The stoichiometry of dissolved organic carbon, nitrogen, and phosphorus release by a planktonic grazer, *Daphnia*

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Abstract

We examined the relationship between *Daphnia* release rates and ratios of dissolved organic carbon (DOC), nitrogen (N), and phosphorus (P) and the elemental composition of algal food. We constructed a three-element (C, N, and P) mass-balance model to explore the relationships between food elemental composition and *Daphnia* release of DOC, N, and P. The model predicts that *Daphnia* DOC:N and DOC:P release ratios will increase with increasing food C:N and C:P ratios, respectively. These effects on DOC:N and DOC:P ratios are dependent, however, on the availability of the third element (P and N respectively) in our model. In addition, we experimentally measured the mass-specific release of DOC, ammonia (NH_4), and soluble reactive phosphorus (SRP) from *Daphnia magna* fed food of contrasting C:N:P ratios. *Daphnia* release rates of DOC were not significantly affected by algal C:N or C:P ratios. However, high C:N and C:P ratios in algal food raised the DOC:NH_4 and DOC:SRP ratios (respectively) released from *Daphnia*. Our results, experimental and modeling, show a strong dependence of DOC: N and DOC:P release ratios by *Daphnia* on food C:N and C:P ratio. Thus, elevated C:N or C:P ratios in food can cause imbalanced release of DOC and NH_4 or SRP, respectively, by *Daphnia* with potentially strong feedback on microbial heterotrophs in planktonic food webs.

Zooplankton can supply a substantial quantity of dissolved organic carbon (DOC) to microbial heterotrophs via sloppy feeding and excretion (Lampert 1978; Strom et al. 1997). This flux of consumer-derived DOC can be an important substrate for heterotrophic microbes in lakes and oceans (Hygum et al. 1997; Richardot et al. 2001). However, bacterial communities may be stimulated less than expected if DOC release is not accompanied by other growth-limiting nutrients (i.e., nitrogen and phosphorus). High ratios of DOC:N and DOC:P have been recently shown to cause N- and P-limited growth of aquatic bacteria (Goldman and Dennett 2000; Vadstein 2000). Here, we examine the release rates and ratios of DOC, N, and P by *Daphnia* as they relate to the elemental composition (C:N:P ratios) of algal food.

Herbivorous zooplankton release inorganic N and P in widely varying ratios (Elser and Urabe 1999). Variable release ratios are produced when consumers retain the element in least supply and discard elements that are in excess (Elser and Urabe 1999). Food N:P ratios that differ from that required for consumer growth and reproduction should either increase or decrease the N:P ratio being released (Sterner 1990). In turn, these higher or lower N:P release ratios feed back to primary producers and enhance potential growth limitation by P or N, respectively (Sterner 1990; Elser and Urabe 1999). Although considerable effort has focused on the release of N and P, the effects of stoichiometric food quality on consumer DOC release and its ratio with N and P have received less attention. One previous study (Darchambeau et al. 2003) has shown that the release rate of DOC by *Daphnia* is sensitive to the food C:P ratio. However, the release of C, relative to N and P, from planktonic herbivores consuming food of varying C:N and C:P content has yet to be addressed.

We examine the effects of high food C:N and C:P ratios on release ratios of DOC, N, and P from *Daphnia*. Our primary objective was to test the hypothesis that elemental food quality (in terms of C:N and C:P ratio) can cause the differential release of C, N, and P from this planktonic herbivore. We created a three-element mass-balance model that estimates the release of DOC, N, and P from *Daphnia* as a function of food C, N, and P content. In addition, we experimentally manipulated the food C:N and C:P ratio consumed by *Daphnia* and measured the rates and ratios of DOC, ammonia (NH_4), and soluble reactive phosphorus (SRP) release. Together, our results show that DOC:N and DOC:P release ratios from *Daphnia* are sensitive to food elemental composition.

Methods

Three-element release model—We derived a model based on mass-balance principles that estimates *Daphnia* release of C, N, and P as a function of the food C:N and C:P ratio. We modified a similar two-element growth model (presented in Sterner 1997 and Frost and Elser 2002) to include a third element and to estimate elemental release. Our current model estimates the release of three elements on the basis of the assumption that the limiting element is retained at a maxi-

Table 1. List of parameters and sources used in the three-element *Daphnia* release model. Note that grazing rates and handling time are mass-specific (per mg C) values for individual *Daphnia* derived from the listed source.

Parameter	Symbol	Unit	Value	Derived from
Grazing rate	g	L mg C^{-1} day^{-1}	7.34	Lampert and Muck 1985
Handling time	T_h	day mg C mg C^{-1}	1.04	Lampert and Muck 1985
Food C:P ratio	$f_{C:P}$	mol C mol P^{-1}	50–1000	Elser et al. 2000
Food C:N ratio	$f_{C:N}$	mol C mol N^{-1}	2–20	Elser et al. 2000
C accumulation efficiency	A_C	None	0.5	
N accumulation efficiency	A_N	None	0.9	
P accumulation efficiency	A_P	None	0.9	
Respiration rate	R_C	mg C mg C^{-1} day^{-1}	0.05	Lampert 1986
C content of *Daphnia* dry mass	Q_C	mg C mg DW^{-1}	0.480	Andersen and Hessen 1991
N content of *Daphnia* dry mass	Q_N	mg N mg DW^{-1}	0.094	Andersen and Hessen 1991
P content of *Daphnia* dry mass	Q_P	mg P mg DW^{-1}	0.014	Andersen and Hessen 1991

mum accumulation efficiency (sensu Sterner 1990), whereas the other two elements are released in excess of demand. Because this approach requires the knowledge of which element (C, N, or P) limits growth as a function of the food C:N and C:P ratio, we first determined, in three-dimen-
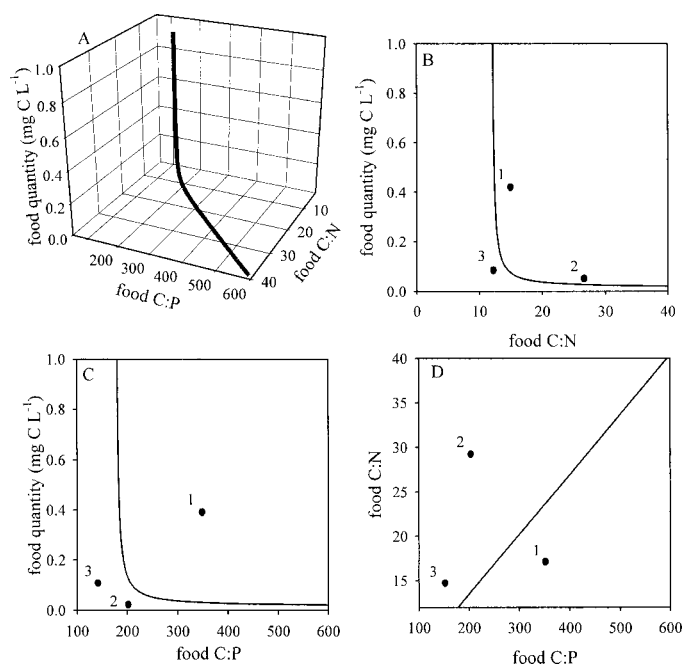

Fig. 1. Model predictions of three-element isocline delineating *Daphnia* growth limitation by food quantity, C:N ratio, and C:P ratio (all ratios are molar). This isocline can be viewed (A) in three dimensions or (B–D) in any two dimensions. Food of a given quality ($f_{C:N}$, $f_{C:P}$) and quantity (C_f) can be plotted in this three-dimensional space and used to predict which element (C, N, or P) likely limits *Daphnia* growth. By sequentially examining the position of a particular food in panels B–D, one can determine which element is limiting. For example, consider three foods (points 1–3) of differing C:N ratio, C:P ratio, and food quantity. Food 1 is located above the C versus N and P isoclines in panels B and C, which indicates that *Daphnia* consuming this food would not be C limited. In panel D, food 1 is below the N versus P isocline, which indicates that P and not N would limit *Daphnia* growth on this food. Using the same sequential method, foods 2 and 3 are shown to be N and C limiting (respectively) for *Daphnia* growth.

sional space, regions of C-, N-, and P-limited growth for *Daphnia*. To do so, we calculated a three-dimensional growth-limitation isocline (*see Table 1* for an explanation of the parameters and values used in this model):

$$C_f^N = \frac{R_C \times Q_N}{g\left(A_C \times Q_N - \dfrac{A_N \times Q_C}{f_{C:N}} - R_C \times T_h \times Q_N\right)} \quad (1)$$

$$C_f^P = \frac{R_C \times Q_P}{g\left(A_C \times Q_P - \dfrac{A_P \times Q_C}{f_{C:P}} - R_C \times T_h \times Q_P\right)} \quad (2)$$

where C_f^N and C_f^P are food quantities that separate regions of N- and P-limited (respectively) growth from regions of C-limited growth by *Daphnia*. Taking the special case of $C_f^N = C_f^P$ and solving for the food C:P ratio gives

$$f_{C:P} = \frac{A_P}{A_N} \times \frac{Q_N}{Q_P} \times f_{C:N} \quad (3)$$

Equation (3) divides the non–C-limited space into regions of N- and P-limitation. Putting together Eqs. 1–3 creates a three-dimensional isocline of "ideal" *Daphnia* growth with respect to food C, N, and P (Fig. 1). The location of a food with a given elemental character ($f_{C:N}$, $f_{C:P}$, and C_f) in this three-dimensional space predicts whether C, N, or P limits growth rates *Daphnia* (*see explanation in Fig. 1*).

With the knowledge of which element (C, N, or P) limits *Daphnia* growth, release rates of these elements can be calculated by rearranging the above equations. Rates for C, N, and P release from *Daphnia* were determined under the assumption that the limiting nutrient governs the amount of any other nutrient's accumulation. For example, under C limitation,

$$C_{release} = C_{ingested} - C_{accumulated} - C_{respiration} \quad (4)$$

where C ingestion is a type II function of food quantity and the C accumulated is a fixed proportion of ingested C. The release rate is then calculated after substitution, as shown in Table 2. N and P release are estimated for a C-limited *Daphnia* by determining the amount of element ingested minus the amount incorporated by assuming a constant body C: nutrient ratio in new body mass (Table 2). This same ap-

Table 2. Equations governing the release of C, N, and P given growth limitation of *Daphnia* by C, N, or P. Parameter values are given in Table 1.

	C limitation	N limitation	P limitation
C release	$\dfrac{C_f \times g}{1+(C_f \times g \times T_h)} \times A_C - R_C$	$\dfrac{C_f \times g}{1+(C_f \times g \times T_h)} - \dfrac{C_f \times g}{1+(C_f \times g \times T_h)} \times \dfrac{Q_C}{Q_N} - R_C \times \dfrac{1}{f_{C:N}} \times A_N$	$\dfrac{C_f \times g}{1+(C_f \times g \times T_h)} - \dfrac{C_f \times g}{1+(C_f \times g \times T_h)} \times \dfrac{Q_C}{Q_P} - R_C \times \dfrac{1}{f_{C:P}} \times A_P$
N release	$\dfrac{C_f \times g}{1+(C_f \times g \times T_h)} \times \dfrac{1}{f_{C:N}} - \dfrac{C_f \times g}{1+(C_f \times g \times T_h)} \times A_C \times \dfrac{Q_N}{Q_C}$	$\dfrac{C_f \times g}{1+(C_f \times g \times T_h)} \times \dfrac{1}{f_{C:N}} - \dfrac{C_f \times g}{1+(C_f \times g \times T_h)} \times A_N$	$\dfrac{C_f \times g}{1+(C_f \times g \times T_h)} \times \dfrac{1}{f_{C:N}} - \dfrac{C_f \times g}{1+(C_f \times g \times T_h)} \times A_P \times \dfrac{Q_N}{Q_P}$
P release	$\dfrac{C_f \times g}{1+(C_f \times g \times T_h)} \times \dfrac{1}{f_{C:P}} - \dfrac{C_f \times g}{1+(C_f \times g \times T_h)} \times A_C \times \dfrac{Q_P}{Q_C}$	$\dfrac{C_f \times g}{1+(C_f \times g \times T_h)} \times \dfrac{1}{f_{C:P}} - \dfrac{C_f \times g}{1+(C_f \times g \times T_h)} \times A_N \times \dfrac{Q_P}{Q_N}$	$\dfrac{C_f \times g}{1+(C_f \times g \times T_h)} \times \dfrac{1}{f_{C:P}} - \dfrac{C_f \times g}{1+(C_f \times g \times T_h)} \times A_P$

Table 3. Elemental composition of *S. acutus* grown with different nutrient media as outlined in Sterner et al. (1993). Note that all ratios are molar.

Food type	%C	%N	%P	C:N ratio	C:P ratio	N:P ratio
MON	44.6	7.56	1.02	7.38	117	15.7
LOP	50.1	6.13	0.19	10.2	691	67.7
LON	47.1	1.94	1.75	30.4	71.7	2.36

proach was used to calculate C, N, and P release when N or P was limiting *Daphnia* growth (Table 2). Because we were interested in the effect of food C:N and C:P ratios on elemental release, we held food quantity (C_f) constant in our model above a level (1 mg C L^{-1}) that saturates *Daphnia's* ingestion rates. In addition, our model calculates total carbon release and does not specifically differentiate between the release of particulate organic carbon and DOC. We converted the total C released into DOC released using the assumption that 10% of released carbon was dissolved.

Experimental procedure—We conducted an experiment to measure the release of DOC, NH_4, and SRP from juvenile *Daphnia magna* consuming algae (*Scenedesmus acutus*) of contrasting C:N and C:P ratios. *Daphnia* were grown in aged tap water in laboratory rearing chambers from birth for 20 d to an approximate dry weight of 70 μg while being provided with high availability of nutrient-rich (low C:N and low C:P ratios) *Scenedesmus* as food. Before the release experiment, *Daphnia* were separated into six 3-liter beakers that contained *Scenedesmus* (at saturating concentrations) of one of three types (using notation as in Sterner et al. 1993): medium on nitrogen (MON), low on nitrogen (LON), and low on phosphorus (LOP). The three types of *Scenedesmus* (MON, LON, and LOP) were grown in batch cultures (2 liters each) that contained growth media with different amounts of N and P (*see* Sterner et al. 1993 for media recipe). *Daphnia* were fed one of these three food types for 3 d before the excretion experiment. Food was replenished daily to maintain saturating concentrations. Samples of MON, LOP, and LON *Scenedesmus* were saved for the analysis of C, N, and P content. The algal food (MON, LOP, and LON) being fed to *Daphnia* had contrasting C:N, C:P, and N:P ratios (Table 3).

On the day of the experiment, we transferred *Daphnia* into excretion chambers and measured their release of DOC, NH_4, and SRP. All *Daphnia* were rinsed three times with distilled water before being placed in the excretion chamber to minimize the concurrent transfer of dissolved nutrients and unconsumed *Scenedesmus*. We placed 14 *Daphnia* into 200 ml of distilled water in each excretion chamber to ensure measurable quantities of DOC, NH_4, and SRP were collected over the 4-h excretion period. No food was given to *Daphnia* during the excretion experiment, to eliminate the possibility of DOC contamination from algal release and sloppy feeding. After 4 h, water from the excretion chamber was filtered through a 0.2-μm polycarbonate filter (prerinsed to remove potential carbon contaminants; Yoro et al. 1999) and refrigerated until chemical analysis. *Daphnia* were collected onto
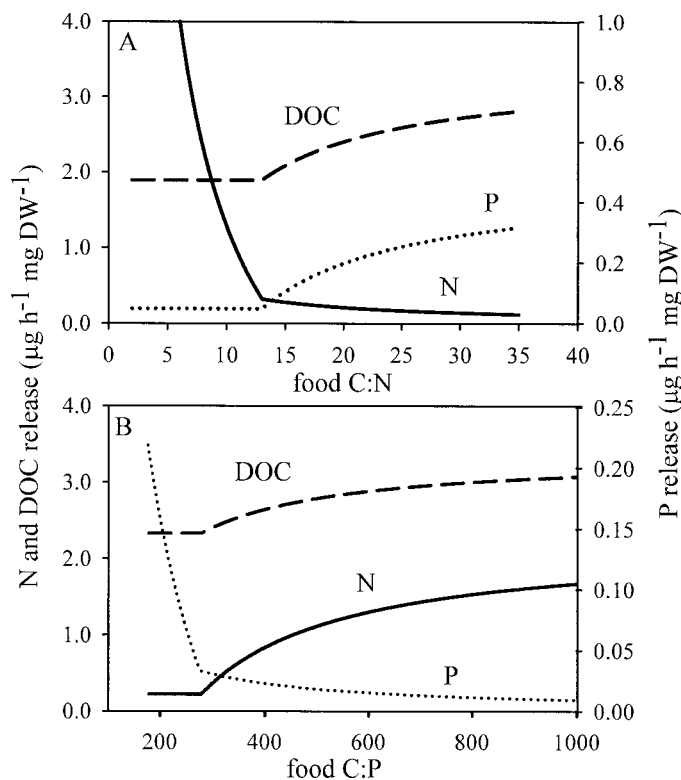
Fig. 2. Predicted release rates by *Daphnia* of DOC, N, and P by our model as a function of (A) food C:N ratio and (B) C:P ratio. Note that, in panel A, the food C:P ratio is held constant at 200, and, in panel B, the food C:N ratio is held constant at 20.

filters, dried at 60°C, and weighed with an electronic micro-balance.

Chemical analysis—DOC was measured with a Shimadzu TOC analyzer after acidification and purging of dissolved inorganic carbon (Sharp et al. 1993). We quantified SRP with a spectrophotometer using the molybdate blue-ascorbic acid technique (American Public Health Association 1992). NH_4 was measured with a spectrophotometer using the phenate method (American Public Health Association 1992). We also measured the C, N, and P content of different algal food (MON, LON, and LOP) provided to *Daphnia.* Particulate carbon and nitrogen were measured on a Costech elemental analyzer (ECS 4010). Particulate phosphorus was measured with the SRP method as described above after digestion of each sample with 5% persulfate in an autoclave (American Public Health Association 1992).

Results

Three-element model results—The three-element release model showed *Daphnia* release of C, N, and P to be a function of food C:N and C:P ratios (Fig. 2). Increasing the food C:N ratio led to lower rates of N release and increased rates of release of P and DOC (Fig. 2). As a consequence, DOC:N release ratios increased with increasing food C:N consumed by *Daphnia* (Fig. 3). At moderate food C:N ratios (10–20), increasing the food C:P ratio reduced DOC:N re-
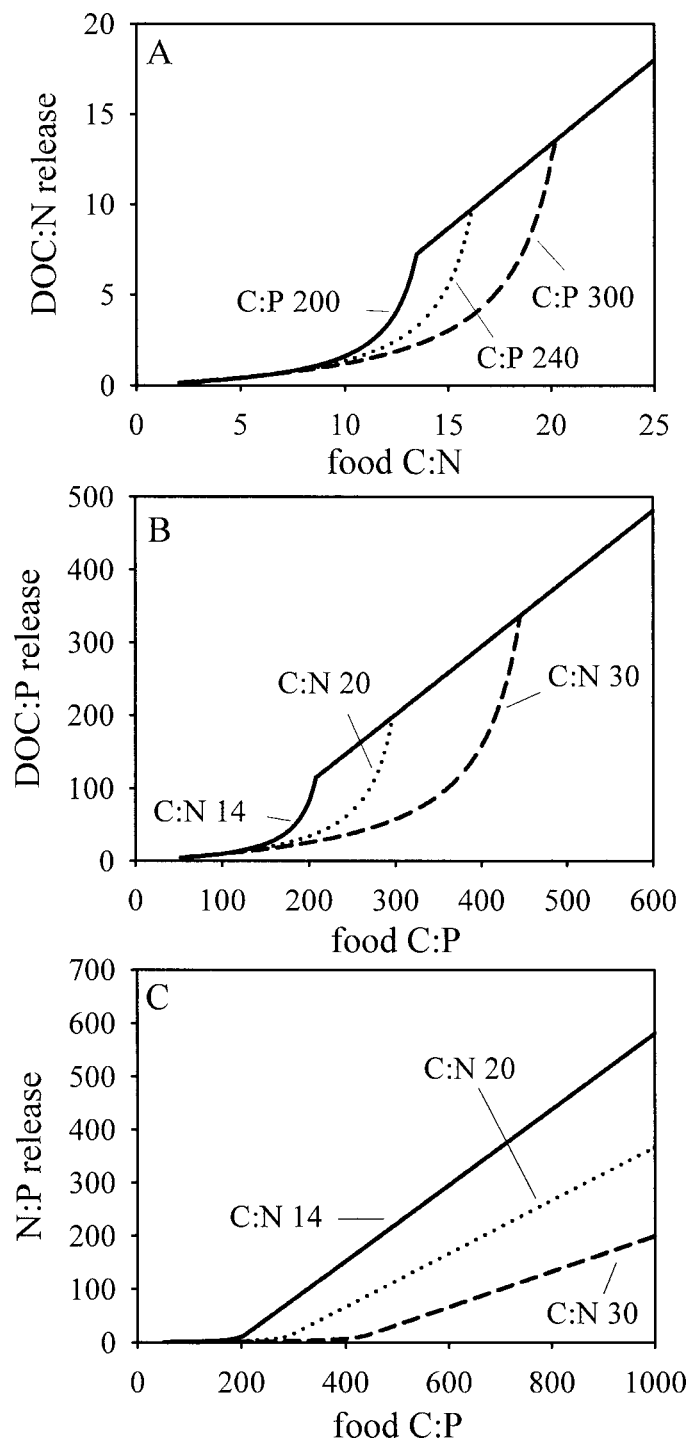

Fig. 3. Predicted release ratios (all molar) by *Daphnia* of (A) DOC:N, (B) DOC:P, and (C) N:P with changing food C:P and C:N ratios, as calculated by our model. Note that, in all cases, C_f is held above the incipient saturating concentration for *Daphnia* (1 mg C L^{-1}).

lease ratios by *Daphnia* (Fig. 3) as N release rates increased, but DOC release rates remained unchanged (results not shown). The release of DOC, N, and P also changed dispro-portionately as a function of the food C:P ratio (Fig. 2). These changes in release rates due to increasing food C:P
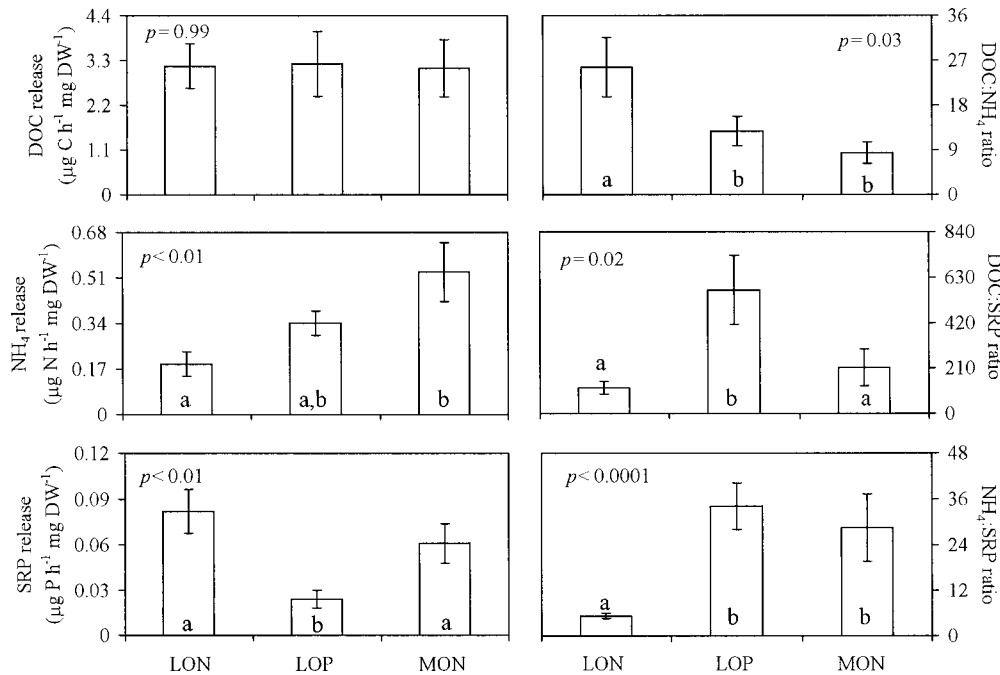
Fig. 4. Effects of food elemental composition on DOC, NH$_4$, and SRP release rates and ratios (all molar) by *Daphnia magna*. Shown are the mean and standard error of six replicate excretion jars of *Daphnia*. See Table 3 for food C:N, C:P, and N:P ratios of LON, LOP, and MON algal food. Treatment (food C:N:P ratio) effects were assessed on mass-specific release rates and ratios (after natural log transformation) with one-way ANOVA on JMP (version 4). Food types with different letters were significantly different from each other at $p < 0.05$ (Tukey's HSD test).

ratio altered the ratios of DOC:P being released by *Daphnia* (Fig. 3). Increasing the food C:N ratio also reduced DOC:P release rates at intermediate food C:P ratios (Fig. 3) because of increased released rates of P but unchanged DOC release rates (results not shown). Similar to previous models (i.e., Sterner 1990), we found that the *Daphnia* N:P release was a linear function of the food N:P ratio (data not shown). As such, increasing food C:P ratio (with C:N ratio fixed) in our model caused increased N:P release by *Daphnia* (Fig. 3).

Experimental results—Mass-specific rates of DOC release from *Daphnia* were not significantly affected by food type (LON, LOP, or MON) in our experiment (Fig. 4). *Daphnia* release of NH$_4$ was significantly reduced by the N-poor LON algae (Fig. 4). Similarly, *Daphnia* released significantly less SRP when eating the P-poor LOP algae (Fig. 4). These changes in nutrient release produced by the different algal foods translated into significant differences in the DOC:NH$_4$ and DOC:SRP ratios released by *Daphnia*. DOC:NH$_4$ release ratios were significantly higher from *Daphnia* eating the LON food, compared with the other two food types (MON or LOP; Fig. 4). Similarly, *Daphnia* released higher DOC:SRP ratios when eating the LOP food (Fig. 4). NH$_4$:SRP release ratios were also affected by the elemental composition of food. *Daphnia* eating MON and LOP (both with high N:P ratios) released higher N:P ratios than *Daphnia* eating the low N:P ratio, LON algal food.

Discussion

Poor elemental food quality strongly affects the growth (Sterner and Schulz 1998), reproduction (Urabe and Sterner 2001), and nutrient release (Elser and Urabe 1999) of zooplankton consumers. Stoichiometric constraints are created when the demand for a particular element (e.g., N or P) by a consumer is not matched to the supply of that nutrient in food (Frost et al. 2002). When ingested carbon is in excess of that needed for growth and reproduction, it must be eliminated through greater respiration, lower assimilation (higher egestion), or higher rates of excretion (Darchambeau et al. 2003). Under these circumstances, the growth-limiting element should be extracted and assimilated with greater efficiency (Elser and Urabe 1999; Sterner and Elser 2002). These physiological adjustments should produce elevated C:nutrient ratios being released from consumers eating food of high C:nutrient ratios. Our modeling and experimental results are consistent with this prediction: *Daphnia* release ratios of DOC:N and DOC:P increased as a function of food C:N and C:P ratios, respectively.

We derived a release model that incorporates information about food elemental composition, consumer ingestion, respiration, and net accumulation of three elements (C, N, and P). This model adjusts the retention and, hence, the release of nonlimiting elements to the growth demand created by the element in least supply. Results from this model are qualitatively and quantitatively consistent with our experimental observations of DOC, N, and P release from the *Daphnia*.

The three-element model further showed DOC:N and DOC:P release ratios to increase as a function of food C:N and C:P ratios, respectively (Fig. 3). These release ratios were also a function of the supply of the other nutrient (N vs. P). For example, increasing food C:N ratios reduced DOC:P release ratios because, under N limitation, there is less demand for P for new biomass construction; thus, more P is left over (even if the food is relatively low in P; Fig. 3). Mass-balance models that incorporate only two elements (e.g., Frost and Elser 2002) miss this interaction, because they assume that all nondefined elements are present in excess of their demand.

One aspect of our model that will require additional refinement is the assumption that a fixed percentage of carbon and nutrients is released in the dissolved form. The percentage of total release in dissolved form likely varies and would reflect two processes not explicitly separated in our model: (1) the assimilation of the ingested element, and (2) the conversion of assimilation into new growth. Future release models and experiments should explicitly consider the efficiency of these two processes separately, because they would allow for improved predictions of dissolved and particulate release as a function of elemental food quality. In this case, it is possible that the form of element released (dissolved or particulate) is dependent on the food C:N or C:P ratio. For example, less assimilation by *Daphnia* of P-poor algal cells would presumably lower the percentage of released carbon found in the dissolved phase. However, there is conflicting evidence about whether changing the P content of food does or does not affect assimilation efficiencies of C, N, and P (Darchambeau et al. 2003). Future work should address these uncertainties and examine how food C:N:P ratios affect the type (particulate vs. dissolved) of carbon and nutrients being released by *Daphnia* and other zooplankton.

In our experiment, we found elevated DOC:N and DOC:P release ratios from *Daphnia* were not caused by higher DOC release rates but instead by decreased N and P excretion. In contrast, Darchambeau et al. (2003) found that increasing the food C:P ratio produced greater DOC release by *Daphnia*. One explanation for our different results is the length of time over which *Daphnia* was exposed to the different food treatments before the release experiment. We fed *Daphnia* the different food types for 3 d, whereas Darchambeau et al. (2003) fed experimental animals for <4 h. During our longer incubation period, *Daphnia* may have reduced its ingestion rate of the N- and P-poor food. Reduced ingestion has been found in *Daphnia* eating P-limited algae during longer incubations (>20 h; Van Donk and Hessen 1993; Sterner and Smith 1993) and may result in less DOC excretion. On the other hand, ingestion rates were not affected by food C:P ratio during experiments with both short (<4 h; Darchambeau et al. 2003) and long (~40 h; DeMott et al. 1998) prerelease feeding periods. Clearly, more work is needed to better quantify how the flux of DOC produced relates to ingestion and assimilation in zooplankton eating food of differing C:N:P ratios for short and long periods of time. Nevertheless, we found that poor elemental food quality altered the balance of elements released from *Daphnia* eating food of contrasting C:N and C:P ratios.

Alternatively, DOC release may not have been affected by food C:N or C:P ratios in our experiments, because excess C was lost through greater rates of respiration, which limited carbon excretion (Sterner 1997). Darchambeau et al. (2003) found greater respiration in *Daphnia* that consumed algae of high C:P ratios. Similarly, low-P algal food increased the appendage beat rates of *Daphnia* and, presumably, their rates of respiration (Plath and Boersma 2001). Such increases in respiratory carbon loss would reduce the likelihood of observing changes in DOC release from *Daphnia* consuming food of high C:N and C:P ratios. However, even no change in DOC release rates could affect DOC:N and DOC:P release ratios, given the sensitivity of N and P excretion to elemental food quality.

Our three-element model approach could, in theory, be used to determine the likelihood of consumer growth limitation by *x* number of elements. This would be accomplished by sequentially eliminating nonlimiting elements using information about their relative supply in food and the demand by an animal for growth and reproduction. For two elements, one equation (i.e., Eq. 1) is needed to determine which element is in relative least supply. For three elements (as shown here), one must use three equations (i.e., Eqs. 1, 2, and 3) to decide which element is likely limiting growth of the consumer. For *x* number of elements, $\sum_{n=1}^{x}(n-1)$ equations are needed to sequentially eliminate the nonlimiting elements. This approach provides a quantitative method to narrow the list of biogenic elements (of which there are 22 in humans; Sterner and Elser 2002) that potentially limit consumer growth and reproduction.

The imbalanced release of carbon, relative to nitrogen or phosphorus, by planktonic grazers has implications for the growth and metabolism of heterotrophic bacteria in pelagic food webs. Aquatic bacteria can have relatively high growth N and P demands (i.e., Chrzanowski and Kyle 1996) and have exhibited limited flexibility in adjusting these demands to the supply of available nutrients (Makino et al. 2003). High DOC:N and DOC:P ratios could thereby place elemental constraints on bacterial growth and cause them to become N or P limited (Goldman and Dennett 2000; Vadstein 2000). Growth limitation of aquatic bacteria by N or P would, in turn, affect their competitive interactions with primary producers (e.g., Olsen et al. 2002). Bacteria can strongly compete with planktonic algae for growth-limiting nutrients and, thereby, may limit algal responses to consumer-driven nutrient recycling. For example, heterotrophic bacteria contributed to severe P limitation in phytoplankton, even at higher levels of P supply, as was seen during a mesocosm experiment in an oligotrophic lake (Elser et al. 2002). As a consequence, interactions between zooplankton and primary producers may be affected by consumer release ratios of DOC:N and DOC:P. As such, the responses of aquatic bacteria to changes in the multiple resource environment (DOC, N, and P) produced by grazers needs to be further investigated. In addition, future work should consider how the imbalanced release of DOC and inorganic nutrients, as shown here, alters consumer–microbe interactions in aquatic systems.

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