplified (forward primer 27f, reverse primer 1492r; Foster & Chrzanowski 2012b), and sequences were aligned (Sequencher 5.0, Gene Codes), matched to known sequences (Ribosomal Database Project, http://rdp.cme.msu.edu/), and assigned to a genus. *Herbaspirillum seropedicae* (ATCC 35892) and *Pseudomonas fluorescens* (ATCC 17386), obtained from the ATCC, served as prey and as internal controls for sequence analyses.

Growth and preparation of *Ochromonas danica*

O. danica was grown in OM in a continuously stirred and aerated 800 ml chemostat (Applikon) operated in the dark at 25°C at a dilution rate of 0.037 h^{-1}. Cells harvested from chemostats were washed 3× with sterile Standard Mineral Base medium (White & Hageman 1998) lacking any source of C, N, or P (hereafter referred to as SMB buffer), concentrated by centrifugation (Sorvall RT6000B, 720 × *g*, 10°C, 7 min), and resuspended in SMB buffer. Concentrated cells were enumerated by direct count using epifluorescence microscopy (Olympus BH2) and acridine orange as the fluorochrome (Hobbie et al. 1977). Aliquots of washed cells were preserved in glutaraldehyde (2% final concentration), stored at 4°C, and later used to determine cell size.

Preparation of bacteria in different growth states

Growth curves for each species were determined from measurements of optical density (OD_{600}). An overnight culture (25°C, 100 rpm, New Brunswick Scientific G10 Gyrotory shaker) was used to inoculate triplicate flasks of R2A broth (Teknova) to an OD_{600} of ~0.01. Cultures were incubated as above, and the OD was recorded at regular time intervals.

Growth curves were used to establish the OD corresponding to the mid-exponential (growing bacteria) and late-stationary phases of growth (non-growing bacteria). The mid-exponential phase of growth was established as the midpoint from the time growth started to the time of transition into the stationary phase. The late-stationary phase was defined as 4 times the time it took a culture to go from mid-exponential phase to transition phase, with time measured from the mid-exponential phase.

For feeding trials, batch cultures of each bacterium (R2A broth) were grown as above until mid-exponential or late-stationary phase. Cells were washed, concentrated, and enumerated as for *Ochromonas danica* (see previous subsection). Aliquots of washed cells were preserved in glutaraldehyde (2% final concentration), stored, and later used to determine cell size.

Cell volume

Bacterial volumes were determined from measures of length and width of cells using epifluorescent microscopy at a magnification of 1250× and SYBR green (Invitrogen) as the fluorochrome. Length and width of individual cells, determined from digital images (Olympus DP70 camera) and imaging software (Simple PCI, Compix), were converted to volume according to the formula given in Chrzanowski & Grover (2008).

Determination of ingestion rates and subsequent growth rate of *Ochromonas danica*

Feeding experiments were conducted in flasks (triplicates) containing SMB buffer. Washed *O. danica* and washed bacteria, both suspended in SMB buffer, were combined to target final concentrations of 4×10^5 and 3×10^7 cells ml^{-1}, respectively. Two sets of controls (duplicates) were used: *O. danica* without bacteria and bacteria without *O. danica*. Predator–prey cultures and controls were gently shaken (60 opm, dark, 25°C). Samples were taken 1 h after predator and prey were combined and every 45 min thereafter for a total elapsed time of 3.25 h. Samples were preserved in ice-cold glutaraldehyde (2% final concentration) and stored (4°C) until processed by flow cytometry. A flow cytometer (BD LSRII, 488 nm argon laser) was used to quantify *Ochromonas danica* and bacteria according to the methods outlined in Foster & Chrzanowski (2012b).

Calculating growth and ingestion rate

The growth rate of *Ochromonas danica* while preying on bacteria was calculated as the slope of a line determined by regressing the natural logarithm of abundance on time. The average growth rate of *O. danica* in 2 control cultures (lacking bacteria) was subtracted from the growth rate in experimental cultures (with bacteria) to obtain the growth rate supported by ingestion of bacteria. The rate at which bacteria were ingested by *O. danica* was determined by dividing the number of bacteria consumed (corrected for controls) by the average *O. danica* concentration during exponential growth (corrected for controls) and dividing by the time of a feeding experiment (Heinbokel 1978).

Element composition of cells

Individual strains of bacteria and *Ochromonas danica* were collected on pre-combusted glass-fiber filters (Whatman GF/F, 0.7 µm nominal retention) for determination of C and N content (Perkin-Elmer Series 2200 CHN Analyzer) and particulate P (Strickland & Parsons 1972). All samples were assayed in triplicate. Element ratios are reported as mole: mole.

Graphical and statistical analyses

Graphical and statistical (descriptive, linear, and non-linear correlations) analyses were performed using Sigma Plot (v11).

RESULTS

Element flux relative to prey size and *Ochromonas danica* growth rate

Growing bacteria spanned a broad range in cell sizes, while non-growing bacteria were small compared to growing bacteria (Table 1). When all data were considered, there was a 20-fold difference between the smallest (non-growing *Sphingomonas*) and largest (growing *Aeromonas*) bacteria offered to *O. danica*. When compared to growing bacteria, smaller non-growing bacteria were characterized by low cell quotas of C, N, and P (Table 2).

Since it seemed likely that the quantity of elements consumed would have a greater effect on *Ochromonas danica*'s growth than would the number of nutrient-bearing cells ingested, we considered how the flux of nutrients (element ingested protozoan^{-1} h^{-1}) varied as a function of prey cell size (Fig. 1) and how *O. danica*'s growth rate varied as a function of nutrient flux (Fig. 2).

Bacteria were supplied to *Ochromonas danica* at a target concentration of 3.0×10^7 cells ml^{-1}. Actual concentrations ranged between 9.20×10^6 to 5.82×10^7 cells ml^{-1} (data not shown). The rates at which *O. danica* ingested growing and non-growing bacteria were similar and typically fell below 20 bacteria protozoan^{-1} h^{-1} (see Fig. 3). However, *O. danica* is a size-selective predator (Chrzanowski & Šimek 1990, Foster & Chrzanowski 2012b); consequently, there was a greater flux of C, N, and P broadly corresponding to the ingestion of bacteria falling within a range of preferred prey sizes (Fig. 1). Non-growing bacteria tended to be small compared to growing bacteria, and spanned a smaller range in cell size; nevertheless, the data suggest that nutrient flux was influenced by prey size even within the limited range of sizes. There was an approximately 10-fold greater peak flux of C, N, and P (based on regression models;

Table 1. Differences in cell sizes of growing and non-growing bacteria. Each value is the mean (±SD) of n (in parentheses) measures

Class Genus	Cell volume (µm^3 cell^{-1}) Growing	 Non-growing
Alphaproteobacteria		
Sphingomonas	0.178 ± 0.112 (212)	0.071 ± 0.036 (515)
Betaproteobacteria		
Aquaspirillum	0.143 ± 0.074 (353)	0.079 ± 0.041 (420)
Herbaspirillum	0.407 ± 0.492 (182)	0.112 ± 0.057 (436)
Ralstonia	0.257 ± 0.163 (154)	0.135 ± 0.069 (302)
Gammaproteobacteria		
Aeromonas	1.355 ± 0.706 (98)	0.277 ± 0.183 (167)
Citrobacter	1.024 ± 0.424 (193)	0.104 ± 0.070 (342)
Escherichia	0.949 ± 0.481 (171)	0.775 ± 0.399 (67)
Pseudomonas	0.244 ± 0.133 (178)	0.094 ± 0.047 (378)
Pseudomonas[a]	0.693 ± 0.333 (207)	0.158 ± 0.073 (509)
Salmonella	0.962 ± 0.220 (123)	0.220 ± 0.101 (261)
Stenotrophomonas	0.479 ± 0.176 (141)	0.103 ± 0.046 (441)
Bacilli/Actinobacteria		
Bacillus	0.778 ± 0.329 (143)	0.361 ± 0.166 (157)
Listeria	0.214 ± 0.107 (276)	0.134 ± 0.062 (324)
Microbacterium	0.124 ± 0.061 (420)	0.309 ± 0.188 (181)
Staphylococcus	0.327 ± 0.187 (119)	0.254 ± 0.131 (83)

[a]*Pseudomonas fluorescens*

Table 2. Element content (quota) of growing and non-growing bacteria. Each value is the mean (±SD) of triplicate measures. C: carbon; N: nitrogen; P: phosphorus

Class / Genus	Quota (fmol cell^{-1})					
	Growing			Non-growing		
	C	N	P	C	N	P
Alphaproteobacteria						
Sphingomonas	85.77 ± 17.37	17.91 ± 8.12	1.56 ± 0.44	4.19 ± 0.94	0.89 ± 0.20	0.08 ± 0.03
Betaproteobacteria						
Aquaspirillum	24.42 ± 1.44	6.79 ± 0.19	0.43 ± 0.06	1.51 ± 0.59	0.55 ± 0.27	0.04 ± 0.01
Herbaspirillum	114.89 ± 11.69	30.98 ± 2.12	1.45 ± 0.29	2.47 ± 0.49	0.62 ± 0.14	0.04 ± 0.01
Ralstonia	102.23 ± 35.88	27.95 ± 9.31	1.26 ± 0.41	1.30 ± 0.03	0.36 ± 0.01	0.03 ± 0.01
Gammaproteobacteria						
Aeromonas	90.83 ± 10.78	16.94 ± 0.40	2.39 ± 0.69	8.53 ± 3.75	1.81 ± 0.80	0.24 ± 0.10
Citrobacter	171.49 ± 61.03	30.20 ± 8.95	2.16 ± 0.40	0.97 ± 0.50	0.21 ± 0.11	0.02 ± 0.01
Escherichia	130.39 ± 15.78	17.15 ± 2.27	1.80 ± 0.26	11.57 ± 8.32	2.41 ± 1.74	0.19 ± 0.11
Pseudomonas	71.15 ± 14.37	16.50 ± 3.73	1.80 ± 0.16	2.18 ± 0.67	0.48 ± 0.14	0.05 ± 0.02
Pseudomonas[a]	61.66 ± 32.95	12.83 ± 6.54	1.81 ± 1.45	3.78 ± 1.19	0.82 ± 0.28	0.15 ± 0.06
Salmonella	100.08 ± 60.55	15.54 ± 9.57	1.97 ± 1.57	10.97 ± 3.91	2.48 ± 0.89	0.21 ± 0.07
Stenotrophomonas	91.25 ± 33.11	24.54 ± 8.74	1.60 ± 0.46	0.24 ± 0.09	0.06 ± 0.02	0.01 ± 0.01
Bacilli/Actinobacteria						
Bacillus	169.52 ± 33.27	45.48 ± 15.14	3.18 ± 0.51	35.07 ± 7.18	9.44 ± 1.81	0.82 ± 0.16
Listeria	45.31 ± 11.45	9.17 ± 2.89	0.73 ± 0.08	47.60 ± 17.80	11.24 ± 4.00	1.30 ± 0.31
Microbacterium	19.41 ± 1.47	4.27 ± 0.27	0.38 ± 0.01	6.46 ± 1.62	1.30 ± 0.34	0.15 ± 0.04
Staphylococcus	43.60 ± 9.44	7.03 ± 1.12	1.23 ± 0.19	14.55 ± 0.81	3.74 ± 0.70	0.35 ± 0.09

[a]*Pseudomonas fluorescens*

Fig. 1) when *O. danica* ingested growing bacteria than when it ingested non-growing bacteria.

With very few exceptions, bacteria offered as prey were ingested by *Ochromonas danica*; however, not all bacteria ingested supported growth, and in some cases the abundance of *O. danica* declined during feeding trials. Consequently, growth rates ranged from as low as −0.20 to as high as 0.29 h^{-1}. Growth rate did not vary as a function of element flux when each element (C, N, or P) was considered independently (Fig. 2). Generally, non-growing bacteria, while having lower quotas of C, N, and P than growing bacteria (Table 2), contained sufficient nutrients to support growth of *O. danica*, often at rates equivalent to that achieved when ingesting growing cells (Fig. 2). This result suggests that *O. danica* frequently consumed nutrients in excess of that required to meet demands of growth.

Ingestion and *Ochromonas danica* growth as a function of prey quality

Food quality was considered as the molar ratios of C:N, C:P, and N:P supplied by each species of bacteria. Element ratios may be indicative of food quality if a consumer is limited by one element of the element pair. For example, if N or P limits consumer growth, as is the tendency for metazoan zooplankton, then the ratio of C:N or C:P would reflect food quality. Low numeric values of C:N or C:P would be indicative of higher food-quality prey. Similar logic may be extended to the N:P ratio depending on which element constrains growth.

The average element ratios for each species of bacteria in each growth state are given in Table 3 and were similar to stoichiometric ratios of bacteria grown under various conditions of nutrient limitation, as well as to bacteria growing under nutrient-sufficient conditions (Nakano 1994, Eccleston-Parry & Leadbeater 1995). The C:N ratios of non-growing bacteria fell largely within a narrow range (3.5 to 5), whereas the C:N ratios of growing bacteria spanned a wider range (3.5 to 7.5). The C:P of non-growing bacteria spanned a wide range (50 to 140), but the C:P ratios of growing bacteria fell within a narrower range (20 to 80). Similar to the C:P ratio, the N:P ratios of non-growing bacteria were broadly distributed (10 to 38), while the N:P ratios of growing bacteria, with a few exceptions, fell within a more limited range (5 to 22; Fig. 3).

There were few relationships between metrics of food quality and the rate at which bacteria were ingested (Fig. 3). However, there were positive relationships between ingestion rate and food quality for both metrics of food quality based on C (C:N and C:P). Ingestion rate was positively correlated to the

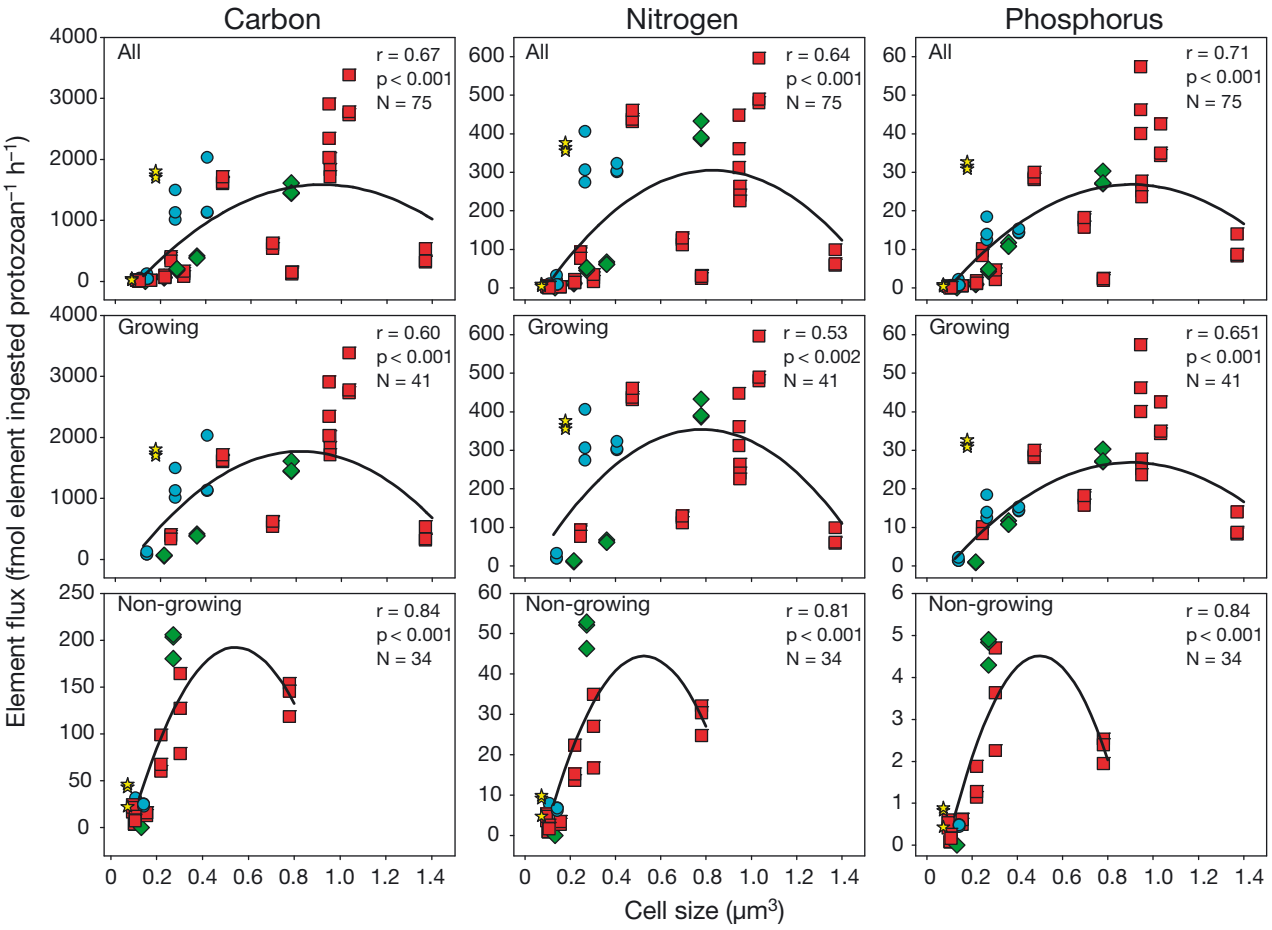

Fig. 1. Element flux as a function of prey size. Top panels depict the relationship when all bacteria were considered; middle row panels, only growing bacteria; and bottom panels, only non-growing bacteria. Left panels depict the flux of carbon (C); center panels, the flux of nitrogen (N); and right panels, the flux of phosphorus (P). Non-linear regression lines fit a quadratic model for C, N, and P fluxes. Attributes describing the full regression models are given in each panel. For each regression, the coefficient describing curvature was significant at $p < 0.001$. Each data point represents an individual experiment. Stars: *Alphaproteobacteria*; circles: *Betaproteobacteria*; squares: *Gammaproteobacteria*; diamonds: *Bacilli* and *Actinobacteria*

C:N ratio when growing and non-growing bacteria were considered together or when growing bacteria were considered alone. Ingestion rate was positively correlated to the C:P content of both non-growing and growing bacteria, but was not correlated to C:P when all data were pooled. These positive relationships suggest that *Ochromonas danica* consumes C-rich prey at higher rates than it ingests prey rich in N or P; this is supported by the lack of a relationship between ingestion rates and the N:P ratios of prey.

While there were few relationships between metrics of food quality and prey ingestion by *Ochromonas danica*, there were several statistically significant correlations between food quality and growth rate (Fig. 4). *O. danica's* growth rate was positively correlated to the C:N ratio of its prey. Growth rate was negatively correlated to the C:P ratio of non-growing bacteria and to the C:P ratio when all bacteria were considered collectively. Growth rate was negatively correlated to the N:P ratio of non-growing, growing, and all bacteria when data were pooled. Further, it appears that the C:P and N:P ratios of *Betaproteobacteria*, and to a lesser extent the C:N ratio, strongly influenced the relationship between growth rate and food quality. These bacteria (*Betaproteobacteria*) had the lowest ratios of C:N and the highest ratios of C:P and N:P of the bacteria supplied to *O. danica*.

Nutrient imbalance and consumer success

The data presented in Figs. 3 & 4 suggest that there were imbalances between nutrients required by *Ochromonas danica* and those supplied by prey and that these imbalances affected *O. danica*'s growth. To further examine this, the element imbalance was

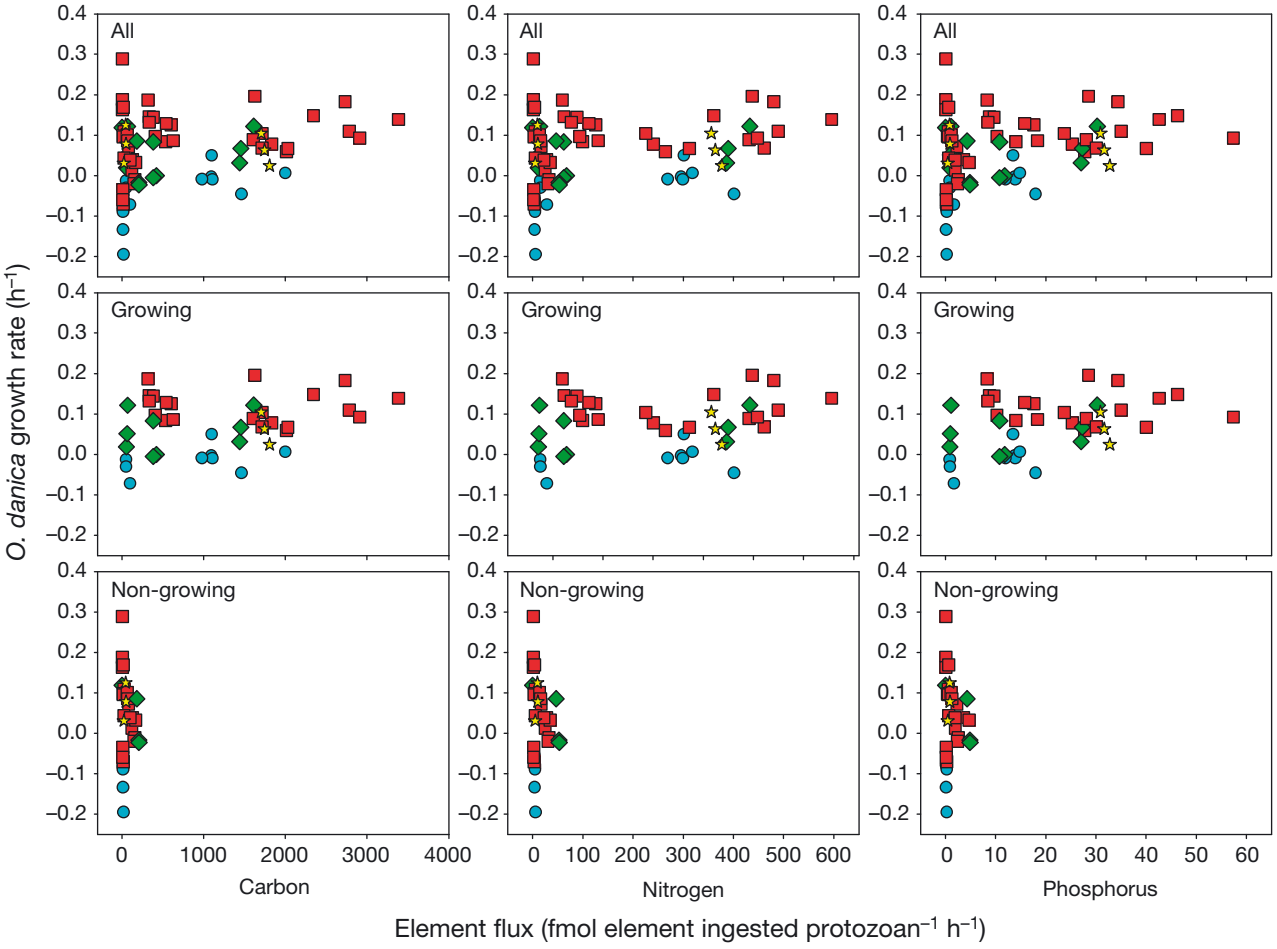

Fig. 2. *Ochromonas danica*. Growth rate of *O. danica* as a function of element flux. Top panels depict the relationship when all bacteria were considered; center panels, only growing bacteria; and bottom panels, only non-growing bacteria. See Fig. 1 for symbol descriptions. Difficult-to-read low values of element flux (bottom panels) are given in the lower panels of Fig. 1

calculated as the log element ratio of *O. danica* less the log element ratio of its prey (for example, log $C:P_{predator}$ – log $C:P_{prey}$) and plotted as a function of *O. danica* growth rate. Depending on the perspective, a negative imbalance would indicate an excess of one nutrient or a deficiency of the other (e.g. log $C:P_{predator}$ < log $C:P_{prey}$ implies an excess of C or a deficiency of P: the ratio could be adjusted by altering the numerator, C, or the denominator, P). A value of zero would indicate a balance between the element ratio of predator and prey and would suggest the point where the potential for nutrient limitation shifts from one element to another (see 'Discussion'). Thus, unimodal curves should result with the apex appearing at the point where limitation shifts from one element to another.

The element imbalance was calculated for the C:N, C:P, and N:P ratios (Fig. 5). The relationship between element imbalance and *Ochromonas danica* fitness could best be described by simple linear models and suggests that experimental conditions did not produce bacteria with a range in element content (particularly P) sufficient to affect *O. danica*'s growth rate. The plots further reveal that prey food quality likely constrains *O. danica*'s growth, particularly when it consumes an excess of C relative to P (Fig. 5, middle panel) or an excess of N relative to C or P (Fig. 5, top and bottom panels, respectively). It appears that *O. danica*, ingesting only bacteria, may consume more N than required to support growth demands. This result is consistent with that of Grover & Chrzanowski (2009) who found high rates of N regeneration (relative to P) when *O. danica* ingested a single type of bacteria whose element composition varied broadly. *Betaproteobacteria* strongly influenced the analyses, as these bacteria were deficient in P (growing or non-growing) and rich in N compared to other bacteria used as prey (Fig. 4).

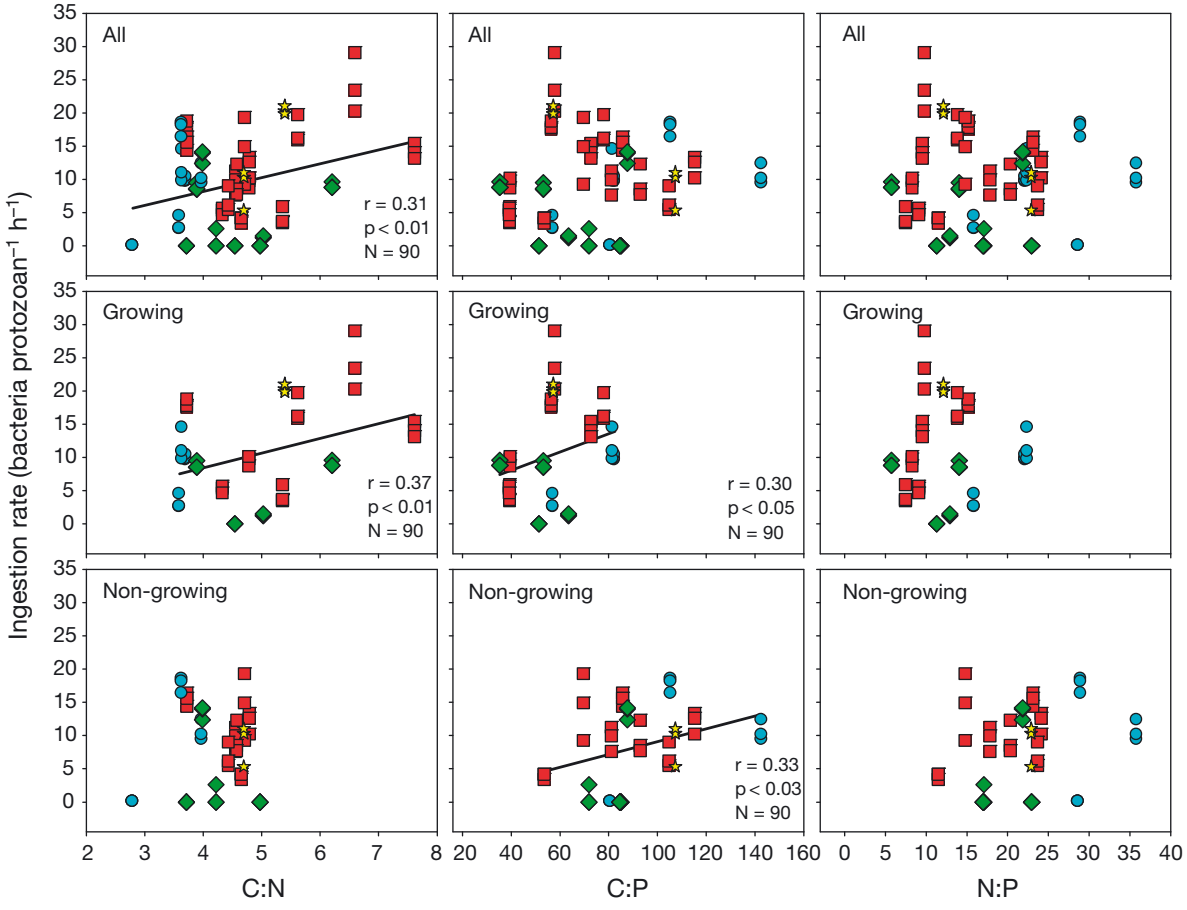

Fig. 3. Ingestion rate of bacteria as a function of prey type, growth state, and element ratio. Top panels depict the relationship when all bacteria were considered; center panels, only growing bacteria; and bottom panels, only non-growing bacteria. Element ratios are mol:mol. Attributes describing linear regression models are given in each panel where correlations were found. See Fig. 1 for symbol descriptions

DISCUSSION

In this study we considered how prey size, nutritional quality, and prey type influenced element flow to, and subsequent growth of, *Ochromonas danica*. Fifteen bacterial species were used as prey and were supplied to *O. danica* at concentrations sufficient to saturate the flagellate's ingestion rate (Grover & Chrzanowski 2009). The array of bacteria included 4 genera of Gram-positive bacteria, 3 within the *Bacilli* and 1 within the *Actinobacteria* (grouped as Gram-positive bacteria), and 11 genera of Gram-negative bacteria spanning 3 classes within the *Proteobacteria* (1 *Alphaproteobacteria*, 3 *Betaproteobacteria*, and 7 *Gammaproteobacteria*). As expected for a diverse suite of bacterial species, each in 2 stages of growth, nearly all measured responses displayed high variability. Patterns in this variability came into focus when separating ingestion rates from growth rates and viewing both through the lenses of size-selective ingestion and prey quality as measured by nutrient element content.

Ochromonas danica is capable of capturing prey spanning a wide-range in size; however, higher ingestion rates have been reported when prey fall between 0.8 and 1.6 µm^3 (Chrzanowski & Šimek 1990). Previously, using the same data set as in the current study, we confirmed size-selective ingestion of bacteria by *O. danica*; bacteria having volumes ranging between 0.6 and 1.0 µm^3 were ingested at higher rates than smaller or larger cells (Foster & Chrzanowski 2012b). Further, we described *O. danica* as an indiscriminant predator in that it readily ingested growing and non-growing bacteria spanning a range of phylogenetic groups. However, some of the strains of ingested bacteria supported growth of *O. danica*, while others, mainly members of the *Betaproteobacteria*, did not. Consequently, we explored in this work the relationship between food quality and prey type to gain insights into factors that

Table 3. Average element ratios of growing and non-growing bacteria and of *Ochromonas danica*. C: carbon; N: nitrogen; P: phosphorus; ND: not determined

Class Genus	Element ratio (mol:mol) Growing C:N:P	 Non-growing C:N:P
Alphaproteobacteria		
Sphingomonas	55:11:1	52:11:1
Betaproteobacteria		
Aquaspirillum	57:16:1	38:14:1
Herbaspirillum	79:21:1	62:16:1
Ralstonia	81:22:1	43:12:1
Gammaproteobacteria		
Aeromonas	38:7:1	36:8:1
Citrobacter	79:14:1	49:11:1
Escherichia	72:10:1	61:13:1
Pseudomonas	40:9:1	44:10:1
Pseudomonas[a]	34:7:1	25:5:1
Salmonella	51:8:1	52:12:1
Stenotrophomonas	57:15:1	24:6:1
Bacilli/Actinobacteria		
Bacillus	53:14:1	43:12:1
Listeria	62:13:1	37:9:1
Microbacterium	51:11:1	43:9:1
Staphylococcus	35:6:1	42:11:1
Chrysophyceae		
Ochromonas	66:10:1	ND

[a]*Pseudomonas fluorescens*

might constrain the growth of *O. danica*. We considered both the flux of elements (C, N, and P) and the quality of food measured as the stoichiometric ratios of elements (C:N, C:P, and N:P).

A careful review of Figs. 1 & 2 indicates that the flux of elements to *Ochromonas danica* was largely a result of prey-size preference (Fig. 1) and that the subsequent growth of *O. danica* did not correlate with the flux of any single element alone (C, N, or P; Fig. 2). The element flux as presented here, represented the gross flux of nutrients, since assimilation efficiencies (and hence excretion rates) of the various elements were not known. Figs. 1 & 2 also show that the strains of *Betaproteobacteria* generally did not support growth of *O. danica*, even though they were readily consumed regardless of growth state. Similarly, Gram-positive bacteria tended to support lower *O. danica* growth rates than did Gram-negative bacteria.

Since *Ochromonas danica*'s growth did not appear limited by the flux of C, N, or P alone, we considered other factors that might constrain *O. danica*'s growth. We considered relationships between food quality and ingestion rate, and between food quality and growth rate (Figs. 3 & 4, respectively).

While there were large differences in food quality among types of bacteria and between growth states, there were no obvious consistent trends in food quality among the various phylogenetic groups (Table 3). Considering the element ratios of bacteria fed to *Ochromonas danica* (Fig. 3, top panels, C:N, C:P, N:P): members of the *Gammaproteobacteria* spanned essentially the entire range of values, suggesting that all bacteria should not be considered equivalent food quality to a consumer regardless of their growth state, even if they are phylogenetically related (see penultimate paragraph; Šimek et al. 2013).

The relationships between the metrics of food quality and ingestion rates are based on a variety of strains of bacteria, the element compositions of which resulted from growth under a single condition (one medium, one temperature). These results are not consistent with the findings of others who have related similar metrics of food quality to consumer ingestion rates using single prey types but grown under different conditions. Shannon et al. (2007), working with a heat-killed strain of *Pseudomonas fluorescens* grown in chemostats at different growth rates and at different temperatures, found negative correlations between the metrics of food quality and the rates at which *Ochromonas danica* ingests the bacterium. Using an approach similar to the one used in this study, John & Davidson (2001) found that the flagellate *Paraphysomonas vestita* ingests low-C:N growing-algal cells at higher rates than it ingests high-C:N stationary-phase cells. Clearly, further study will be necessary before the relationship between food quality measured by element ratios and prey selection/ingestion by flagellate consumers can be fully developed.

Studies of element imbalances, the difference between the element ratio of a consumer and that of its resource, have proven useful in identifying food-quality constraints on consumer success. For example, using such an approach, Tao & Hunter (2012) identified P-limitation of herbivorous insects, and Laspoumaderes et al. (2010) linked the element ratio of resources to copepod development. When applied to adult metazoan zooplankton, element ratios serve well as metrics of food quality and element imbalances are indicative of constraints on growth. For this group, low values of resource C:N or C:P indicate high-quality foods rich in the nutrients generally limiting to growth (N or P relative to C).

When applied to the flagellate *Ochromonas danica*, element ratios of prey bacteria may not adequately reflect their quality as food, particularly with respect to N. Imbalance plots (Fig. 5) and other data (Grover

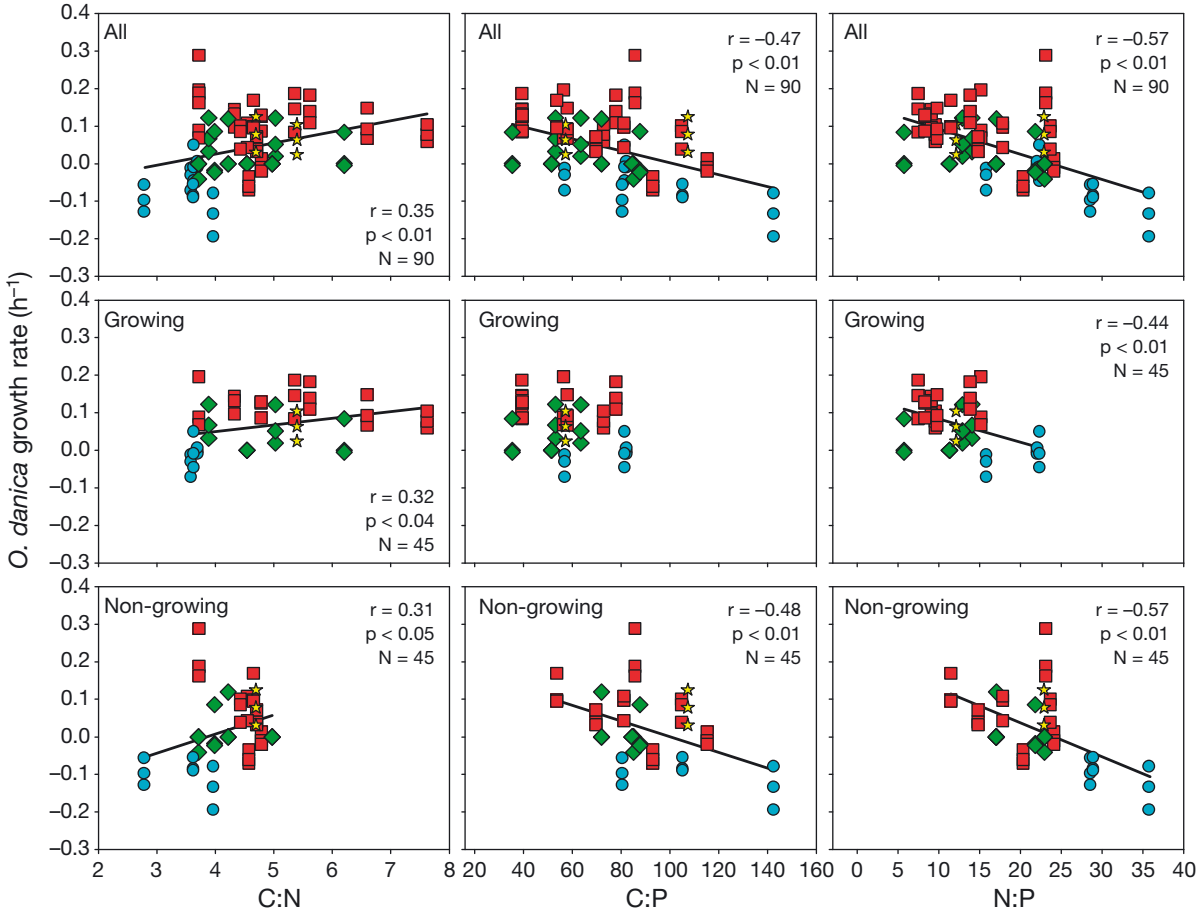

Fig. 4. *Ochromonas danica*. Growth rate of *O. danica* as a function of prey type, growth state, and element ratio. Top panels depict the relationship when all bacteria were considered; center panels, only growing bacteria; and bottom panels, only non-growing bacteria. Element ratios are mol:mol. Attributes describing linear regression models are given in each panel where correlations were found. See Fig. 1 for symbol descriptions

& Chrzanowski 2009) suggest that *O. danica* obtains sufficient N from bacteria to meet metabolic demands and that consumption of excess N relative to C or P has a cost to fitness (depressed growth rate). Alternatively, and similar to use with metazoan zooplankton, low values of prey C:P or N:P appear to be metrics indicative of high-quality prey.

Simple comparisons between the element ratio of a consumer and resource may mask more subtle factors influencing consumer fitness. For example, an element imbalance may not account for differences in the digestibility of individual prey (as suggested by the data in Figs. 1 & 2), for the efficiency at which a consumer assimilates individual elements, for potential prey toxicity (Matz et al. 2004), or for the potential for a prey item to supply an essential biochemical (Jónasdóttir 1994, Müller-Navarra et al. 2004). Nevertheless, a large imbalance, as a result of either nutrient in a pair, may point to decreased consumer growth as the excess nutrient still must be processed in some way (stored, excreted) (Eccleston-Parry & Leadbeater 1995, Hessen & Anderson 2008, Wang et al. 2009).

Metazoan zooplankton may compensate for an element imbalance by adjusting grazing rates. Consequently, the quantity of food available may modulate potential nutrient limitation and simultaneously exacerbate consumption of excess elements. The data presented in Fig. 3 (C:N and C:P) suggest this may also be true for *Ochromonas danica*. It appears that *O. danica* (as with all phagotrophic flagellates) faces something of a dilemma when preying upon bacteria. The flagellate consumes C, N, and P simultaneously, and element quotas of prey are likely to be correlated to each other. For the bacteria used here, there were strong positive correlations among the element pairs: C-N, $r = 0.95$, $n = 90$, $p < 0.001$; C-P, $r = 0.92$, $n = 90$, $p < 0.001$; N-P, $r = 0.87$, $n = 90$, $p < 0.001$.

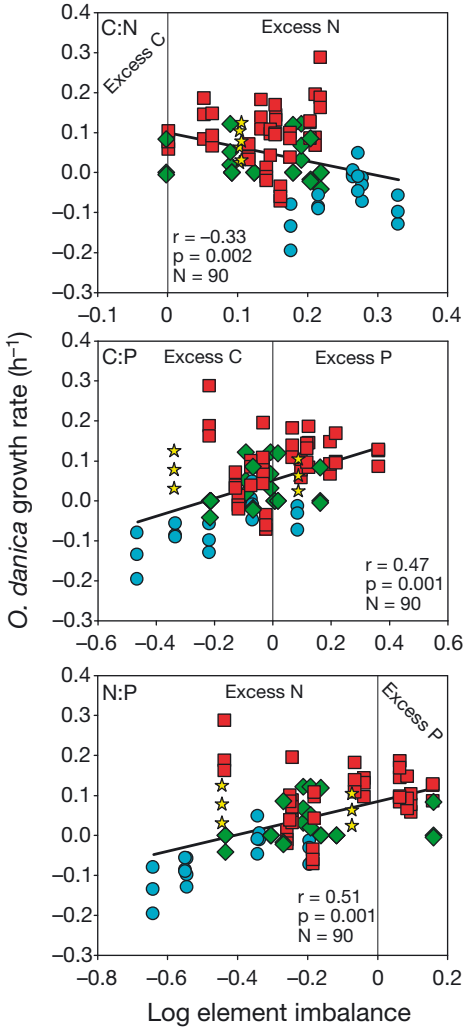

Fig. 5. *Ochromonas danica*. Growth rate of *O. danica* as a function of element imbalance between *O. danica* and prey. The imbalance is shown for C:N (top panel), C:P (center panel), and N:P (bottom panel) ratios. Attributes describing linear regression models are given in each panel. See Fig. 1 for symbol descriptions

Thus, consumption of a bacterium with a high C quota also meant consumption of a bacterium with a high N or P quota. So, even though the ratio of C:N may suggest a food of low quality with a cost to fitness, sufficient P may have been consumed to allow for improved growth (not necessarily positive) and, in part, compensate for the metabolic demands of processing excess N.

Ochromonas danica has been characterized as being weakly homeostatic; its element stoichiometry varies somewhat depending on the element stoichiometry of its resources, its prey (Chrzanowski et al. 2010), or the composition of the medium and the conditions under which it was grown (Simonds et al. 2010). The element stoichiometry (C:N:P) of *O. danica* harvested from chemostats averaged 66:10:1 (Table 3) and varied slightly depending upon batches of medium fed to chemostat reactors (data not shown).

Bacteria also do not appear to be strictly homeostatic with respect to their element content. *Vibrio splendidis* and *Pseudomonas fluorescens* have been shown to vary their element content (element μm^{-3}) based upon the medium in which they are grown (Løvdal et al. 2008, Grover & Chrzanowski 2009). *P. fluorescens* also varies its element content as a function of growth rate and temperature (Chrzanowski & Grover 2008).

In the experiments described here, the bacteria serving as prey were grown at a single temperature on a single type of medium considered to be a low-nutrient medium (R2A). *Ochromonas danica* was also grown in a chemostat to minimize variability in its macromolecular composition. These artificial conditions allowed us to demonstrate how an element imbalance between a consumer and its resource may constrain consumer fitness. In particular, the *Betaproteobacteria*, as grown under the conditions in this study, were prey deficient in P relative to C or N, which may explain why members of this group failed to support the growth of *O. danica* (Foster & Chrzanowski 2012b). It seems reasonable to ask if similar outcomes might be expected when nutrient or environmental conditions (resource pool, temperature) vary. Given that *O. danica* is weakly homeostatic, nutrient imbalances between it and its prey may play less of a role in determining fitness when not restricted by limited resources (i.e. a single prey item). A diet, broad in prey types, prey growth states, and perhaps element contents may lead to improved consumer fitness. The recent work of Šimek et al. (2013) seems to support this argument.

In their work, several strains of *Betaproteobacteria* isolated from freshwaters were used as prey for a diverse group of flagellates enriched from lake water. In their experiments (typically lasting up to 60 h), flagellate growth rates varied depending upon the strain of *Betaproteobacteria* used as prey. While lacking a quantifiable metric of food quality, they describe a strain of bacteria as 'high-quality food' if it supported rapid growth of some component of the flagellate community. Different strains of *Betaproteobacteria* allowed for differential growth rates among flagellate populations and brought about a shift in flagellate community structure. Our data imply that prey stoichiometry has little impact on predator grazing rate, but a large impact on predator

fitness (as growth rate). When considered together (i.e. Šimek et al. 2013 and the present study), the results suggest that different strains of bacteria are of different nutritional values to consumers.

We conclude from our work and from that of Šimek et al. (2013) that bacteria (sensu lato) should not be considered to be of similar nutritional quality to all members of the flagellate community. When extended to aquatic systems, these findings suggest that shifting resources bring about shifts in bacterial community structure (Buck et al. 2009, Newton et al. 2011), with concomitant shifts in the quality of bacteria as food. This shift in bacterial community structure likely cascades upward, bringing about shifts in the community of bacterivores and, perhaps, shifts in nutrient-element regeneration (Wang et al. 2009).

Acknowledgements. We thank Walton Lee for his assistance with the technical work. Many of the ideas presented in this study were developed during long conversations with Jim Grover (University of Texas Arlington). His contributions to this work are gratefully acknowledged. The thoughtful comments of several anonymous reviewers improved the presentation of this work.

LITERATURE CITED

Aaronson S, Baker HA (1959) A comparative biochemical study of two species of *Ochromonas*. J Protozool 6: 282–284

Anderson TR, Hessen DO, Elser JJ, Urabe J (2005) Metabolic stoichiometry and fate of excess carbon and nutrients in consumers. Am Nat 165:1–15

Barlett MA, Leff LG (2010) The effects of N:P ratio and nitrogen form on four major freshwater bacterial taxa in biofilms. Can J Microbiol 56:32–43

Bratbak G (1985) Bacterial biovolume and biomass estimations. Appl Environ Microbiol 49:1488–1493

Buck U, Grossart HP, Amann R, Pernthaler J (2009) Substrate incorporation patterns of bacterioplankton populations in stratified and mixed water of a humic lake. Environ Microbiol 11:1854–1865

Cardinale BJ, Hillebrand H, Harpole WS, Gross D, Ptacnik R (2009) Separating the influence of resource 'availability' from resource 'imbalance' on productivity–diversity relationships. Ecol Lett 12:475–487

Cebrian J, Shurin JB, Borer ET, Cardinale BJ, Ngai JT, Smith MD, Fagan WF (2009) Producer nutritional quality controls ecosystem trophic structure. PLoS ONE 4:e4929

Chrzanowski TH, Grover JP (2008) Element content of *Pseudomonas fluorescens* varies with growth rate and temperature: a replicated chemostat study addressing ecological stoichiometry. Limnol Oceanogr 53: 1242–1251

Chrzanowski TH, Šimek K (1990) Prey-size selection by freshwater flagellated protozoa. Limnol Oceanogr 35: 1429–1436

Chrzanowski TH, Šimek K (1993) Bacterial growth and losses due to bacterivory in a mesotrophic lake. J Plankton Res 15:771–785

Chrzanowski TH, Lukomski NC, Grover JP (2010) Element stoichiometry of a mixotrophic protist grown under varying resource conditions. J Eukaryot Microbiol 57: 322–327

Dickman EM, Newell JM, González MJ, Vanni MJ (2008) Light, nutrients, and food-chain length constrain planktonic energy transfer efficiency across multiple trophic levels. Proc Natl Acad Sci USA 105:18408–18412

Eccleston-Parry JD, Leadbeater BSC (1995) The regeneration of phosphorus and nitrogen by four species of heterotrophic nanoflagellates feeding on three nutritional states of a single bacterial strain. Appl Environ Microbiol 61:1033–1038

Edgar SM, Andersen RA (2003) The systematics of *Ochromonas* (Chrysophyceae). J Phycol 39(Suppl):14

Foster BLL, Chrzanowski TH (2012a) A simple defined medium for growth and maintenance of the mixotrophic protist *Ochromonas danica*. J Microbiol Methods 88: 322–324

Foster BLL, Chrzanowski TH (2012b) The mixotrophic protist *Ochromonas danica* is an indiscriminant predator whose fitness is influenced by prey type. Aquat Microb Ecol 68:1–11

Gebre-Mariam Z, Taylor WD (1989) Heterotrophic bacterioplankton production and grazing mortality rates in an Ethiopian rift-valley lake (Awassa). Freshw Biol 22:369–382

Grover JP (2004) Predation, competition and nutrient recycling: a stoichiometric approach with multiple nutrients. J Theor Biol 229:31–43

Grover JP, Chrzanowski TH (2009) Dynamics and nutritional ecology of a nanoflagellate preying upon bacteria. Microb Ecol 58:231–243

Heinbokel JF (1978) Studies on the functional role of tintinnids in the southern California bight. 1. Grazing and growth rates in laboratory cultures. Mar Biol 47:177–189

Hessen DO, Anderson T (2008) Excess carbon in aquatic organisms and ecosystems: physiological, ecological, and evolutionary implications. Limnol Oceanogr 53: 1685–1696

Hessen DO, Færøvig PJ, Andersen T (2002) Light, nutrients, and P:C ratios in algae: grazer performance related to food quality and quantity. Ecology 83:1886–1898

Hillebrand H, Borer ET, Bracken MES, Cardinale BJ and others (2009) Herbivore metabolism and stoichiometry each constrain herbivory at different organizational scales across ecosystems. Ecol Lett 12:516–527

Hobbie JE, Daley RJ, Jasper S (1977) Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl Environ Microbiol 33:1225–1228

Jiang L, Morin PJ (2005) Predator diet breadth influences the relative importance of bottom-up and top-down control of prey biomass and diversity. Am Nat 165:350–363

John EH, Davidson K (2001) Prey selectivity and the influence of prey carbon:nitrogen ratio on microflagellate grazing. J Exp Mar Biol Ecol 260:93–111

Jónasdóttir SH (1994) Effects of food quality on the reproductive success of *Acartia tonsa* and *Acartia hussonica*: laboratory investigations. Mar Biol 121:67–81

Kemp PF, Lee S, LaRoche J (1993) Estimating the growth rate of slowly growing bacteria from RNA content. Appl Environ Microbiol 59:2594–2601

Laspoumaderes C, Modenutt B, Balseiro E (2010) Herbivory versus omnivory: linking homeostasis and elemental imbalance in copepod development. J Plankton Res 32: 1573–1582

Løvdal T, Skjoldal EF, Heldal M, Norland S, Thingstad TF (2008) Changes in morphology and elemental composition of *Vibrio splendidus* along a gradient from carbon-limited to phosphate-limited growth. Microb Ecol 55:152–161

Matz C, Deines P, Boenigk J, Arndt H, Eberl L, Kjelleberg S, Jürgens K (2004) Impact of violacein-producing bacteria on survival and feeding of bacterivorous nanoflagellates. Appl Environ Microbiol 70:1593–1599

Mitra A, Flynn KJ, Fasham MJR (2007) Accounting for grazing dynamics in nitrogen–phytoplankton–zooplankton models. Limnol Oceanogr 52:649–661

Müller-Navarra DC, Brett MT, Park S, Chandra S, Ballantyne AP, Zorita E, Goldman CR (2004) Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. Nature 427:69–72

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

Newton RJ, Jones SE, Eiler A, McMahon KD, Bertilsson S (2011) A guide to the natural history of freshwater lake bacteria. Microbiol Mol Biol Rev 75:14–49

Okamura T, Mori Y, Nakano S, Kondo R (2012) Abundance and bacterivory of heterotrophic nanoflagellates in the meromictic Lake Suigetsu, Japan. Aquat Microb Ecol 66: 149–158

Salcher MM, Hofer J, Hornák K, Jezbera J and others (2007) Modulation of microbial predator–prey dynamics by phosphorus availability: growth patterns and survival strategies of bacterial phylogenetic clades. FEMS Microbiol Ecol 60:40–50

Shannon SP, Chrzanowski TH, Grover JP (2007) Prey food-quality affects flagellate ingestion rates. Microb Ecol 53: 66–73

Šimek K, Kasalický V, Jezbera J, Horňák K and others (2013) Differential freshwater flagellate community response to bacterial food quality with a focus on *Limnohabitans* bacteria. ISME J 7:1519–1530

Simonds S, Grover JP, Chrzanowski TH (2010) Element content of *Ochromonas danica*: a replicated chemostat study controlling growth rate and temperature. FEMS Microbiol Ecol 74:346–352

Sinsabaugh RL, Hill BH, Folstad-Shah JJ (2009) Eco-enzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462: 795–798

Starr RC (1978) The culture collection of algae at the University of Texas at Austin. J Phycol 14:47–100

Sterner RW, Elser JJ (2002) Ecological stoichiometry. Princeton University Press, Princeton, NJ

Strickland JD, Parsons TR (1972) A practical handbook of seawater analysis, 2nd edn. Bull Fish Res Board Can 167

Tao L, Hunter MD (2012) Does anthropogenic nitrogen deposition induce phosphorus limitation in herbivorous insects. Glob Change Biol 18:1843–1853

Wang H, Jiang L, Weitz JS (2009) Bacterivorous grazers facilitate organic matter decomposition: a stoichiometric modeling approach. FEMS Microbiol Ecol 69: 170–179

Watson SW, Novitsky TJ, Quimbly HL, Valois FW (1977) Determination of bacterial number and biomass in the marine environment. Appl Environ Microbiol 33: 940–946

White D (2007) The physiology and biochemistry of prokaryotes, 3rd edn. Oxford University Press, New York, NY

White D, Hageman GD (1998) Microbial physiology and biochemistry laboratory: a quantitative approach. Oxford University Press, New York, NY

Editorial responsibility: Karel Šimek, České Budějovice, Czech Republic

Submitted: April 18, 2013; Accepted: November 11, 2013
Proofs received from author(s): January 24, 2014