# Consumer nutrient stoichiometry: patterns, homeostasis, and links with fitness.

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**Abstract**

The linkages between food webs and nutrient cycles are heterogeneous and often influenced by human activities. Ecological stoichiometry provides one framework for understanding and predicting these linkages. Yet, as it has been extended underlying assumptions are often not evaluated. This dissertation shows that examination of implicit and explicit assumptions reveals unknown mechanisms, interactions, and linkages. For instance, theory assumes that invertebrate stoichiometry does not vary with diet stoichiometry (i.e., strict homeostasis), even though many invertebrates are not strictly homeostatic. Chapters one and two examine the role of stoichiometric homeostasis in shaping the fitness of *Daphnia* species. Chapter one shows that the long-term phosphorus (P) use efficiency of stoichiometrically flexible *Daphnia* species is higher in habitats with temporally variable diets, resulting in higher fitness relative to strictly homeostatic species. Chapter two shows that the P cost of a unit of growth increased with growth rate and structures tradeoffs among growth rate, sensitivity to P limitation, and stoichiometric flexibility.

Stoichiometric theory can be extended to novel ecosystems, such as streams, to predict the role of consumers in food web and nutrient cycles. To do this, the balance between consumer and diet stoichiometries is a logical starting point. Chapter three examines intra-specific variation in consumer-resource stoichiometries at a suite of sites within a river network. In contrast to previous work, this chapter describes wide intra-specific variation in consumer stoichiometry, similar in magnitude to the variation among invertebrate taxa. Intra-specific variation in nitrogen and phosphorus content was related to both ontogeny and diet. These results suggest that the role of a species in stream nutrient cycles could vary spatially with diet and temporally through ontogeny. Chapter four examines the influence of diet stoichiometry on nutrient release ratios of four stream detritivores. Predictions of nutrient release ratios from bulk diet stoichiometries were misleading for these detritivores, which selectively consumed a

nutrient rich portion of the bulk diet. Selective feeding greatly reduced stoichiometric mismatches between these consumers and their diets. Taken together, this dissertation demonstrates that examination of stoichiometric assumptions improves our understanding of consumer-resource dynamics, competition, and the role of consumers in nutrient cycles.

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## INTRODUCTION

The natural world is characterized by a dazzling diversity of organisms, dynamics, and elemental flow pathways. One goal of ecologists is to develop predictive theories that simplify and explain this complexity. Ecological stoichiometry is one promising approach. It uses elemental mass balances to understand competition, consumer-resource dynamics, and nutrient cycles (Sterner and Elser 2002). This work commonly focuses on carbon (C) as a surrogate for energy as well as nitrogen (N) and phosphorus (P) because these elements are two common constraints on autotrophic production in terrestrial and aquatic systems (Elser et al. 2009).

A key trait in these mass balance models is the carbon to nutrient ratio (C:X, where X is N or P) of the consumer. The C:X of the consumer is thought to reflect nutrient demand and, therefore, is used to predict the nutrient requirements of growth, sensitivity to nutrient-limitation, as well as nutrient regeneration rates and ratios (Sterner and Hessen 1994, Elser and Urabe 1999). Simultaneously, consumer C:X reflects allocation of nutrients to structure and growth machinery. For example, growth requires investment in P-rich ribosomal RNA for protein synthesis. The growth rate hypothesis (GRH) predicts a tripartite linkage among growth, consumer P content, and percent r-RNA (Elser et al. 2003). Thus, theory predicts P-rich consumers should have rapid maximum growth rates and high P requirements that should lead to increased sensitivity to P-limitation of growth. The C:X of the consumer has been used to

understand or predict species distributions, consumer-resource dynamics, and the role of consumer in nutrient cycles (Sterner and Elser 2002).

Stoichiometric mass balances almost universally assume strict homeostasis for the consumer (Andersen et al. 2004). Homeostasis is defined as the regulation of consumer stoichiometry in response to variation in resource stoichiometry, when life stage and other environmental factors are constant. Strict homeostasis occurs when consumer stoichiometry does not vary in response to wide variation in resource stoichiometry. Although the N or P homeostasis of invertebrates is not as weak as autotrophs, not all invertebrates are strictly homeostatic for N or P (Persson et al. 2010). Violation of the strict homeostasis assumption leads to two important questions. First, how does among species variation in degree of homeostasis influence competitive ability? Second, does violation of the strict homeostasis assumption influence the application of stoichiometric predictions? The former question is the primary motivation behind the first two chapters of this dissertation. The last chapters examine how underlying assumptions influence the application of stoichiometric prediction to stream ecoystems.

*Stoichiometric homeostasis and competitive ability*

Chapter one examines how variation in stoichiometric homeostasis influences competitive ability in experimental environments with different levels of temporal variation in dietary P content. There is a long history of linking physiological flexibility

with a competitive advantage in variable environments (Sommer 1985, Raubenheimer and Jones 2006), although this linkage has not been examined with respect to stoichiometric homeostasis. In this chapter, I examine this hypothesis with theoretical and experimental approaches, using species of the pelagic Cladoceran *Daphnia* as model organisms. This work shows that stoichiometrically flexible *Daphnia* use excess P from high P diets to supplement growth on low P diets. This integration of P across diets results in higher P use efficiencies and growth rates over the long-term than predicted by a time-weighted average of component growth rates (i.e., low P or high P growth rates). Thus, all else being equal, stoichiometrically flexible individuals should grow more rapidly in environments with variable diet P concentrations. To our knowledge, this is the first study to link stoichiometric homeostasis with a trait linked with competitive ability.

In chapter two, I examined the linkages among four key traits associated with population dynamics: carbon and phosphorus growth rates, stoichiometric homeostasis, and sensitivity to P-limitation of growth. For the first time, I present a single framework for understanding these linkages. Phosphorus and carbon growth rates were tightly correlated across diets and treatments. The relationship between these rates provides information about the P cost of a unit of growth, an important term central to stoichiometric predictions but not addressed by the growth rate hypothesis. Chapter two shows that while the P cost of a unit of growth varies among treatments and species, it can be predicted by a simple linear equation. The P cost of a unit of growth

increases with growth rate. As a result, rapidly growing species are more sensitive to P limitation but are also more stoichiometrically flexible. Taken together, chapters one and two indicate that *Daphnia* species trade off maximizing growth against sensitivity to P-limitation; however, stoichiometric flexibility can diminish the negative impact of a low P diet when small patches of P rich food are available.

*Extending ecological stoichiometry out of the pelagic zone*

In general, the principles of ecological stoichiometry regarding consumers have been developed and tested in pelagic systems. Pelagic systems and consumers are well suited for testing theory. There is a wealth of information known about zooplankton feeding behavior, growth, and natural history. In addition, zooplankton diets (i.e., suspended algae, microbes, and detritus) are well mixed, relatively simple, and digestible. In contrast, less is known about the feeding behavior, growth, and natural history of consumers in other freshwater habits. In benthic systems (i.e., streams and lake bottoms), it is often a challenge to even identify organisms to the species level. Furthermore, benthic diets can be spatially heterogeneous, widely variable in digestibility (e.g., algae versus terrestrial leaf litter), and often difficult to even identify.

There is great interest in using ecological stoichiometry to understand and predict processes in benthic habitats (Cross et al. 2005). Chapters three and four contribute to this work by examining patterns of consumer stoichiometry and nutrient release in streams. These chapters show that a simple extension of stoichiometric

principles to stream ecosystems can be misleading; however, analysis of the underlying assumptions improves understanding of stream ecosystems and furthers the development of ecological stoichiometry.

Chapter three examines intra-specific patterns of stoichiometry in a species of *Psychoglypha* (hereafter: *Psychoglypha* ;Trichoptera) among natural populations distributed along a stream network. Variation in stream size is associated with changes in multiple biological and environmental factors predicted to influence consumer stoichiometry. The dominant organic matter switches from terrestrial leaf litter to algae, which is relative more nutrient rich. Water temperatures also increase as the canopy opens. Our results show that *Psychoglypha* C, N, and P contents varied widely both among and within sties. Surprisingly, the degree of variation in *Psychoglypha* stoichiometry was similar in magnitude to the variation among invertebrate taxa. We attribute this variation to two factors. First, *Psychoglypha* N and P content both declined with body mass, indicating that ontogeny provides a template upon which other factors might influence stoichiometry. Second, after accounting for variation due to size, *Psychoglypha* N content decreased with diet C:N, indicating that this species may be N-limited at some sites within this watershed. This work suggests that within this network of streams *Psychoglypha* may play a variety of roles in stream nutrient cycles. These roles are dependant upon life stage and diet.

Chapter four examines the influence of diet stoichiometry on the nutrient release rates and ratio of four stream consumers. Stoichiometric theory predicts that

nutrient release by a consumer will increase with diet stoichiometry with a non-zero intercept and a slope greater than one (Sterner 1990). Comparisons of nutrient release by zooplankton to diet nutrient content support these predictions (Elser and Urabe 1999); however, tests of the predictions in benthic systems offer mixed support (Frost and Tuchman 2005, Balseiro and Albarino 2006). I found little support for stoichiometric predictions. In general, nutrient release rates and ratios did not vary with diet stoichiometry. These results do not arise from failure of stoichiometric principles. Instead, chapter four shows that selective feeding by these consumers drastically decreases the stoichiometric mismatch between the consumers and their diet and, therefore, explains patterns of nutrient release. Chapter four suggests that extension of stoichiometric predictions to stream ecosystems should proceed with caution and careful consideration of the feeding behavior of the focal species, information which is rarely known or collected. These results also suggest that stream detritivores may play a very different role in small detrital-based streams than predicted based on the extreme mismatch between detritivores and bulk diet stoichiometry.

**CHAPTER 1:**

# Diet mixing: Do animals integrate growth or resources across temporal heterogeneity?

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Animals commonly experience spatial and temporal variation in resource quality, thus experiencing temporally variable diets. Methods for scaling up growth in component patches to long-term growth across heterogeneity are seldom explicitly considered. Long-term growth is sometimes considered to be a weighted average of growth rates on component diets (growth integration). However, if animals integrate resources across high- and low-quality diets, their long-term growth may be greater than predicted from diet-specific growth rates (resource integration). We measured biomass growth rates of seven *Daphnia* species exposed to different types of diel variation in algal phosphorus (P) content. Support for resource integration was found for four of the seven species, which achieved near maximal growth when high P-food was available for at least 12 hours. In contrast, no support for resource integration was found for the other three species. These three species achieved only one-half maximal growth rate under the same conditions and could be considered growth integrators. The type of integration could be predicted from the degree of stoichiometric homeostasis. Species with weak homeostatic regulation exhibited a capacity for resource integration. Resource integrators should have an advantage in heterogeneous environments.

## INTRODUCTION

Animals experience spatial and temporal variation in resource quality, which influences multiple aspects of their ecology including: movement (Jones et al. 2006), competition (Chesson 2000), and fitness (DeMott et al. 2004a). To succeed in nutritionally complex environments, animals must locate and acquire nutrients while balancing their nutritional demands against other biotic and abiotic factors such as predation or temperature. When they are able to move without restrictions among habitats or diets, animals can be remarkably adept at mixing diets to optimize the intake of multiple nutrients (Behmer 2009). But, biotic and abiotic factors often preclude intake of the optimal mixture (e.g., Power et al. 1989, Scrimgeour and Culp 1994, Lewis 2001, Bakker et al. 2005, Maclean et al. 2005, Hansson and Hylander 2009). When choices are restricted, strategies for integrating across heterogeneity may play key roles in ecological success and evolutionary fitness. In these situations, the long-term fitness of an individual is related to patch-specific fitness; however, the nature of integration across patches has seldom been explicitly considered.

Two approaches have been used to specify the relationships between long-term fitness and patterns of patch use. The most parsimonious approach is to experimentally identify how critical abiotic and biotic factors influence patch choice and shape long-term fitness (e.g., Power et al. 1989). This approach estimates long-term fitness within complex landscapes; however, it can be very labor intensive and case-specific. It does not reliably extrapolate to novel landscapes because the relationship between patch use

and fitness is not explicitly determined. The more common alternative is to identify the abiotic and biotic determinants of patch quality (Iversen 1974, Sterner 1993, Vos et al. 2002) and use the relationship between patch quality and fitness to predict long-term fitness in novel heterogeneous environments, scaling up from knowledge of individual patches to predict success in a complex environment. This inductive approach is built upon a mechanistic consideration of the characteristics of quality; however, when scaling component results to heterogeneous environments, diet-specific growth rates and patch use frequency must be combined to estimate long-term growth.

When animals cannot store resources from high quality patches and use them in low quality patches long-term growth may be a simple linear weighting of growth in individual patches (proportion of time in each patch times growth rate in that patch, summed over patches). Such animals are described here as "growth integrators" because they integrate growth across patches. However, patch quality is often determined by nutrients such as protein, carbohydrates, or phosphorus that animals may store and transport among patches. When excess resources are stored in one patch and subsequently used to supplement growth in low quality patches, long-term fitness is likely to be greater than predicted by simple linear weighting of growth across patches. These animals are termed here "resource integrators". Growth integration is most easily scalable theoretically across different types of variation, but the preponderance of storage molecules among animals (Woods et al. 2002, Lee et al. 2006, Raubenheimer and Jones 2006) indicates that temporal resource mixing may often

influence long-term fitness. A means to understand and predict these two different types of population dynamics is needed.

To determine how temporal diet mixing affects long-term growth, it is necessary to explicitly track the availability and incorporation of limiting resources. Ecological stoichiometry (Sterner and Elser 2002) uses a mass balance and elemental ratios to determine how a stoichiometrically imbalanced diet influences fitness and food web dynamics. A critical parameter in ecological stoichiometry is the degree of elemental regulation, or homeostasis. Variation in stoichiometric homeostasis reflects the degree of elemental depletion during nutrient limitation as well as a species' capacity for elemental storage during periods of excess. Few stoichiometric studies have examined spatial or temporal heterogeneity. Sterner and Schwalbach (2001) examined how temporal mixing of high and low phosphorus (P) diets influenced the growth rate of *Daphnia magna*. They showed that what we call here growth integration underestimates long-term dynamics on temporally mixed diets, presumably because *D. magna* temporally mixes P across diets.

Members of the genus *Daphnia* often play a keystone role in lakes (Elser et al. 1988, Rudstam et al. 1993) and are an excellent model species for this type of study. They are generalist feeders that commonly experience both temporal and spatial variation in food quality, which is determined by a number of factors including food particle size, toxicity, essential fatty acids, and P content (Sterner and Schulz 1998). "Seston" refers to the collection of particles suspended in lake waters. The seston

phosphorus to carbon (P:C) ratio is one determinant of food quality for *Daphnia* (Sterner 1993, DeMott and Pape 2005), which actively identify and congregate within patches having high food P:C ratios (Schatz and McCauley 2007). Seston P:C is both spatially and temporally heterogeneous (Sterner et al. 1997, Hessen et al. 2005, Berger et al. 2006), varying seasonally as well as over time spans of hours to days (Cunningham and Maas 1978, Dickman et al. 2006). Seston P:C varies in response to gradients of nutrient mixing (Hall et al. 2005), light (Sterner et al. 1997), and grazing (Tessier et al. 2001). During diurnal migrations, vertically migrating zooplankton often consume P-rich algae in cold, deep waters during daytime hours and P-poor food in warm, shallow waters at night (DeMott et al. 2004a). The wealth of information available on P stoichiometry and homeostasis for members of this genus make *Daphnia* an ideal model group.

Given the many potential complexities of spatial and temporal heterogeneity of food quality in natural environments and the unknown nature of biotic response to this heterogeneity, it might seem difficult, if not impossible, to define a framework to scale up from the patch to the landscape scale. In this article, we suggest that scaling of growth from individual patches to long-term dynamics in complex landscapes may be achievable using simple principles of stoichiometric homeostasis. We first evaluate this hypothesis with two stoichiometrically explicit models of population growth. Then, because *Daphnia* species have different degrees of stoichiometric homeostasis (DeMott and Pape 2005), we test this hypothesis using a comparative framework. *Daphnia* are a well-suited model organism for this study because juvenile body mass growth rates,

which can be measured in short-term (< 1 week) experiments, are closely related to population growth rates. The relationship between juvenile growth rates and population growth rates holds across species, within a species by genotype, and across feeding treatments (Lampert and Trubetskova 1996, Ferrao Filho et al. 2005). Specifically, we use seven *Daphnia* species to ask if the ability to temporally mix resources across diets is linked to their stoichiometric homeostasis.

*Theoretical Expectations*

We argue that the two integration strategies described here are fundamentally linked to the degree of stoichiometric homeostasis exhibited by a species. If a species strictly balances nutrient homeostasis through intake regulation or assimilation, instantaneous growth will vary instantaneously with food quality (Fig. 1a). When this response is summed over multiple periods of variability, long-term growth will be the temporally weighted average of diet-specific growth rates. Thus, it is growth that is integrated across patches. This strategy produces a positive, linear relationship between growth ($\mu$) and the proportion of time spent in high quality food ($F$) (Fig. 1b). Note that these species grow slower over the long-term than they would on a mixed diet of similar mean quality (Fig. 1b). Thus, an attempt to measure overall habitat quality by a spatially or temporally weighted sum of within-patch qualities would produce an incorrect picture.

In contrast, a weakly homeostatic species can utilize resources obtained in rich patches to supplement growth in poor patches, and in such cases shifts in instantaneous growth will lag behind shifts in food quality (Fig. 1a). Specifically, as a resource integrator moves from a rich to a poor patch, stored nutrients may be used to maintain rapid growth. Following the transition to the low quality patch, growth slowly declines through time, as stored resources are incorporated into new growth and eventually exhausted. The shape and duration of the decline in growth rate depends upon the relative magnitude of nutrient stores as well as how rapidly stores are utilized. When summed over days, the long-term growth of a resource integrator produces a curvilinear, saturating relationship between $\mu$ and $F$ (Fig. 1b). The most effective resource integrators would transfer all ingested resources in excess of the requirements of maximum growth from the rich patch to the poor patch. For these species, the non-linear relationship should approach that seen between long-term growth rate and a mixed diet with the same mean resource level (Fig. 1b). Thus, there is in theory a gradient of ability to integrate resources; beginning with species incapable of integrating any resources, a condition termed growth integration, and ending with very efficient resource integrators, species integrating all of a resource across diets. Hypothetically, a species' capacity for resource integration is determined by its degree of stoichiometric homeostasis.

To explore the mechanisms linking P balance and the type of integration, we first constructed and analyzed a population growth model for a homeostatic and plastic

grazer. We then measured the resource integrative ability of seven *Daphnia* species, exploring relationships among stoichiometric homeostasis and growth patterns in habitats with variation in nutritional quality.

## METHODS

*Population Growth Models*

We used a suite of models (described in detail below) to examine the influence of P homeostasis on the short- and long-term patterns of population growth in habitats with heterogeneous resource quality. We start with a base model (Homeostatic), similar to other stoichiometrically explicit models (Loladze et al. 2000, Hall 2004), in that the grazer instantaneously uses ingested nutrients for new biomass with a fixed stoichiometric ratio. The next model (Plastic), an extension of a model presented by Grover (2003), allows for variable grazer stoichiometry. Parameters for both models are listed in Table 1.

Because our focus is on animal production dynamics the models do not simulate algal dynamics; algal densities ($A$) and carbon biomass ($Q_{A,C}$) are maintained at constant levels. Variation in resource quality was imposed by varying algal P content ($Q_{A,P}$) in a square-wave fashion between $Q_{A,P\text{-}LP}$ and $Q_{A,P\text{-}HP}$ as a function of time based on the parameter $S$, the proportion of each day the grazer consumes high quality algae ($Q_{A,P\text{-}HP}$).

*Homeostatic model*: The homeostatic model describes the population dynamics of a grazer with a fixed P:C stoichiometry of growth:

$$dZ/dt = \mu_{diet} Z - mZ \quad (2)$$

where $Z$ is the zooplankton density (ind $L^{-1}$), $\mu_{diet}$ is the grazer's reproductive rate ($d^{-1}$) for a given diet either HP or LP algae, and $m$ is mortality rate ($d^{-1}$).

*Plastic model:* The plastic model, based on Grover (2003), uses two differential equations to describe the dynamics of a grazer population with a variable P:C stoichiometry. The first is the equation for zooplankton dynamics:

$$dZ/dt = \mu Z - mZ \quad (3)$$

where $\mu$ is the reproductive rate ($d^{-1}$) of the grazer population. Reproductive rate ($\mu$) is dependant upon the grazer's stores of limiting nutrients:

$$\mu = \mu_{\max}\left[1 - \max_i\left(\frac{Q_{Z,i}^{\max} - Q_{Z,i}}{Q_{Z,i}^{\max} - Q_{Z,i}^{\min}}\right)\right] \quad (4)$$

where $\mu_{max}$ is the maximum reproductive rate of the grazer ($d^{-1}$), $i$ = C or P, $Q_{Z,i}$ is body elemental content or quota of the grazer (mol nutrient $ind^{-1}$), $Q_{Z,i}^{\min}$ and $Q_{Z,i}^{\max}$ are respectively the minimum and maximum grazer elemental content. DeMott et al. (1998) describes a similar positive, linear relationship between growth and body P for P-limited Daphnia. The model assumes a similar relationship for C as for P, though potential variation in $Q_{Z,C}$ is tightly constrained and therefore has limited influence on dynamics (Table 1). The grazers' degree of P homeostasis is modified by $\Delta_i$, defined as the difference between $Q_{Z,i}^{\max}$ and $Q_{Z,i}^{\min}$.

The second differential equation is for the grazer's elemental content, which is a function of intake and losses to either growth or release:

$$dQ_{Z,i}/dt = aIQ_{A,i} - \mu Q_{Z,i} - R_i \quad (5)$$

where nutrient ingestion follows a linear functional response $aI$, $a$ is the algal density (cells $L^{-1}$) and $I$ is the clearance rate of the grazer (L $ind^{-1}$ $day^{-1}$), $R_i$ represents the grazer's P or C loss rates (mol $ind^{-1}$ $day^{-1}$). Nutrient loss (C or P) depends upon nutrient ingestion ($aIQ_{A,i}$) and the demand for those elements based on the relative nutrient content of the grazer:

$$R_i = aIQ_{A,i}\left[1 - e_i\left(\frac{Q_{Z,i}^{\max} - Q_{Z,i}}{Q_{Z,i}^{\max} - Q_{Z,i}^{\min}}\right)\right] \quad (6)$$

where $e_i$ is the maximum accumulation efficiency for nutrient $i$.

Simulations were conducted with Berkeley Madonna (Macey and Oster 2006). We used the Runge-Kutta 4 method to integrate the homeostatic model. The plastic model is computationally intensive, so we used the auto-stepsize method. We calculated integral growth rate and homeostasis as described in the *Homeostasis and Model Fitting* section using the initial and final (t=10) results of simulations.

*Taxa and Culture Conditions*

We used the experimental framework developed by Sterner and Schwalbach (2001) to examine the integrative ability of seven *Daphnia* species: *D. lumholtzi, D. magna, D. mendotae, D. obtusa, D. parvula, D. pulicaria,* and *D. pulex*. These seven

species fall into all four of the major North American *Daphnia* phylogenetic groups identified by Colbourne and Herbert (1996): the subgenus *Ctenodaphnia* (2 species: *D. lumholtzi* and *D. magna*), the *pulex* group (3 species: *D. obtusa, D. pulicaria, D. pulex*), the *longispina* group (1 species: *D. mendotae*), and the "orphan taxa" (1 species: *D. parvula*). Six of seven species had been maintained in culture in the RWS lab in Saint Paul, MN for several years. *Daphnia lumholtzi* was obtained from L. Wieder (University of Oklahoma).

Twenty-liter stock cultures of *Daphnia* were maintained at room temperature in a COMBO medium (Kilham et al. 1998) modified to contain 40 μM P and 500 μM nitrogen (N). Batch cultures were fed a combination of *Scenedesmus obliquus* and ground dried alfalfa. For the experimental diets, *Scenedesmus obliquus* was grown in chemostats under P-limited (LP: 1000 μM N, 5 μM P, dilution = 0.1 $day^{-1}$) or N-limited (HP: 400 μM N, 80 μM P, dilution = 0.5 $day^{-1}$) conditions. In experiment one, LP algae had a mean C:P of 588 while the mean C:P of HP algae was 78 (Fig. 2). In experiment two, LP algae had a mean C:P of 1325 while the mean C:P of HP was 94. Algal C and P concentrations were determined with a FOSS systems NIRS spectrometer. Calibration equations and validation procedures are described by Hood *et al.* (2006).

*Experiment One*

Twenty-four hours before initiating these experiments, gravid *Daphnia* were removed from batch cultures with a pipette and placed in a jar containing COMBO

medium, N (1000 μM N), and P (80 μM P). Mothers were fed HP *ad libitum*. This experiment began with < 24 hr old neonates and ended at the first sign of ovary development. Neonates were distributed as follows. Fifteen neonates were placed on glass slides, dried (60˚ C), weighed, and analyzed for P content as described below. The remaining individuals were distributed among seven treatments. Daphniids were allowed to feed on HP for a predetermined number of hours each day (0, 1, 3, 5, 9, 12, or 24) and fed upon LP for the remaining time. Four replicate jars were created for the 100% LP and HP treatments (0 and 24 hr treatments), used to measure P homeostasis, while the intermediate treatments had two replicates. When this experiment was repeated with *D. magna* and *D. parvula* (run two) only two replicate jars were created for the 100% LP and HP treatments. Jars were stocked with 10 - 20 animals per jar. We varied the density of animals per jar to achieve approximately equal final biomass. Often, there were not enough neonates to start an entire experiment in one day; therefore, the initiation of each experimental bottle was randomized. Neonate samples were collected each day bottles were initiated. Each experimental bottle contained 250 mL of COMBO medium (lacking N and P) and 500 μg C $L^{-1}$ of *S. obliquus*.

To standardize the impact of transferring, animals receiving a constant diet of LP or HP algae were also transferred to a new jar after 12 hours. To remove excess algae during transfers, animals were first placed in a rinse beaker containing basal COMBO and no algal food for at least one minute. Experimental jars were kept on a tissue culture roller table to keep algae in suspension, and were maintained at 20˚ C under low

light in an environmental chamber. Species were run in pairs, with the exception of *D. obtusa* (Fig. 2).

Animals were allowed to grow until the first individuals in each container exhibited signs of ovary development (3 to 6 days). At harvest, animals were removed, placed on a glass slide, and dried at 60˚ C. Each dried individual was removed from the slide and weighed three times to the nearest 0.1 µg on a microbalance (Mettler UMT2). Several precautions were taken to improve determination of mass in these small individuals. The microbalance was kept in a basement room on a marble table anchored in sand. A Static Master Ionizer (Amstat industries) was used to neutralize static electricity charges. Juvenile growth ($\mu$) was calculated as

$$\mu = \frac{\ln(mass_{final}) - \ln(mass_{initial})}{time}, \qquad (7)$$

where time was in days.

After weighing, *Daphnia* were transferred to a borosilicate glass tube (2-10 individuals tube$^{-1}$), ashed (550˚ C), and analyzed for P content using the molybdate-absorbate method described by DeMott *et al.* (1998). Phosphorus analyses were run in a 10 or 20 mL reaction tube. To estimate daphniid P:C (molar), we used measured daphniid % P and assumed that *Daphnia* were 45% C (Andersen and Hessen 1991).

*Experiment Two*

We used the results of experiment two only to help determine the degree of homeostatic regulation for these seven *Daphnia* species. This experiment began with < 24 hour old neonates and ended after 72 hours. Neonates were distributed as follows. Fifteen neonates were placed on glass slides, dried (60˚ C), weighed, and analyzed for P content as described above. The remaining individuals were distributed between two treatments, with three replicates per treatment, and 10-20 animals per container. Each experimental bottle contained 250 mL of COMBO medium (lacking N and P) and 1 mg C/L of algae. Daphniids received either HP or LP *S. obliquus*. Daily, animals were transferred to a new bottle containing fresh basal COMBO and algae. During transfers, animals were rinsed in a beaker containing basal COMBO and no algae. Experiments were run on a roller table, under low light, in an environmental chamber at 20˚ C. *Daphnia* growth and P content were determined as described above, although a Mettler UMX2 microbalance was used in this experiment.

*Homeostasis and Model Fitting*

The homeostasis parameter *H* (eta) describes the degree to which a species regulates body elemental content in response to variation in diet (Sterner and Elser 2002). Here, we describe results in terms of 1/ *H*, which measures stoichiometric flexibility. Higher values of 1/*H* indicate weaker homeostasis (less variation in consumer nutrient content relative to resource nutrient content). We calculated 1/*H* as the slope of the regression line between natural log *Daphnia* P:C and natural log algal P:C. Only

*Daphnia* % P values from 100% LP or HP treatments were used. Differences in 1/*H* among species were examined with a homogeneity of slopes test (Statistica).

The parameter *H* quantifies the stoichiometric regulatory ability of a species for a given resource under a given set of conditions. The value of *H* measured in any given study might vary with the range of variables considered, the growth conditions, and other environmental factors. For the purposes of this study, we wanted to know the maximum homeostasis that the species might exhibit under conditions similar to our experiments. We therefore chose to use the maximum estimate of stoichiometric homeostasis (*H*) from our two experiments. Since the relationship between natural log *Daphnia* P:C and natural log algal P:C is linear, differences in algal C:P between experiments are not expected to influence homeostasis estimates (DeMott et al. 1998, Sterner and Elser 2002).

Resource integrators exhibit a saturating relationship between $\mu$ and *F*, which can be fit empirically by a square-root function:

$$\mu = \beta_0 + \beta_1 F + \beta_2 F^{1/2} \tag{8}$$

The coefficient $\beta_2$ is of particular interest. It defines the curvilinear nature of this nonlinear function. When this square-root function is fit to the linear, growth integrator response (Fig. 1b: growth integrator) $\beta_2$ is near zero. $\beta_2$ increases as the relationship between $\mu$ and *F* becomes more curvilinear. We use $\beta_2$ as a metric of a species ability to integrate P across diets. The square-root model was fit in Statistica (Statsoft, Inc).

When comparing $\beta_2$ among species (or runs) we must assume all species have a similar $\beta_2$ for the relationship between long-term growth and the mean food quality of a mixed diet (Fig. 1b). Statistical tests used to make additional comparisons are noted in the Results. These tests were conducted in Statistica with $\alpha = 0.05$.

## RESULTS

*Population Growth Model*

Comparison of dynamics from the two models supports our hypothesis that the type of integration is linked to stoichiometric homeostasis. The homeostatic model generates symmetry between the dynamics of population growth and algal P (Fig. 3a), as predicted for growth integrator (Fig. 1). In contrast, the plastic model produces asymmetry between population growth and algal P, as predicted for resource integrators. In the plastic model, temporal variation in growth is characterized by a saturating response following the LP to HP shift and a slow, near linear decline when shifting from HP to LP algae (Fig. 3a).

When model results are averaged over the long-term (ten days), the homeostatic model produces a linear relationship between $\mu$ and $F$ (Fig. 3b). In contrast, over the long-term the plastic model produces a nonlinear relationship between $\mu$ and $F$ (Fig. 3b). The curvilinear nature of this relationship increases with the plasticity ($\Delta_P$) of the grazer (Fig. 4a), resulting in a positive, saturating relationship

between resource integration ($\beta_2$) and stoichiometric flexibility (1/*H*, Fig. 4b). This relationship plateaus at a 1/*H* of approximately 0.4.

*Growth and Dietary Phosphorus Integration*

All *Daphnia* species grew slower on LP than HP (factorial ANOVA diet effect: $F_{1,34}$ = 23.6, P < 0.0001; Tukey HSD post-hoc: all species: P < 0.05, Table 2). Dietary P had the greatest impact on the growth of *D. mendotae* ($\mu_{LP}\ \mu_{HP}^{-1}$ = 0.06) and the least impact on *D. pulicaria* (0.68).

Visually, the relationship between juvenile growth ($\mu$) and *F* for each species can clearly be categorized as linear or nonlinear (Fig. 5). Three of the seven species (*D. lumholtzi, D. mendotae,* and *D. parvula* two runs) exhibited a near linear relationship between $\mu$ and *F* (Fig. 5). For these species, estimates of $\beta_2$ were not significantly different from zero (Table 3). These species have little to no capacity for resource integration and could be considered growth integrators. The other four species (*D. magna,* two runs*, D. obtusa, D. pulicaria, and D. pulex)* exhibited a nonlinear relationship between $\mu$ and *F* (Fig. 5). Estimates of $\beta_2$ were significant for these five datasets (Table 3) indicating support for resource integration. The capacity for resource integration varied among these four species (Fig. 5, Table 3).

Both *D. magna* and *D. parvula* runs were replicated in time. The C:P of HP and LP did not differ between runs (HP: mean C:P = 75 v. 77; t-test: df=14, P = 0.56; LP: mean C:P = 517 v. 619; t-test: df=14, P = 0.12). These two species exhibited qualitatively

similar relationships between $\mu$ and *F* in that *D. magna* was found to be a resource integrator in both runs and *D. parvula* was found to be a growth integrator in both runs (Fig. 5, Table 3). Coefficients fits, however, differed between the runs. The parameter $\beta_2$ was higher in the second *D. magna* run (0.0261 v. 0.1048, Fig. 5). The intercept differed between *D. parvula* runs (Fig. 5). These differences across runs presumably are due to some unidentified experimental condition or had to do with pre-experimental feeding history.

*Daphnia P Content and Homeostasis*

In both experiments one and two, *Daphnia* percent P was generally within the range of previous studies, excluding measurements for *D. pulex* and *D. obtusa* in HP as well as *D. mendotae* in LP (Table 4). Daphniid P content differed among species in both the LP and HP treatments (Table 4). The diet-specific P content of *D. parvula* and *D. magna* did not differ significantly between runs (t-test; *D. magna* (HP): n=4, P = 0.13; *D. magna* (LP): n=3, P = 0.09; *D. parvula* (HP): n = 4, P = 0.76; *D. parvula* (LP): n = 4, P = 0.47).

These seven *Daphnia* species differed in their degree of P flexibility (1/*H*) which ranged from -0.031 to 0.197 (homogeneity of slopes: $F_{6,35} = 22.2$, P = < 0.0001, Table 4). For three of the seven species (*D. lumholtzi*, *D. mendotae*, and *D. parvula*) estimates of 1/*H* were not significantly different from zero (Table 4). These species could be considered strictly homeostatic (1/*H* = 0). Although *D. magna* exhibited the weakest

homeostasis (1/*H* = 0.197), all four species with relaxed homeostasis (P < 0.05) varied little in 1/*H* (range: 0.155 – 0.197).

Two species (*D. obtusa* and *D. mendotae*) showed large differences in their degree of P homeostasis between experiments one and two (Table 4). In experiment one, *D. mendotae* exhibited weak homeostasis (1/*H* = 1.210) while in experiment two this species proved to be strongly homeostatic (1/*H* = 0.112, P > 0.05). In contrast, *D. obtusa* exhibited a negative 1/*H* in experiment one (1/*H* = -0.643) and a positive 1/*H* in experiment two (1/*H* = 0.166). The potential implications of these differences are discussed later.

*Integrative Strategies, Homeostasis, and Life History Parameters*

Simulations of the plastic model suggest that resource integration ($\beta_2$) increases linearly with stoichiometric flexibility then plateaus (Fig. 4b). The experimental dataset suggests, in contrast, that resource integration ($\beta_2$) increases nonlinearly with stoichiometric flexibility (1/H, Fig. 6). Inspection of this dataset suggests that the seven daphniids can be categorized into two groups with regard to integration: species with $\beta_2$ estimates similar to zero (growth integrators) and species with a capacity for resource integration (Fig. 6, Table 3). Species with a capacity for resource integration were more stoichiometrically flexible than those categorized as growth integrators (t-test: t = -3.79, df = 5, P = 0.013; Fig. 6). Exclusion of *D. obtusa* and *D. mendotae*, species with greatly

divergent 1/*H* estimates, does not influence the nature of this relationship (t-test: t = -5.90, df = 3, P = 0.010).

Resource integrators did not differ from growth integrators in terms of $\mu_{HP}$ (t-test n = 5, P = 0.98), $\mu_{LP}$ (t-test n = 5, P = 0.60), $\%P_{HP}$ (t-test n = 5, P = 0.92), $\%P_{LP}$ (t-test n = 5, P = 0.97), or sensitivity to P-limitation ($\mu_{LP}\ \mu_{HP}^{-1}$: t-test n = 5, P = 0.52). There was a suggestion that growth integrators were smaller than resource integrators although the difference was not significant at $\alpha = 0.05$ (GI: 11.0 ± 4.2 μg, RI: 29.2 ±18.2 μg; t-test log mass: n = 5, P = 0.07). Furthermore, the homeostasis parameter 1/*H* was not linearly related (P > 0.05) to $\mu_{HP}$, $\mu_{LP}$, $\%P_{HP}$, $\%P_{LP}$, or sensitivity to P limitation.

## DISCUSSION

Animals must often consume diets of varying quality, resulting in a temporally heterogeneous diet. However, there has been little consideration of how long-term growth in a heterogeneous environment relates to component growth rates associated with patches of different nutritional quality. The most common approach uses diet-specific growth rates to predict long-term growth (Iversen 1974, White 1993, Urabe et al. 1997); thus, implicitly assuming that animals integrate instantaneous growth rates across diets. Alternatively, animals may temporally mix resources across diets (i.e., integrate resources not growth) and exhibit higher growth rates than expected based on component diets (Fig. 1).

The seven *Daphnia* examined in this study exhibited wide variation in their capacity for resource integration. Three species (*D. lumholtzi, D. mendotae, and D. parvula*) exhibited little to no capacity for resource integration (Fig. 6). For simplicity, we consider these species growth integrators because their estimates of $\beta_2$ are not statistically different from zero (Table 3). The other four species (*D. magna, D. obtusa, D. pulicaria,* and *D. pulex*) exhibited variation in their capacity for resource integration. We found a positive nonlinear relationship between resource integration and stoichiometric flexibility (Fig. 6). Growth integrators were more homeostatic than resource integrators. These results may suggest that resource integration requires a threshold level of stoichiometric flexibility. Yet, our model simulations suggest that resource integration should increase linearly with stoichiometric flexibility to a plateau (Fig. 4b).

The prevalence of nutrient storage and wide variation in N and P homeostasis observed in invertebrates (Persson et al. 2010), suggests that strategies across a continuum of resource integration efficiency may be common. Thus, understanding how to scale growth rates (and by implication, fitness) from component growth rates in individual habitats up to complex mosaics of high and low quality patches could potentially be extremely difficult but our results indicate that a relatively simple parameter, the degree of stoichiometric homeostasis, will be useful in determining how to scale fitness from patches to the landscape.

Another approach for determining the influence of patch heterogeneity on fitness is provided by the geometric framework developed by Raubenheimer, Simpson and colleagues (see review in Behmer 2009). This framework uses state-space plots to determine how animals mix complementary diets (e.g., a high protein diet and a high carbohydrate diet) to maximize fitness. This body of work clearly demonstrates that many diverse animals can identify high quality food and act to mix diets to optimize nutrient intake as well as growth, when allowed to feed *ad-libitum*. This approach does not, however, explicitly identify relationships between patch use and long-term fitness. In this study, *Daphnia* were not allowed to choose or optimize diet mixtures. Instead, we examined a wide range of predetermined mixtures (i.e., hours in HP), allowing us to predict the relationship between patch use and long-term fitness across a wide range of landscapes. Terrestrial insects may use both growth and resource integrator strategies, although not the same strategy for all nutrients. For example, Raubenheimer and Jones (2006) fed German Cockroaches (*Blatella germanica*) diets high either in carbohydrates or proteins. German Cockroaches store excess carbohydrates and proteins, when the nutrients were available, suggesting the potential for integration of both nutrients. Furthermore, Raubenheimer and Jones (2006) argue that the ability to store excess carbohydrate is common among insect species while the ability to store excess proteins may be rare. Resource integration may thus be a common strategy for coping with environmental heterogeneity in carbohydrates but not proteins.

*Phosphorus Homeostasis*

Our results show that regulation of P homeostasis varies widely among *Daphnia* species, supporting DeMott and Pape's (2005) findings. In our study, three species (*D. lumholtzi*, *D. mendotae,* and *D. parvula*) were identified as strongly homeostatic while four other species (*D. magna*, *D. obtusa*, *D. pulex*, and *D. pulicaria*) exhibited weaker P homeostasis. Our measurements are, in general terms, consistent with several studies, although we did identify one discrepancy. Published laboratory studies with *D. magna, D. pulex,* and *D. pulicaria* also indicate weak P homeostasis (1/H: 0.1 - 0.2, DeMott and Pape 2005; Ferrao Filho et al. 2007; Plath and Boersma 2001) while DeMott and Pape (2005) describe *D. mendotae* as strongly homeostatic. In contrast, *D. parvula* exhibited weak P homeostasis in one laboratory study (1/H = ~0.1, DeMott and Pape 2005) while our results suggest strong homeostasis. Discrepancies among studies are not particularly surprising. Phosphorus homeostasis is not a species-level trait; instead, it varies within species among clones (Jeyasingh et al. 2009) and with food quantity (Ferrao Filho et al. 2007).

Our decision to use the maximum homeostasis from experiment one and two affects the interpretation of our results though the main conclusions do not rest on this point. The homeostasis parameter 1/*H* only differed greatly between experiments for two species: *D. mendotae* and *D. obtusa*. Exclusion of these two species does not significantly alter our central finding that the capacity for resource integration increases

with stoichiometric flexibility. Furthermore, the mathematical models also support the link between nature of integration and stoichiometric homeostasis.

*Mechanistic Explanations for Resource Integration*

Two mechanisms could be responsible for resource integration: luxury uptake or variation in nutrient use efficiency (NUE). These mechanisms are not mutually exclusive and may at times work in concert to maximize resource integration. Luxury uptake has been defined in multiple ways (Agren 2008). Here we define luxury uptake operationally as nutrient assimilation in excess of immediate growth requirements (Elrifi and Turpin 1985).

Organisms with the capacity for luxury uptake and the subsequent storage of excess nutrients can use nutrient reserves to supplement growth on a low quality diet, allowing an organism to be a resource integrator. A wide variety of organisms ranging from autotrophs to invertebrates and mammals are capable of resource (i.e., protein, carbohydrate, phosphorus) storage and therefore resource integration (Barboza and Parker 2006; Elrifi and Turpin 1985; Khoshmanesh et al. 2002; Lee et al. 2006; Raubenheimer and Jones 2006; Woods et al. 2002), though the capacity for storage varies widely among both groups and resources.

Luxury uptake may not always be available as a strategy. Some species may not have a significant capacity for luxury uptake or may reside in environments where resources are never available in excess. However, resource integration can also occur in

the absence of luxury uptake, when the NUE of a nutrient-limited species negatively scales with diet quality and growth rate. Here, we define NUE as the carbon to nutrient ratio of an organism's body (e.g., C:P), which will reflect the carbon to nutrient ratio of new growth. It is common for NUE to vary with dietary or environmental variables (Sterner and Elser 2002). Phosphorus use efficiency, for example, can be a growth-rate dependant parameter. When P is limiting, PUE is inversely related to growth rate (Elser et al. 2003). This relationship results from the tripartite linkage between growth, RNA, and body P described by the growth rate hypothesis (Elser et al. 1996). This tripartite linkage can lead to resource integration.

In heterogeneous environments with low and high quality patches these linkages allow for resource integration. When a species with a variable NUE moves from a high quality to low quality patch (e.g., HP to LP) its high nutrient content and growth rate are not sustainable in the new patch. Over time, NUE increases, as the organisms' growth rate and nutrient content declines. In this scenario, the difference between the organism's nutrient content in the high quality and low quality patches can be considered excess nutrients, although those nutrients were not excess when incorporated. These newly excess nutrients; made available by diet-induced changes in NUE, supplement growth on the low quality patch, leading to resource integration. This mechanism of resource integration may be common among weakly homeostatic species, at least when P is the focal resource. A wide variety of organisms, ranging from bacteria to both aquatic and terrestrial invertebrates (DeMott et al. 1998; Elser et al.

2003; Fink and Von Elert 2006), exhibit the positive relationship between growth and body P required for this resource integration mechanism.

The two physiological mechanisms described above appear to be widespread across heterotroph groups; suggesting, although not proving, that resource integration may be a common response to environmental heterogeneity. It is more difficult to estimate the prevalence of the growth integration strategy, which requires strict homeostasis, since estimates of 1/*H* are rare for heterotrophs other than *Daphnia* (Persson et al. 2010). Nevertheless, the distribution of strategies within the genus *Daphnia* could suggest that both strategies are common.

*Integration Strategies, Competition, and Ecosystem Dynamics*

The strategic and physiological differences between growth and resource integrators provide a framework for understanding competition in heterogeneous systems. When temporally mixing diets, resource integrators use nutrients available across complex landscapes more efficiently than do growth integrators. This increased efficiency should allow resource integrators to potentially outcompete growth integrators in variable environments or heterogeneous landscapes. Yet, the ability to integrate a resource instead of growth does not inherently lead to dominance in variable environments. Rapidly growing species or those insensitive to nutrient limitation may dominate in both homogeneous and heterogeneous landscapes, regardless of integration strategy. We did not identify any clear tradeoffs between

resource integration and growth rates in constant environments. In the absence of any tradeoff, resource integration would be favored in any heterogeneous environment. It has no clear drawback while there appears to be no tradeoff associated with weak homeostasis.

The linkage between integration strategy and stoichiometric homeostasis implies that resource and growth integrators may play different roles in food webs and nutrient cycles. Strictly homeostatic growth integrators will release nutrients when available in excess while retaining nutrients when scarce, consistent with past theoretical treatments (Sterner 1990). Resource integrators fit differently into nutrient cycles. These species store nutrients when available in excess, only releasing nutrients once storage reservoirs are full. By sequestering nutrients in tissue, resource integrators may decrease, relative to growth integrators, the availability of labile nutrients as well as the quality of their own food. Storage could lengthen the periods of autotroph and heterotroph nutrient limitation in consumer-resource cycles (Andersen et al. 2004). Furthermore, since the nutrient stoichiometry of resource integrators may be highly variable, the presence of these species in food webs could more likely lead to nutrient limitation of predators (Malzahn et al. 2007).

*Significance*

In spite of widespread acknowledgement of the occurrence of spatial and temporal heterogeneity, few studies have examined how an animal's instantaneous

response to patch quality scales into its long-term performance within a complex landscape. Here, using mathematical simulations and *Daphnia* as a model group, we explored the function linking patch quality and frequency of patch use with long-term growth. We suggest there is a continuum of responses, beginning with growth integrators and ending with resource integrators capable of mixing all nutrients in excess across diets. Resource integrators should have higher long-term resource use efficiency than growth integrators. Thus, all else being equal, resource integrators should outcompete growth integrators in heterogeneous environments, where resource quality varies among patches. A species' capacity for resource integration can be predicted by the degree of stoichiometric flexibility. Furthermore, the degree of resource integration will influence a species' fitness as well as the role it plays in food webs and nutrient cycles.

## TABLES

Table 1. Notation for the homeostatic and plastic models.

| Symbol | Definition | Units | Assigned value |
|---|---|---|---|
| Index: | | | |
| $i$ | Index for elements (C, P) | | |
| *diet* | Index for algal types LP and HP | | |
| State variables: | | | |
| $Z$ | Grazer density | ind $L^{-1}$ | |
| $Q_{Z,i}$ | Quota of nutrient *i* for grazer | mol nutrient $ind^{-1}$ | |
| Functions: | | | |
| $\mu_Z$ | Per capita reproductive rate of grazer | $d^{-1}$ | |
| $R_i$ | Loss rate of nutrient *i* | mol $ind^{-1}$ $day^{-1}$ | |
| Parameters: | | | |
| $m$ | mortality rate of grazer | $d^{-1}$ | 0.3 |
| $a$ | Algal density | cells $L^{-1}$ | $8.3 \times 10^{7}$ |
| $I$ | Filtration rate of grazer | L $ind^{-1}$ $day^{-1}$ | 0.2 |
| $\mu_{diet}$ | Growth rate for algal type *diet* | $d^{-1}$ | 0.05 for LP; 0.70 for HP |
| $\mu_{max}$ | Maximal reproductive rate of grazer | $d^{-1}$ | 0.7 |
| $Q_{A,C}$ | Algal quota for C | mol nutrient $cell^{-1}$ | $1 \times 10^{-12}$ |
| $Q_{A,P\text{-}LP}$ | P quota for LP algae | mol nutrient $cell^{-1}$ | $1 \times 10^{-15}$ |
| $Q_{A,P\text{-}HP}$ | P quota for HP algae | mol nutrient $cell^{-1}$ | $1 \times 10^{-14}$ |
| $Q_{Z,i}^{\min}$ | Minimal quota of nutrient *i* for grazer | mol nutrient $ind^{-1}$ | $1.7 \times 10^{-6}$ for C; varies, see Δ |
| $Q_{Z,i}^{\max}$ | Maximal quota of nutrient *i* for grazer | mol nutrient $ind^{-1}$ | $1.9 \times 10^{-6}$ for C; $0.3 \times 10^{-7}$ for P |
| $\Delta_i$ | Difference between $Q_{Z,i}^{\min}$ and $Q_{Z,i}^{\max}$ | | varies |
| $e_i$ | Maximal accumulation efficiency for nutrient *i* by grazers | Dimensionless | 0.65 for C; 1.0 for P |
| $S$ | Proportion of day spent in HP | Dimensionless | varies |

Note – The index *diet* and the parameter $\mu_{diet}$ are specific to the homeostatic model.

Table 2. Initial neonate mass as well as the influence of diet on *Daphnia* mass and growth rate ($\mu$).

| | | HP | | LP | |
|---|---|---|---|---|---|
| Species | Neonate mass | Mass | $\mu$ ($d^{-1}$) | Mass | $\mu$ ($d^{-1}$) |
| *D. lumholtzi* | 3.03 (3.37) | 12.44 (0.28) | 0.26 (0.00) | 7.49 (3.85) | 0.12 (0.06) |
| *D. magna* (run 1) | 10.27 (2.21) | 55.53 (5.49) | 0.28 (0.02) | 28.17 (3.08) | 0.17 (0.01) |
| *D. magna* (run2) | 4.83 (1.69) | 46.6 (0.91) | 0.37 (0.02) | 12.81 (3.55) | 0.15 (0.05) |
| *D. mendotae* | 2.71 (1.56) | 14.50 (0.77) | 0.29 (0.02) | 3.37 (0.50) | 0.02 (0.01) |
| *D. obtusa* | 2.11 (0.91) | 14.07 (1.55) | 0.33 (0.01) | 3.98 (0.83) | 0.11 (0.01) |
| *D. parvula* (run 1) | 1.38 (0.48) | 6.06 (0.89) | 0.43 (0.04) | 2.61 (0.56) | 0.21 (0.03) |
| *D. parvula* (run 2) | 1.42 (0.72) | 6.03 (n.a.) | 0.36 (n.a.) | 2.46 (0.56) | 0.11 (0.01) |
| *D. pulex* | 1.88 (1.15) | 22.52 (3.07) | 0.41 (0.01) | 5.07 (1.75) | 0.15 (0.04) |
| *D. pulicaria* | 5.28 (2.41) | 24.79 (2.27) | 0.30 (0.02) | 15.58 (1.77) | 0.21 (0.01) |

Note. — Neonate and final masses (μg) ± 1 standard deviation. Growth rates on LP (one-way ANOVA $F_{7,19}$=14.5, P<0.0001) and HP (one-way ANOVA $F_{7,19}$=6.04, P=0.0008) differed among taxa.

Table 3. Estimates of the relationship between long-term growth ($\mu$) and hours in HP.

| Species | Adjusted $R^2$ | $\beta_0$ | $\beta_1$ | $\beta_2$ |
|---|---|---|---|---|
| *D. lumholtzi* | 0.702 | 0.1254 | 0.0099 | -0.0210 |
| | | (0.0166)* | (0.0033)* | (0.0164) |
| *D. magna* (run 1) | 0.775 | 0.1666 | 0.0009 | 0.0261 |
| | | (0.0109)* | (0.0019) | (0.0100)* |
| *D. magna* (run 2) | 0.775 | 0.1687 | -0.0134 | 0.1048 |
| | | (0.0199)* | (0.0034)* | (0.0177)* |
| *D. mendotae* | 0.799 | 0.0418 | 0.0079 | 0.0087 |
| | | (0.0195)* | (0.0038) | (0.0192) |
| *D. obtusa* | 0.888 | 0.1131 | -0.0119 | 0.1030 |
| | | (0.0155)* | (0.0027)* | (0.0143)* |
| *D. parvula* (run 1) | 0.649 | 0.2186 | 0.0087 | -0.0010 |
| | | (0.0266)* | (0.0050) | (0.0258) |
| *D. parvula* (run 2) | 0.866 | 0.1234 | 0.0071 | 0.0102 |
| | | (0.0156)* | (0.0031) | (0.0145) |
| *D. pulicaria* | 0.846 | 0.2093 | -0.0047 | 0.0458 |
| | | (0.0098)* | (0.0019)* | (0.0096)* |
| *D. pulex* | 0.936 | 0.1474 | -0.0046 | 0.0759 |
| | | (0.0117)* | (0.0023) | (0.0115)* |

Note— Coefficient estimates for the polynomial relationship between $\mu$ and $F$ (slope ± 1 standard error) for each run

* $p < 0.05$

Table 4. Stoichiometric flexibility (1/*H*) and the influence of diet on *Daphnia* P content.

| | Experiment 1 | | | Experiment 2 | | | |
|---|---|---|---|---|---|---|---|
| Species | $\%P_{HP}$ | $\%P_{LP}$ | slope | $\%P_{HP}$ | $\%P_{LP}$ | slope | 1/H |
| *D. lumholtzi* | 1.58 (0.12) | 1.45 (0.04) | 0.036 (0.034) | 1.88 (0.17) | 1.25 (0.13) | 0.111(0.042)* | 0.036 |
| *D. magna* (run 1) | 1.57 (0.06) | 0.89 (0.00) | 0.293 (0.026)* | 1.79 (0.16) | 0.93 (0.07) | 0.197 (0.035)* | 0.197 |
| *D. magna* (run2) | 1.18 (0.30) | 1.90 (0.54) | -0.231 (0.194) | | | | |
| *D. mendotae* | 1.06 (0.15) | 0.10 (0.02) | 1.210 (0.150)* | 1.23 (0.06) | 1.12 (0.17) | 0.112 (0.057) | 0.112 |
| *D. obtusa* | 0.64 (0.24) | 2.02 (0.23) | -0.643 (0.207)* | 1.36 (0.09) | 0.95 (0.05) | 0.166 (0.056)* | 0.166 |
| *D. parvula* (run 1) | 1.94 (0.09) | 2.06 (0.14) | -0.031 (0.044) | 1.34 (0.14) | 1.17 (0.15) | 0.038 (0.079) | -0.031 |
| *D. parvula* (run 2) | 1.83 (n.a.) | 1.80 (0.40) | 0.005 (0.170) | | | | |
| *D. pulex* | 2.45 (0.15) | 0.81 (0.14) | 0.572 (0.101)* | 1.66 (0.19) | 1.01 (0.08) | 0.155 (0.022)* | 0.155 |
| *D. pulicaria* | 1.50 (0.10) | 1.00 (0.06) | 0.193 (0.038)* | 1.36 (0.02) | 0.92 (0.02) | 0.207 (0.046)* | 0.193 |

Note. – *Daphnia* P content (µg P µg dry mass$^{-1}$, ± 1 standard error) in the LP and HP treatments and the slope (± 1 standard error) of the relationship between ln *Daphnia* P:C and ln algal P:C. Results from both experiment one and two are shown. The degree of stoichiometric flexibility (1/*H*) is the maximum degree of homeostasis (*H*) exhibited between experiments. Daphnid P content differed among species when fed both LP (one-way ANOVA: $F_{7,18} = 28.9$, $P<0.0001$) and HP ($F_{7,19} = 11.5$, $P < 0.0001$).

* $p < 0.05$

## FIGURE LEGENDS

Figure 1. Schematic showing the short- (a) and long-term (b) responses of growth (GI) and resource integrators (RI) to heterogeneity in diet P:C. a) Instantaneous growth rate as a function of time for an animal temporally mixing LP and HP. Two potential responses exist. Growth could immediately respond to diet quality, producing synchrony between growth and diet, resulting in the square function (dashed line). Alternatively, asynchrony between growth and diet results when P is mixed across diets (dotted line). b) Long-term growth rate for animals consuming a diet which either switches, with variable frequency, between LP and HP (broken lines) or is a mixture of LP and HP (solid line). Mean daily food quality was normalized by the P content of the diet and ranges from 100% LP (0) to 100% HP (1). Under constant food supply, a saturating relationship exists between long-term growth rate and mean food quality (solid line labeled mixed diet). When food supply varies, long-term growth rate could respond to mean food quality following two potential classes of relationships. The dashed line (labeled GI) in b is the long-term result of growth described by the square function in a; whereas, the dotted line (labeled RI) corresponds to the curvilinear line (dotted) in a. The nonlinear relationship exhibited by RI varies between the GI response (i.e., no resource integration) and the mixed diet curve as a function of how efficiently excess P is mixed across diets.

Figure 2. Composite C:P of HP and LP *S. obliquus* used in experiment one. *Daphnia* species labels indicate which species were examined in the five runs. Note break in x-axis between 27 Feb 07 and 28 Apr 07.

Figure 3. Short- and long-term dynamics of the homeostatic and plastic models. a) Temporal variation in instantaneous growth rate from simulations of the homeostatic (squares) and plastic (circles) models. For reference, temporal variation in algal P (gray line) is shown ($S$ = 0.5). b) Relationship between long-term growth rate ($\mu$) and the hours out of 24 in HP ($F$) from simulations of the homeostatic (squares) and plastic (circles) models.

Figure 4. In simulations of the plastic model, the ability to integrate resources ($\theta_2$) increases with the plasticity of body P ($\Delta_P$ = $Q_{Z,P}^{\max}$ - $Q_{Z,P}^{\min}$). a) Relationships between long-term growth rate and hours out of 24 in HP ($F$) for model species varying in P plasticity ($\Delta_P$, $\rho$mol P cell$^{-1}$). b) The relationship between resource integration ($\theta_2$) and stoichiometric flexibility (1/$H$) from multiple runs of plastic model where only $\Delta_P$ varied among runs. The homeostasis parameter (1/$H$) was calculated from simulations where animals consumed 100% LP or HP.

Figure 5. Relationship between long-term growth rate ($\mu$, $d^{-1}$) and hours out of 24 in HP (*F*) for seven *Daphnia* species. *Daphnia parvula* and *D. magna* runs were replicated in time. Runs are designated with run numbers. Only run 1 was used in comparisons.

Figure 6. Resource integration ($\beta_2$) increases nonlinearly with stoichiometric flexibility (1/*H*). Species with a significant capacity ($\beta_2$ p-value < 0.05) for resource integration were the most stoichiometrically flexible (t-test: n = 5, P = 0.013). Filled circles indicate species (*D. magna*, *D. obtusa, D. pulex,* and *D. pulicaria*) with significant model fits (P < 0.05) for both resource integration and stoichiometric flexibility. Open circles indicate species (*D. lumholtzi, D. mendotae,* and *D. parvula*) with insignificant fits for both models (P > 0.05). Error bars are 1 standard error.

Figure 1.

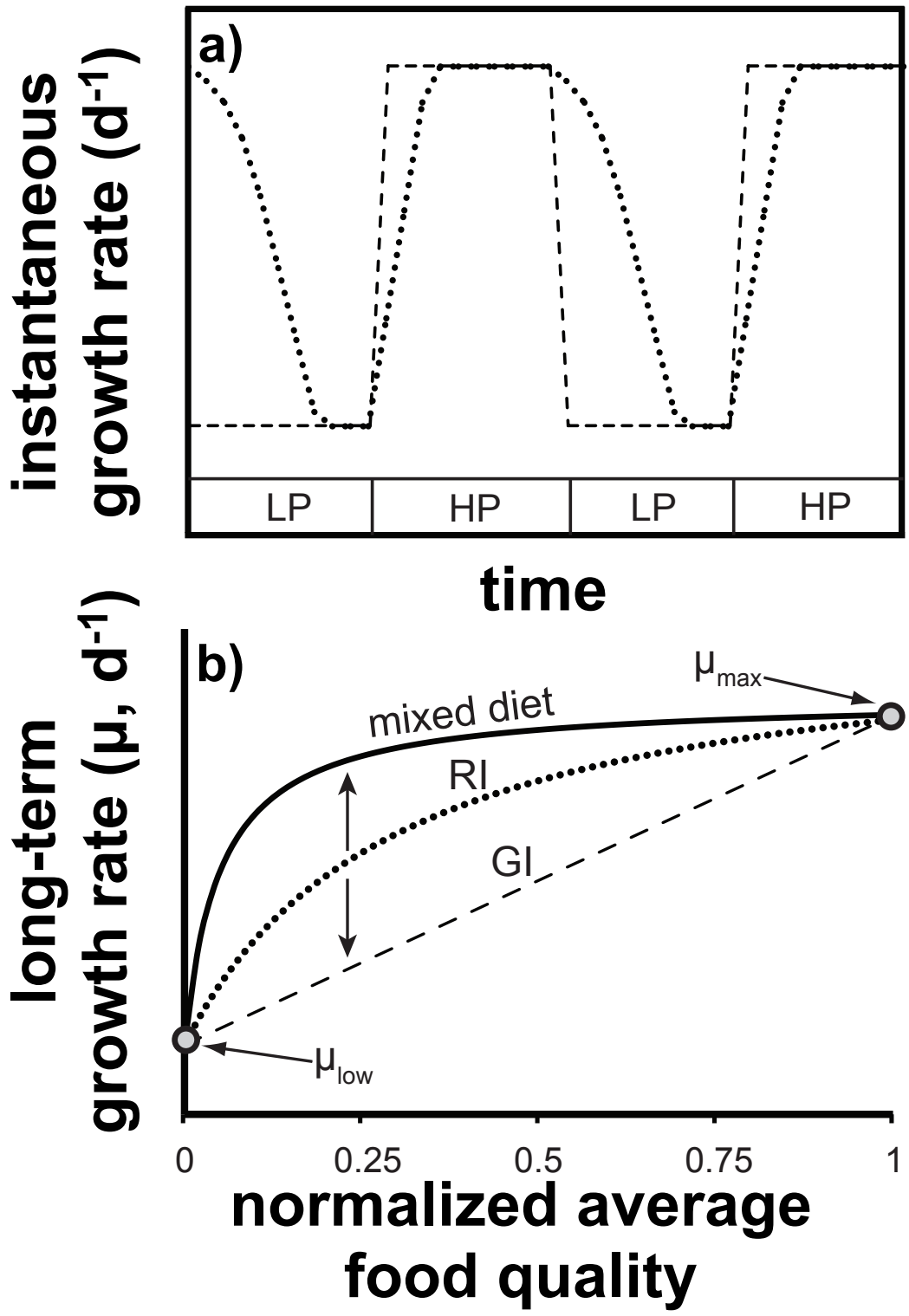

a)
instantaneous growth rate (d-1)
LP
HP
LP
HP
time
b)
long-term growth rate (μ, d-1)
μmax
mixed diet
RI
GI
μlow
0
0.25
0.5
0.75
1
normalized average food quality

Figure 2.

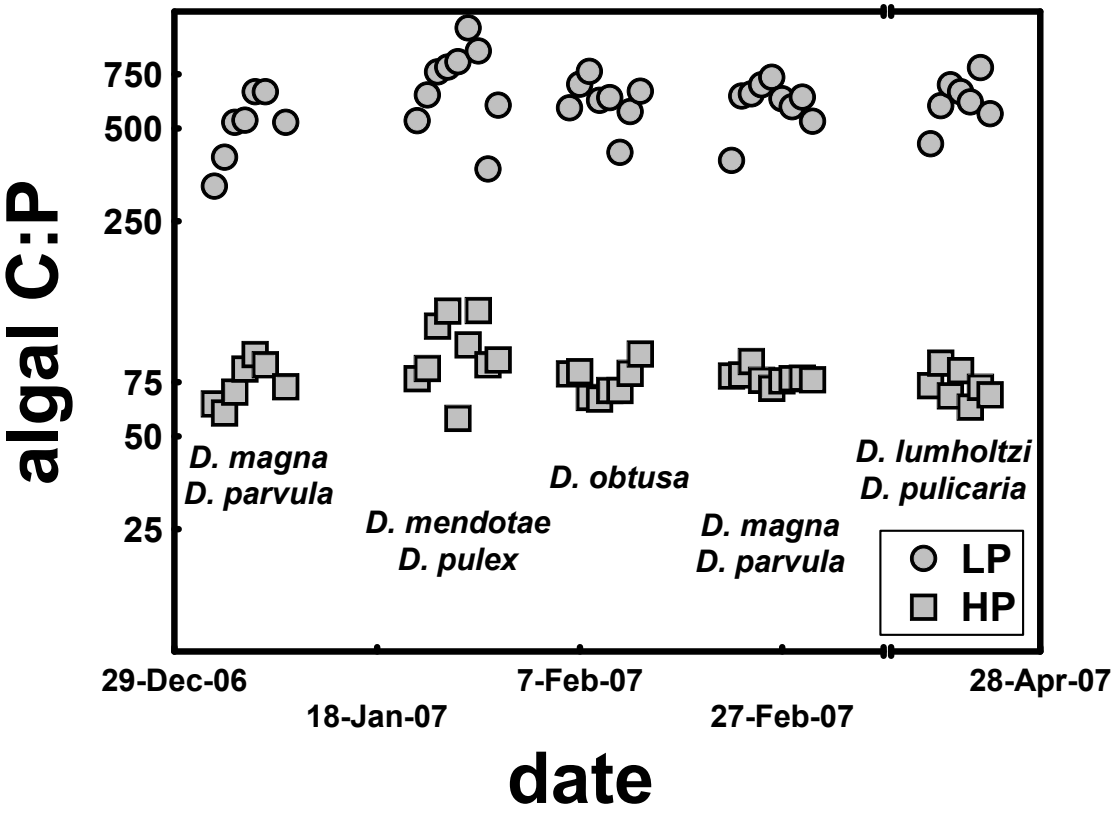

algal C:P
750
500
250
75
50
25
D. magna
D. parvula
D. mendotae
D. pulex
D. obtusa
D. magna
D. parvula
D. lumholtzi
D. pulicaria
LP
HP
29-Dec-06
18-Jan-07
7-Feb-07
27-Feb-07
28-Apr-07
date

Figure 3.

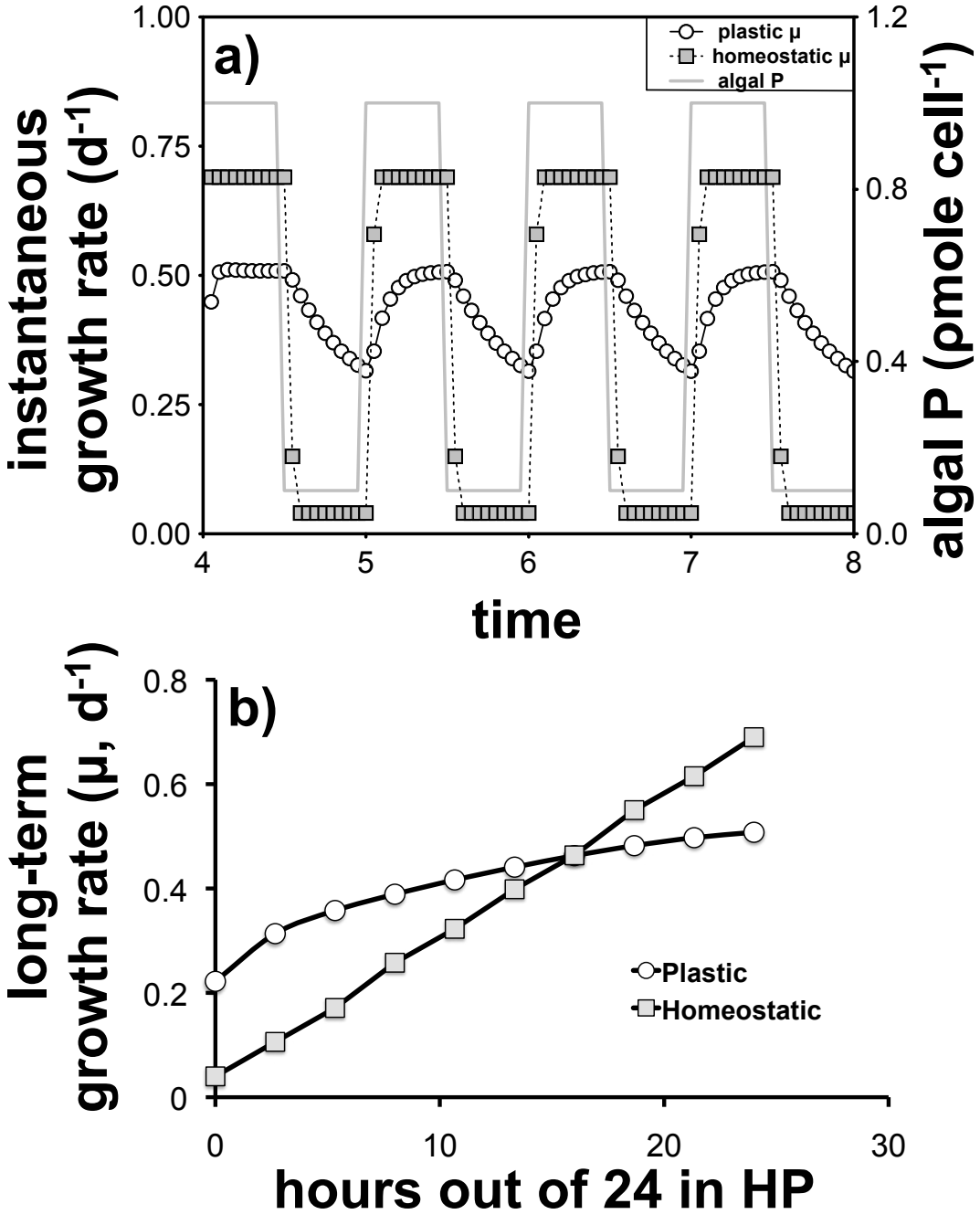

a)
plastic μ
homeostatic μ
algal P
instantaneous growth rate (d-1)
algal P (pmole cell-1)
time
b)
long-term growth rate (μ, d-1)
Plastic
Homeostatic
hours out of 24 in HP

Figure 4.

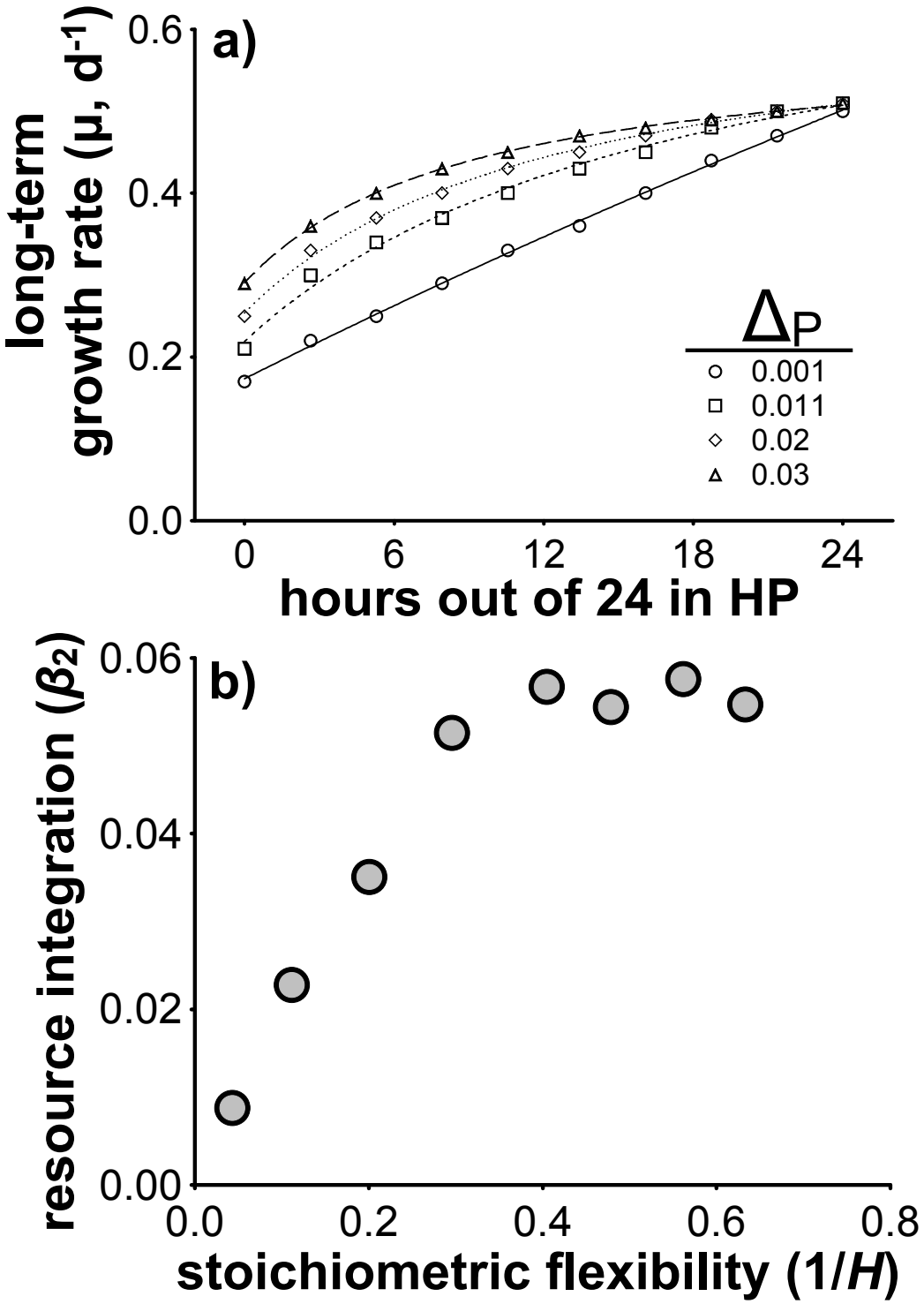

a)
long-term growth rate (μ, d-1)
0.6
0.4
0.2
0.0
0
6
12
18
24
hours out of 24 in HP
ΔP
0.001
0.011
0.02
0.03
b)
resource integration (β2)
0.06
0.04
0.02
0.00
0.0
0.2
0.4
0.6
0.8
stoichiometric flexibility (1/H)

Figure 5.

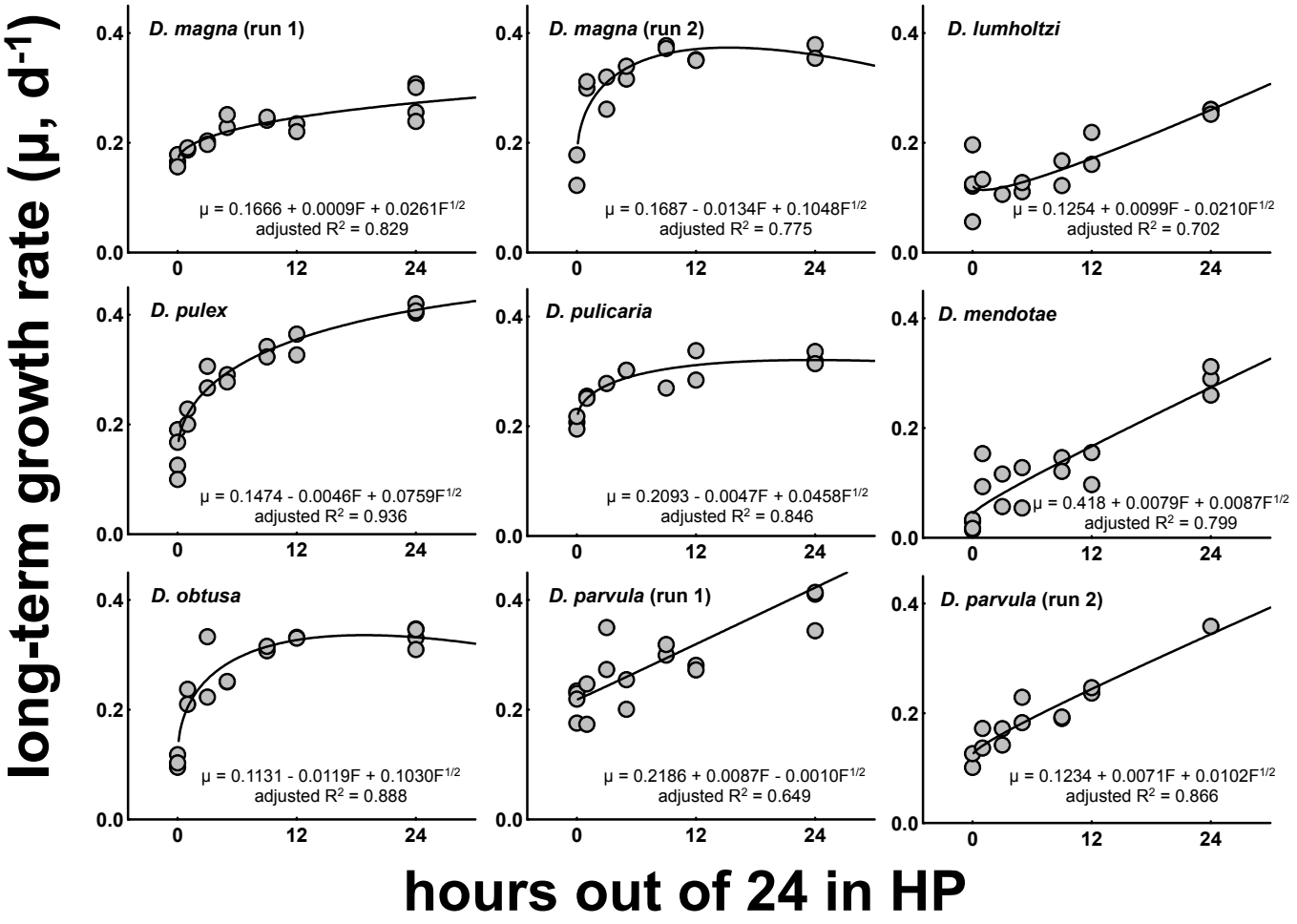
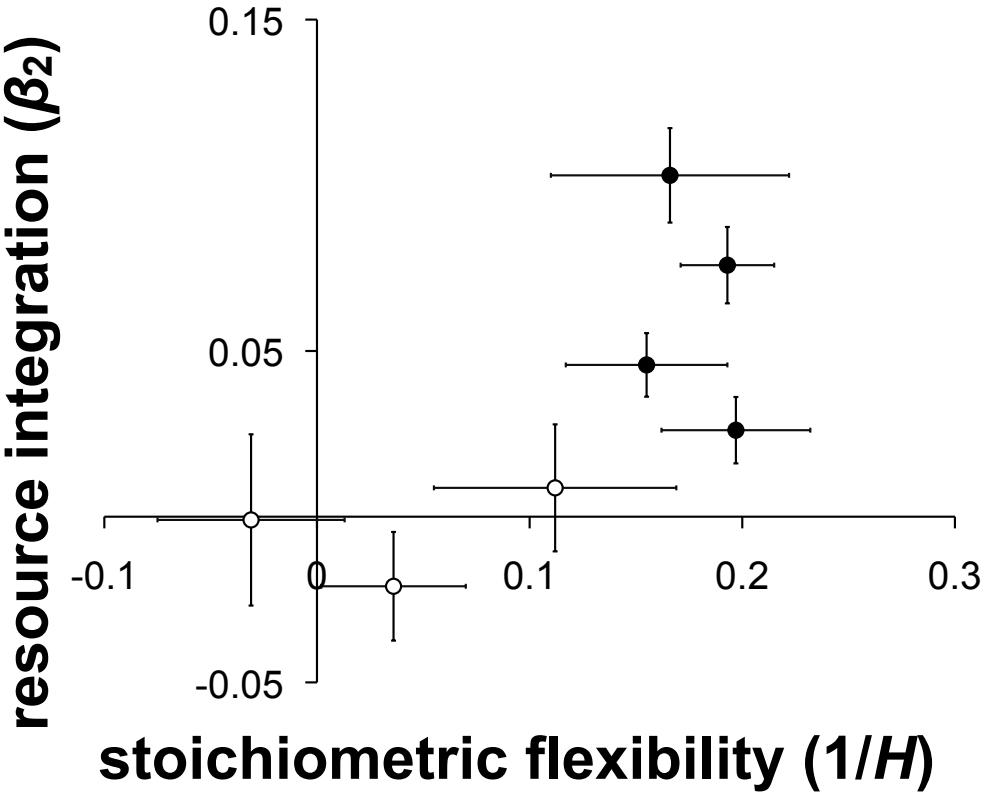

long-term growth rate (μ, d-1)
D. magna (run 1)
μ = 0.1666 + 0.0009F + 0.0261F1/2
adjusted R2 = 0.829
D. magna (run 2)
μ = 0.1687 - 0.0134F + 0.1048F1/2
adjusted R2 = 0.775
D. lumholtzi
μ = 0.1254 + 0.0099F - 0.0210F1/2
adjusted R2 = 0.702
D. pulex
μ = 0.1474 - 0.0046F + 0.0759F1/2
adjusted R2 = 0.936
D. pulicaria
μ = 0.2093 - 0.0047F + 0.0458F1/2
adjusted R2 = 0.846
D. mendotae
μ = 0.418 + 0.0079F + 0.0087F1/2
adjusted R2 = 0.799
D. obtusa
μ = 0.1131 - 0.0119F + 0.1030F1/2
adjusted R2 = 0.888
D. parvula (run 1)
μ = 0.2186 + 0.0087F - 0.0010F1/2
adjusted R2 = 0.649
D. parvula (run 2)
μ = 0.1234 + 0.0071F + 0.0102F1/2
adjusted R2 = 0.866
0.0
0.2
0.4
0
12
24
hours out of 24 in HP

Figure 6.


0.15
0.05
-0.05
-0.1
0
0.1
0.2
0.3
resource integration ($\beta_2$)
stoichiometric flexibility (1/$H$)

**CHAPTER 2:**

# Tradeoff between maximum growth and sensitivity to P-limitation of growth.

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Ecological stoichiometry offers a framework for predicting the sensitivity of a species to P-limitation and maximum growth rate, two important traits shaping the fitness of *Daphnia* species. Phosphorus mass balances suggest that sensitivity of a species to P-limitation should increase with P content. The growth rate hypothesis (GRH) predicts that among species maximum growth rate should increase with P content, because rapid growth requires more P-rich ribosomal RNA. Tests using *Daphnia* species did not support these predictions, even though the predictions of the GRH have wide support when comparisons are made across genera or higher taxonomic groups. Here, we use the relationship between dry mass and P growth rates to gain a better understanding of the factors shaping sensitivity to P-limitation and maximum growth rate. We grew seven *Daphnia* species on constant and variable algal diets differing in P content. Contrary to predictions, we found no relationship between the P content of a species and maximum growth rate or sensitivity to P-limitation. Sensitivity to P-limitation of growth was related to both dry mass and P growth rates, suggesting that growth rate may be a better indicator of P demand than P content. Carbon and P growth rates were tightly correlated ($R^2 = 0.83$) across all treatments and species with a positive intercept and slope less than one. Thus, the P cost of a unit of new carbon growth increases with growth rate. The disproportionate increase of P demand with growth rate potentially explains why rapidly growing species are most sensitive to P-limitation. Taken together, our results show that species face a tradeoff between maximum growth and sensitivity to P-limitation.

## INTRODUCTION

Ecologists seek to identify traits and linkages that can be used to understand the complexity of the natural world and make predictions about dynamical outcomes. Ecological stoichiometry is one framework. It uses elemental mass balances to understand the connections among nutrients, consumer performance, and food web dynamics (Sterner and Elser 2002, Andersen et al. 2004). The phosphorus stoichiometry of consumers and resources is generally the focus of freshwater studies because this nutrient is the primary constraint on algal production (Schindler et al. 2008) and can determine food quality for many aquatic grazers (Frost and Elser 2002, Elser et al. 2005a, Seidendorf et al. 2010).

Stoichiometric theory predicts linkages among invertebrate P content, sensitivity to P-limitation, and maximum growth. These predictions are partially based on the growth rate hypothesis (GRH; Main et al. 1997, Elser et al. 2003), which posits a tripartite linkage among growth, body P content, and RNA content both among and within species in response to diet. Rapid growth requires high levels of protein synthesis driven by P-rich ribosomal RNA; P in r-RNA constitutes 25 to 75% of the total P pool in invertebrates (Elser et al. 2003, Acharya et al. 2004). Thus, rapidly growing animals are predicted to have high levels of r-RNA and therefore P. This tripartite linkage has been observed in a diversity of groups including bacteria, fruit flies, and zooplankton (Elser et al. 2003). The high P requirement of rapid growth also suggests

that P-rich species should be the most sensitive to P-limitation of growth (Sterner and Hessen 1994, Sterner and Elser 2002). These linkages make stoichiometry a useful tool for predicting the outcome of competition and suggest key ecological traits affecting fitness.

In freshwater systems, algal C:P is often both temporally and spatially variable (Sterner et al. 1997, Hessen et al. 2005, Berger et al. 2006). Algal C:P varies over periods of hours to days in response to resource availability and ambient light levels (Cunningham and Maas 1978, Dickman et al. 2006). Spatial variation in resource availability shapes small and large scale patchiness in algal C:P. For instance, algae in epilimnetic zones with high light are often P-poor in comparison to algae in hypo- or metalimnetic waters with low light (Sterner et al. 1997, DeMott et al. 2004a). Vertically migrating zooplankton graze in both regions during diel migrations (DeMott et al. 2004a).

Thus, it is important to understand how individuals respond to short-term variation in diet C:P. Zooplankton utilize a suite of factors to mitigate the negative influence of P poor diets. Some species are not sensitive to P-limitation of growth. A low-P diet has little impact on the performance of these species (DeMott and Pape 2005, Seidendorf et al. 2010). Species with a relatively high sensitivity to P-limitation exhibit several strategies for coping with poor quality diets. Some zooplankton species can identify and actively seek out P-rich algae (Schatz and McCauley 2007) or use P stores to supplement growth while feeding in low quality patches (Chapter 1, Sterner

and Schwalbach 2001). Finally, at least some *Daphnia* species are able to quickly recover lost mass and phosphorus when a P-poor diet is replaced with a P-rich diet (DeMott 2003).

The percent P and percent RNA of *Daphnia* decline in response to a P-poor diet (DeMott and Pape 2005, Persson et al. 2010). The magnitude of intra-specific variation in stoichiometry (e.g., μg P · μg dry mass$^{-1}$) of a consumer in response to variation in resource stoichiometry is defined as the degree of stoichiometric homeostasis, when life stage and environmental conditions are held constant (Sterner and Elser 2002). Homeostasis is considered strict when consumer stoichiometry does not vary in response to resource stoichiometry. Theoretical studies have generally assumed strict stoichiometric homeostasis for consumers; yielding insights into competition, food web dynamics, and nutrient cycling (Elser and Urabe 1999, Andersen et al. 2004). Yet, many invertebrates to which this theory has been applied do not maintain strict nitrogen or P homeostasis (Persson et al. 2010), even though the stoichiometry of these invertebrates does not vary as much as their resource. The degree of stoichiometric homeostasis likely influences patterns of consumer growth, assimilation, and excretion.

The relationship between P and C absolute and specific growth rates provides fundamental information about the P economy of growth that, while central to stoichiometric theory, is rarely examined (but see: DeMott 2003). For instance, the relationship between P and C absolute growth rates ($g_P$:$g_c$) indicates the P:C of new growth and how it is related to growth rate (Fig. 1a). The P:C of growth plays an

important role in stoichiometric mass balance models. It is thought to reflect the P cost of a unit of growth, which is linked with nutrient release rates and sensitivity to P-limitation. Yet $g_P$:$g_c$ only reflects the P cost of a unit of growth when growth is fueled by ingested P. If internal P stores are used to fuel growth, which occurs for some P-limited species (DeMott 2003), $g_P$:$g_c$ would underestimate the P cost of a unit of growth. Both internal and external sources of P can be accounted for by examining the specific P and C growth rates (Fig. 1b). The ratio of specific P and C growth rates ($\mu_P$:$\mu_C$) indicates the change in P:C relative to the initial P:C. Here, we refer to $\mu_P$:$\mu_C$ as the relative P cost of new growth and argue that examination of this term improves our understanding of the influence of food quality and growth rate on the P cost of a unit of growth (Fig. 1b).

The P cost of a unit of growth is perhaps one of the most fundamental aspects of the P economy of a consumer; yet, relatively little is known about this trait. The GRH predicts that the P cost of a unit of growth should increases with growth rate, since rapid growth requires more P-rich r-RNA. To our knowledge, it is not known if the P cost of a unit of growth increases proportionately or disproportionately with growth rate. The nature of this increase would influence competition among species as well as the evolution of rapid growth. A better understanding of the P economy of growth will improve our understanding of consumer growth and competition as well as provide the information required to better integrate variation in degree of homeostasis into stoichiometric theory.

Here, we used seven *Daphnia* species to gain a better understanding of the

principles linking growth, P demand, and stoichiometric homeostasis. Animals were grown under three different feeding regimes: high P food, low P food, and a transition from low P to high P food. First, we examined how these seven species differ in their ability to recover from P-limitation, a first step towards understanding how diet heterogeneity may influence performance. Second, we examined whether our dataset supports predictions about linkages between growth, P content, and sensitivity to P-limitation. Third, we show how examining the relationship between C and P growth rates can improve our understanding of the linkages between growth, P demand, and stoichiometric homeostasis.

## METHODS

*Taxa and culture conditions*

This study examines elemental growth coupling under constant and non-constant diet conditions using seven *Daphnia* species (*D. lumholzi, D. magna, D. mendotae, D. obtusa, D. parvula, D. pulex, D. pulicaria*). With the exception of *D. lumholtzi*, R.W. Sterner (University of Minnesota – Twin Cities) has maintained these species in culture for several years.

Twenty-liter stock cultures of *Daphnia* were maintained at room temperature (~20˚ C) in a COMBO medium (Kilham et al. 1998) modified to contain 40 µM P and 500 µM N. Batch cultures were fed a combination of *Scenedesmus obliquus* and ground

alfalfa. For the experimental diets, *Scenedesmus obliquus* was grown in chemostats under P-poor (LP: 1000 µM N, 5 µM P, dilution = 0.1 $day^{-1}$) and P-rich (HP: 400 µM N, 80 µM P, dilution = 0.5 $day^{-1}$) conditions. LP algae had a mean C:P of 1325 while HP algae's mean C:P was 94. Algal C, N, and P concentrations were determined with a FOSS systems NIRS spectrometer. Calibration equations and validation procedures are described by Hood et al. (2006).

*Experimental design*

All experiments began with <24 hour old neonates and ended after 72 hours. For each species, neonates were distributed as follows. Fifteen neonates were immediately placed on glass slides for mass and P content determination. The remaining neonates were distributed between three treatments varying in diet: 72 hrs LP, 48 hrs LP then 24 hrs HP (switch treatment, SP), and 72 hours HP. We created three replicate bottles per treatment and stocked jars with 10-20 animals per container. The density of animals per container varied to standardize the final biomass. Often, there were not enough neonates to start an entire experiment in one day; therefore, the initiation of each bottle was randomized. Neonate samples were collected each day bottles were initiated.

Each experimental bottle contained 250 mL of COMBO medium (lacking N and P) and 1 mg C/L of algae. Daily, animals were transferred to a new bottle containing fresh basal COMBO and algae. During transfers, animals were "rinsed" in a beaker containing

basal COMBO (containing no N or P) for at least one minute. Experiments were run on a roller table, under low light, in an environmental chamber at 20˚ C. At the end of each experiment, each dried individual was removed from the slide and weighed in triplicate to the nearest 0.1 µg on a Mettler microbalance (Mettler UMX2). Several precautions were taken to reduce environmental variability. The microbalance was kept in a basement room on a marble table anchored in sand. A Static Master Ionizer (Amstat industries) was used to neutralize static electricity charges. Specific dry mass and P growth rates were calculated as

$$\mu_i = \frac{\ln\left(mass_{final}\right) - \ln\left(mass_{initial}\right)}{time},$$

where *i* indexes dry mass or mass of P, and time was in days. Juvenile *Daphnia* exhibit exponential growth in mass (DeMott 2003), so specific rates are used to evaluate patterns of growth and calculate the metrics described below.

The relationship between P and C absolute and specific growth rates provides information about the P:C of growth and the change in P:C through the experiment, respectively. To estimate daphniid C mass, we assumed that *Daphnia* were 45% carbon (Andersen and Hessen 1991). Absolute C and P growth rates were calculated as

$$g_i = \frac{mass_{final} - mass_{initial}}{time},$$

where *i* indexes mass of C or P, and time was in days. We explore daphniid P economy by examining the relationship between C and P specific and absolute growth rates. Fits of the relationship between C and P growth rates were estimated with standardize

major axis regression using SMATR (Falster et al. 2003). We used a α of 0.1 to test whether the intercept was significantly greater than zero and a α of 0.05 to test whether the slope was different from one. These tests were conducted in SMATR (Falster et al. 2003).

After weighing, *Daphnia* were transferred to a borosilicate glass tube, ashed (550˚ C), and analyzed for P content using the molybdate-ascorbate method described by DeMott et al. (1998). Phosphorus analyses were run in a 10 or 20 mL reaction. Daphnia numbers per reaction tubes were adjusted to maximize the number of samples analyzed per container while achieving sufficient phosphorus levels to provide good precision.

*Modeling and parameter calculation*

The homeostasis parameter *H* (eta) describes the degree to which the elemental content of a species responds to variation in diet elemental content (Sterner and Elser 2002). Higher values of *H* indicate greater homeostasis (less variation in consumer nutrient content). We calculated *H* for *Daphnia* body P:C by first regressing the natural log of *Daphnia* body P:C against the natural log of algal P:C. We used only the treatments where food was held constant (LP and HP). The homeostasis parameter *H* is calculated as the inverse of the slope of this regression line. When the regression line is shallow *H* approaches infinity. To avoid values of infinity we used 1/*H* (the slope of the regression line) for statistical purposes. Differences in the degree of stoichiometric

homeostasis among species were examined by testing for differences in the slope of the regression line with a homogeneity of slopes test (Statistica, STATSOFT). Other tests were conducted in Statistica with $\alpha = 0.05$.

Here, we were interested in quantifying both 1) differences among species in sensitivity to P-limitation and 2) recovery from this condition; therefore, we calculate indexes of sensitivity to P-limitation and recovery from P-limitation. We calculate sensitivity to P-limitation ($S_{DM}$) using final mass

$$S_{DM} = 1 - \frac{mass_{HP}}{mass_{LP}}$$

where mass is either the final dry mass in HP or LP. We also calculated the relative recovery of each species from P-limitation in the SP treatment. This index is calculated for both dry mass and phosphorus. To examine the recovery of phosphorus, we focus on total body P mass (μg P $ind^{-1}$) and not body P content as variation in %P can result from changes in P atoms as well as other constituents of dry mass. Relative recovery was calculated in terms of both dry mass ($R_{DM}$) and μg P ($R_P$) as:

$$R_i = \frac{\left(mass_{i,SP} - mass_{i,LP}\right)}{\left(mass_{i,HP} - mass_{i,LP}\right)}$$

where *i* indexes either dry mass or mass of P atoms.

In the final day of the SP treatment, animal growth rates might specificly switch from LP to HP growth rates or differ from HP. We did not directly measure growth during the final day of the switch treatment. Instead, we use two approaches to estimate dry mass and P growth rates for the last day of the SP treatment. These

approaches use different assumptions to estimate dry or P masses after 48 hours of LP and likely bound the actual growth rate. If growth during the first 48 hours of the LP treatment is exponential, then we can estimate catch-up growth ( $\mu^{A}_{i,SP(24)}$ ) as:

$$\mu^{A}_{i,SP(24)} = \frac{\ln\left(mass_{i,SP}\right) - \ln\left(mass_{i,initial} e^{\mu_{i,LP}\,2}\right)}{time}$$

where $\mu_{i,LP}$ is the mean LP specific growth rate in terms of *i*, $mass_{i,SP}$ is the final mass of *i* in SP, $mass_{i,initial}$ is the neonate mass of *i*, and time is in days. Alternatively, *Daphnia* may not grow exponentially when consuming a P-poor diet. DeMott (2003) shows that the dry mass and P growth rates of P-limited *D. magna* are initially high then decline to near zero over 48 hours; suggesting that the first approach may under-estimate mass in SP at 48 hours and over-estimate catch-up growth rates. A more conservative estimate of growth rates during the last 24 hours of SP assumes that *Daphnia* stop growing in the LP treatment after 48 hours (i.e., $mass_{LP(48\ hrs)} = mass_{LP(72\ hrs)}$). This approach calculates catch-up growth ( $\mu^{B}_{i,SP(24)}$ ) as:

$$\mu^{B}_{i,SP(24)} = \frac{\ln\left(mass_{i,SP}\right) - \ln\left(mass_{i,LP}\right)}{time}$$

where $mass_{i,LP}$ is the final mass of *i* in LP.

## RESULTS

*Dry mass growth*

In general, the P-poor diet (LP) decreased *Daphnia* growth and final dry mass (Fig. 2a, Fig. 3a) relative to the high P (HP) treatment. Five of seven species fed LP grew more slowly and achieved a lower final mass, compared to HP (Figs. 2a, Fig. 3a). Growth varied widely among species in the HP treatment from 0.29 (*D. mendotae*) to 0.56 (*D. lumholtzi*) and in the LP treatment from 0.13 (*D. mendotae*) to 0.30 (*D. pulex*, Fig. 2a).

*Phosphorus content and homeostasis*

Diet had a significant effect on P content (Fig. 2b); however, few species-specific contrasts between treatments were significant ($P > 0.05$). *Daphnia* P content (% dry mass) varied widely among species in the HP treatment from 1.3% (*D. parvula*) to 1.9% (*D. lumholtzi*) and in the LP treatment from 0.9% (*D. pulicaria*) to 1.3% (*D. lumholtzi*; Fig 2b). *Daphnia* P mass (μg $ind^{-1}$) also varied among treatments (Fig. 3b).

These daphniids differed in their stoichiometric flexibility ($1/H$), which is measured as the slope of the relationship between log (*Daphnia* P:C) and log (resource P:C). Two of seven *Daphnia* species (*D. mendotae* and *D. parvula*) exhibited no significant relationship between log (body P:C) and log (algal P:C) (Table 1) and were classified as strictly homeostatic. Body P content of the five other species increased with dietary P; these species were classified as plastic. The five plastic species exhibited a statistically similar degree of homeostatic regulation (mean $1/H = 0.16$, Table 1). Stoichiometric flexibility (1/H) was not linearly related to $\mu_{DM}$, $\mu_P$, %P, or mass in any treatment ($P > 0.05$).

*Growth rate hypothesis (GRH)*

The GRH posits a positive relationship between growth rate and body P content. We tested the GRH on an intra-specific basis with only the HP and LP treatments. We found no relationship between $\mu_{DM}$ and P content for the strictly homeostatic species, which by definition do not vary in P content in response to diet. Among the plastic species, *D. pulicaria* and *D. magna* both exhibited significant, positive relationships between growth and body P, while the three other plastic species exhibited positive relationships of marginal significance ($0.05 < P < 0.1$, Table 2). We also examined the GRH on an inter-specific basis for maximally growing individuals. There was no relationship between dry mass growth rates and P content in HP (Table 2).

*Sensitivity to P-limitation*

Sensitivity to P-limitation of growth ($S_{DM}$) is expected to reflect the P demand of a species. Contrary to past predictions (Sterner and Hessen 1994), $S_{DM}$ was not related to the simple measurement of %P, but could be predicted by stoichiometric principles. Sensitivity to P-limitation of growth ($S_{DM}$) varied widely among species (Fig 4). *D. lumholtzi* was most sensitive and *D. pulicaria* was least sensitive. Contrary to predictions, $S_{DM}$ was not related to the P content of optimally growing individuals (HP, Fig 4a); however, it was positively related to both specific dry mass and P growth rates in HP (Fig 4b and 4c). These results suggest that growth rate may be a better indicator

of P demand than P content.

*Recovery from P limitation*

*Daphnia* masses in the SP treatment were intermediate between masses in the LP and HP treatments (Fig 2a), although diet did not significantly influence *D. mendotae* and *D. pulicaria* mass. Relative dry mass recovery ($R_{DM}$) varied among species. Most species exhibited $R_{DM}$ around 50% but *D. pulicaria* and *D. pulex* exhibited near-complete recovery (Fig. 5a). *D. pulex* and *D. pulicaria* do not stand out as being especially insensitive to P-limitation ($S_{DM}$ = 0.60 and 0.75, respectively) and $R_{DM}$ was not linearly related to sensitivity to P-limitation, 1/*H*, or any other trait from the HP treatment examined including $\mu_{DM}$, $\mu_P$, %P, mass ($P > 0.05$).

We did not measure catch-up growth rates ($\mu_{SP(24)}$) during the last 24 hours of the SP treatment. Instead, we present two approaches for estimating $\mu_{SP(24)}$, which likely bound the actual rate. If we assume animals grow exponentially when consuming LP (approach A), growth rates during the last 24 hours of the SP treatment are estimated to be approximately two times higher than those in the HP treatment (Fig 6a). Alternatively, if we assume that animals stop growing after consuming LP for 48 hours (approach B) growth rates during the last 24 hours are lower than in HP for all species except *D. lumholtzi* (Fig 6a inset).

Excluding the strictly homeostatic species (*D. mendotae* and *D. parvula*), P masses were similar between SP and HP for all species except *D. magna*, even though

exposure to P in food was less in SP than HP (Fig 3b). Both approaches for estimating P growth rate during the last 24 hours of SP, predict higher rates than observed in HP for all species except the strictly homeostatic *D. parvula* (Fig 6a).

Phosphorus mass recovery ($R_P$) varied widely from 0.34 (*D. magna*) to one (*D. parvula*, Fig.5b). Strictly homeostatic species had the highest $R_P$ ($F_{1,5} = 8.18$, $P = 0.035$) because P content did not differ between LP and HP for these species. Phosphorus mass recovery was not linearly related to any trait we examined ($S_{DM}$, $\mu_{DM\text{-}HP}$, $\mu_{P\text{-}HP}$, or $mass_{HP}$). However, there is a suggestion that $R_P$ declines with stoichiometric flexibility ($y = 0.29 - 0.27*x$, $R^2 = 0.54$, $P = 0.061$), particularly when the possible outlier *D. pulicaria* is removed (without *D. pulicaria*: $y = 0.31 - .34*x$, $R^2 = 0.94$, $P = 0.001$). Finally, species with high $R_P$ added more P than C during the final 24 hours of SP ($R_P = 0.45 \_ 0.74* \mu_{C:P,\ SP\text{-}24}$, $R^2 = 0.77$, $P = 0.010$).

*Relationship between P and C absolute and specific growth rates*

Our results reveal that the P requirements of growth create functional linkages among C and P growth dynamics, P content, and stoichiometric homeostasis that transcend species and diets. Across all species and diets, specific dry mass growth rates were positively related to P content ($\mu_C = -0.03 + 0.27 \cdot \%P$, $P < 0.001$, $R^2 = 0.467$). Phosphorus content also increased with specific P growth rates ($\%P = 0.88 + 1.35 \cdot \mu_P$, $P < 0.001$, $R^2 = 0.69$) with an intercept of 0.88, indicating the P content at zero growth.

This suggests that rapid growth requires higher P concentrations, as predicted by the GRH.

Absolute growth rates in terms of P or C (e.g., dC/dt) indicate the magnitude of P or C accumulation during the experiment $\left( \frac{dP}{dt}\frac{dt}{dC} = \frac{dP}{dC} \right)$. The ratio of these rates ($g_P$:$g_C$) is the P:C of new growth and is thought to indicate the P cost of a unit of C growth. Phosphorus and C absolute growth rates were positively correlated across treatments and species with an intercept less than zero (β = 0 t-test: $t_{19}$ = -2.87, P = 0.01; Fig. 7a). The non-zero intercept indicates that rapid growth requires disproportionately more P than slow growth. Inspection of the residual variation around this line indicates that the equation under-predicts P growth rates at low growth (Fig. 7a). Within treatments, the P:C of new growth did not vary with growth rate (LP: P = 0.98; HP: P = 0.11; SP: P = 0.90). The P:C of new growth (molar) in the HP and SP treatments was 0.02 and 0.01, respectively. In the LP treatment the C:P of growth was 0.006.

Specific growth rates reflect the change in an element per unit time relative to the initial concentration of that element (dC/Cdt). The ratio of P and C specific growth rates $\left( \frac{dP}{Pdt}\frac{Cdt}{dC} = \frac{dP}{dC}\frac{C}{P} \right)$ indicates the change in daphniid P:C during the experiment as well as the P cost of a unit of growth relative to the initial P:C. The ratio of P and C specific growth rates ($\mu_P$:$\mu_C$) varied widely from 0.33 to 1.47 across all treatments. In the HP and SP treatments $\mu_P$:$\mu_C$ was near balanced (mean ± 1 S.D. ; HP: 1.07 ± 0.28; SP: 0.92 ± 0.22) and P and C specific growth rates were related with a single slope (n = 14,

test statistic = 0.49, P = 0.52) greater than unity (HP: y = -0.22 + 1.64*x, $R^2$ = 0.77, P = 0.009; SP: y = -0.19 + 1.49*x, $R^2$ = 0.74, P = 0.012). In the LP treatment, $\mu_P$:$\mu_C$ was low (LP: 0.65 ± 0.26) and varied widely among species from 0.33 (*D. mendotae*) to 1.06 (*D. parvula*). The LP treatment decoupled C and P growth rates (P > 0.05), though the small range in values decreased power.

Since $\mu_P$:$\mu_C$ indicates the relative P cost of a unit of growth, the nature of the relationship between specific P and C growth rates provides information on the influence of growth rate on the relative P cost of growth. Across treatments and species, specific P and C growth rates were tightly coupled (Fig. 7b) with a slope significantly greater than unity (β = 1, t-test: $t_{19}$ = -6.67, P < 0.001) and a non-zero intercept (β = 0, t-test: $t_{19}$ = 6.88, P < 0.001). Thus, rapid growth requires a disproportionately higher amount of P than slow growth. The relative P cost of growth when growing optimally (HP) appears to influence the degree of homeostatic flexibility. Species with the greatest P demand when growing optimally were the most stoichiometrically flexible (Fig. 7c).

## DISCUSSION

Dietary P content has a strong influence on *Daphnia* fitness (Urabe et al. 1997, Seidendorf et al. 2010) and is thought to influence competition among zooplankton species (Andersen et al. 2004). A framework is required for documenting how

important traits such as maximum growth rate and sensitivity to P-limitation covary and shape fitness. Previous approaches predict that across species, maximum growth rate and sensitivity to P-limitation should increase with P content, a proxy for the P requirements of growth (Sterner and Hessen 1994, Elser et al. 2003). Yet, tests fail to support these predictions (DeMott and Pape 2005, Kyle et al. 2006). Here we take an alternate approach by looking directly at the relationship between P and C growth rates; in so doing we can directly access a species' P requirements for maximal growth. Our results demonstrate a fundamental dependency of growth on P acquisition, as predicted by the GRH, but by directly examining P growth rates we also reveal a tight linkage among growth, sensitivity to P-limitation, and P homeostasis.

*Linkages among P demand, sensitivity to P-limitation, and P homeostasis*

Across all species and diets, P and C specific growth rates were tightly correlated ($R^2 = 0.92$). A positive relationship is predicted by the GRH, but the strong linear trend, with slope less than one and positive intercept reveals underlying linkages between P and C dynamics. This shows that the P requirements of growth, though not constant across species and diets, correspond to a simple linear equation. Tight coupling of P and C growth is surprising in light of previous work that uses P content as a metric of the P requirements of growth. Several studies, including ours, found no relationship between the maximal growth rate and P content (DeMott and Pape 2005, Kyle et al. 2006). Kyle et al. (2006) suggests that P content is a poor predictor of the P requirements of growth

and, therefore, growth rates across *Daphnia* species because of differences in the amount of P involved in maintenance processes. The strength of the relationship between P and C specific growth rates suggests that, though P allocation to maintenance processes might vary among species, the P requirements of new growth are described by a simple linear equation. Our results show that the ratio of P and C specific growth rates is a better predictor of the relative P requirements of growth than P content.

The slope of the relationship between C and P specific growth rates can be used to determine the relative P cost of a unit of growth. The GRH predicts that rapid growth requires more P-rich r-RNA, but to our knowledge makes no predictions about the P cost of a unit of growth (Elser et al. 2003). The P cost of a unit of growth is an important parameter that ultimately shapes the cost of maximizing growth through evolution. By analyzing the relationship between P and C specific growth rates, we show that the relative P cost of a unit of growth increases with growth rate. Thus, as growth rates increase growth becomes disproportionately more expensive in terms of P. To our knowledge, this has not been previously observed; however, it helps explain patterns of sensitivity to P-limitation and stoichiometric homeostasis.

Mass balance models suggest that if P content is a reliable measure of the P requirements of growth, then sensitivity to P-limitation should increase with P content. Several studies, including ours, did not find support for this hypothesis (DeMott and Pape 2005, Seidendorf et al. 2010), suggesting again that P content may not be a

reliable measure of the P requirements of growth. Here we show that sensitivity to P-limitation increases with growth rate, supporting previous work by Seidendorf et al. (2010). No mechanism has been proposed for this relationship; yet, the relationship between P and C specific growth rates helps explain this relationship. Rapidly growing species have a disproportionately higher P requirement for growth than slowly growing species and, therefore, we would expect them to be more sensitive to P-limitation of growth. These results suggest that species in the genus face a tradeoff between maximizing growth and avoiding P-limitation.

Our results suggest that patterns of stoichiometric homeostasis are due to the P requirements of optimal growth. Species with the highest relative P cost of a unit of growth ($\mu_P$:$\mu_C$) when growing optimally (HP) were the most stoichiometrically flexible (i.e., highest 1/*H*, Fig. 7c). Initially, it is surprising that the way species grow optimally is linked to stoichiometric flexibility; yet, examination of the relationship between P and C specific growth rates can be used to understand this linkage. Here, we show that the relative P cost of a unit of growth increases with growth rate, suggesting that rapidly growing individuals invest disproportionately more P into r-RNA and other growth machinery. Since, individuals consuming a low P diet experience a decline in percent RNA and percent P, rapidly growing species, which must invest heavily in P-rich growth machinery, would be expect to exhibit the greatest decline in total P concentrations and, therefore, the greatest stoichiometric flexibility.

The low P diet led to highly imbalanced growth; that is, $\mu_P$:$\mu_C$ was low, indicating C growth at the expense of internal P stores. The P:C of growth in LP was 0.006 for all species whereas the P:C of growth in HP was 0.02 (Fig. 7a). Carbon or more specifically dry mass growth at the expense of P homeostasis has been previously observed in daphniids (DeMott et al. 1998, DeMott 2003), although this is the first study to demonstrate this for a number of species.

*Growth on variable diets*

After consuming HP for 24 hours, all species recovered at least 45% of lost mass, relative to the HP treatment. *Daphnia pulex* and *D. pulicaria* recovered greater than 80% of lost mass, while recovery was more similar among the remaining species (0.49 – 0.66%, Fig 4a). DeMott (2003) also reported rapid resumption of growth for P-limited *D. manga* following a switch to a P-rich diet. No parameter we examined, including sensitivity to P-limitation, was related to the recovery rate of all species. Recovery rates may be a function of interactions between sensitivity to P-limitation, capacity for ingesting and assimilating P, and the rate of modification of ingestion and assimilation rates to HP.

The higher recovery rates of *D. pulex* and *D. pulicaria*, two closely related daphniids, suggests that these species may be well-suited to take advantage of P pulses. If true, these species would have a competitive advantage in systems with spatially or temporally heterogeneous algal P. Although closely related, *D. pulex* and *D. pulicaria*

are commonly found in very different habitats, fishless ponds and large lakes respectively (DeMott and Pape 2005).

Relative recovery rates indicate that dry mass and P growth rates rapidly increase when P-rich food becomes available. Our two approaches for estimating dry mass and P growth rates during the last 24 hours of SP place wide bounds on these growth rates. Dry mass growth rates could vary between slightly less than the rates observed in HP to nearly twice these rates. Both approaches suggest that *D. lumholtzi* grew more rapidly during the last 24 hours of SP than in the HP treatment. DeMott (2003) also observed a rapid resumption of *D. magna* growth following a switch from a P-poor to a P-rich diet. Measured growth rates of *D. manga* in DeMott's switch and P-rich treatments were nearly identical. Phosphorus growth rates estimated for this period were higher than the growth rates observed in HP for all species except for the strongly homeostatic *D. parvula*.

Additional research on temporal diet mixing by *Daphnia* (Chapter 1, Sterner and Schwalbach 2001), indicate that plastic *Daphnia* species benefit greatly from short-term pulses of high P food. This work shows that plastic *Daphnia* species required P-rich food for less than 25 % of each day to obtain one-half the growth differential between HP and LP. The ability to take advantage of small quantities of high P food increased with stoichiometric flexibility. In this study, the magnitude of recovery from P-limitation in the SP treatment was not related to homeostasis or any other parameter we examined. This finding supports Hood and Sterner's (Chapter 1) contention that the response of

resource integrators is predicated upon the transition from HP to LP, instead of the transition from LP to HP which we examined in this study.

*Significance*

To our knowledge, this is the first study to examine the coupling of dry mass and P growth rates among a suite of species grown under constant and variable diets. This approach demonstrated that the P requirements of growth create tight linkages among dry mass and P growth rates, sensitivity to P-limitation, and stoichiometric homeostasis that transcend species and diets. The P cost of a unit of growth increased with growth rate, leading to greater sensitivity to P limitation and greater stoichiometric flexibility among rapidly growing species. These results demonstrate that members of this genus tradeoff the capacity for maximum growth against the potential for P limitation of growth.

## TABLES

Table 1. Degree of stoichiometric flexibility (1/*H* ± 1 SE). Species with significant regression equations have a similar degree of homeostasis (Heterogeneity of slopes: species · log algal P:C, $F_{4, 20} = 0.47$, P = 0.76).

| species | slope (1/*H*) | $R^2$ | n | p-value |
|---|---|---|---|---|
| *D. lumholtzi* | 0.170 (0.061) | 0.665 | 6 | 0.048 |
| *D. magna* | 0.197 (0.035) | 0.890 | 6 | 0.005 |
| *D. mendotae* | 0.053 (0.074) | 0.114 | 6 | 0.512 |
| *D. obtusa* | 0.146 (0.037) | 0.794 | 6 | 0.017 |
| *D. parvula* | 0.066 (0.076) | 0.157 | 6 | 0.437 |
| *D. pulex* | 0.152 (0.045) | 0.738 | 6 | 0.028 |
| *D. pulicaria* | 0.132 (0.010) | 0.978 | 6 | < 0.001 |

Table 2. Regression coefficients from the relationship between growth rate ($\mu_{DM}$) and P content. The growth rate hypothesis predicts a positive relationship between growth and P content. The SP treatment was not included in the intra-specific comparisons.

| Species | intercept | slope | $R^2$ | n | p-value |
|---|---|---|---|---|---|
| Intra-specific comparisons | | | | | |
| *D. lumholtzi* | -0.169 (0.244) | 0.351 (0.151) | 0.573 | 6 | 0.081 |
| *D. magna* | -0.017 (0.051) | 0.170 (0.036) | 0.850 | 6 | 0.009 |
| *D. mendotae* | 0.204 (0.177) | 0.024 (0.149) | 0.006 | 6 | 0.883 |
| *D. obtusa* | -0.120 (0.136) | 0.413 (0.116) | 0.761 | 6 | 0.023 |
| *D. parvula* | 0.170 (0.175) | 0.078 (0.138) | 0.074 | 6 | 0.601 |
| *D. pulex* | 0.145 (0.088) | 0.173 (0.063) | 0.649 | 6 | 0.053 |
| *D. pulicaria* | -0.119 (0.109) | 0.384 (0.094) | 0.807 | 6 | 0.015 |
| Inter-specific comparisons | | | | | |
| HP | 0.113 (0.239) | 0.187 (0.156) | 0.225 | 7 | 0.282 |

## FIGURE LEGENDS

Figure 1. Conceptual diagrams illustrating how the relationship between P and C absolute (a) and specific (b) growth rates provides information about the P economy of growth. Absolute growth rates in terms of P or C (e.g., dC/dt) indicate the magnitude of P or C accumulation during the experiment $\left( \frac{dP}{dt}\frac{dt}{dC} = \frac{dP}{dC} \right)$. The ratio of these rates ($g_P$:$g_C$) is the P:C of new growth and is thought to reflect the P cost of a unit of growth. If the line has a zero intercept, the P:C of new growth does not vary with growth rate (lines 1a-c). If the intercept is less than zero, the P:C of growth increases with growth rate (line 2). This approach does not account for the initial P:C and, therefore, $g_P$:$g_C$ may provide a misleading estimate of the P cost of a unit of growth when growth is fueled by internal P stores. In contrast, specific growth rates reflect the change in an element per unit time relative to the initial concentration of that element (e.g., dC/Cdt). The ratio of specific P and C growth rates $\left( \frac{dP}{Pdt}\frac{Cdt}{dC} = \frac{dP}{dC}\frac{C}{P} \right)$ indicates the change in daphniid P:C over the measurement period and is what we call here the relative P cost of a unit of growth ($\mu_P$:$\mu_C$). When the intercept is at the origin (line 3) $\mu_P$:$\mu_C$ does not vary with growth rate. When the intercept is not at the origin (lines 4 and 5), $\mu_P$:$\mu_C$ varies with growth rate. In this case, if the slope is less than one $\mu_P$:$\mu_C$ decreases with growth rate (line 4); that is, rapid growth requires disproportionately less P than slow growth. An unlikely scenario. In contrast, when the slope is greater than one $\mu_P$:$\mu_C$ increases with

growth rate, indicating that rapid growth requires disproportionately more P than slow growth (line 5).

Figure 2. Diet P strongly influences *Daphnia* growth rate and P content (growth: $F_{14,42}$ = 36.32, P < 0.001; %P: $F_{14,42}$ = 5.45, P < 0.001). Mean dry mass growth ($d^{-1}$, a) and percent P (b) for all seven species grown on 3 diets. Error bars are 1 standard error. Letters represent significant differences within a species among diets (Tukey HSD, P < 0.05). Species are sorted by $\mu_{DM}$ or %P in the HP treatment.

Figure 3. Diet strongly influences *Daphnia* dry mass and P mass (mass: : $F_{14,42}$ = 17.81, P < 0.001; P mass: $F_{14,42}$ = 23.2, P < 0.001). Mean dry mass (μg $ind^{-1}$, a) and phosphorus mass (μg P $ind^{-1}$, b) for all seven species grown on 3 diets. Error bars are 1 standard error. Letters represent significant differences within a species among diets (Tukey HSD, P < 0.05). Species are sorted by dry or phosphorus mass in the HP treatment.

Figure 4. Sensitivity to P-limitation ($S_{DM}$) was not related to $\%P_{HP}$ (a), but increased with $\mu_{DM}$ (b) and $\mu_P$ (c) in HP.

Figure 5. Recovery of dry (a) and phosphorus (b) mass in SP.

Figure 6. Dry mass (a) and phosphorus growth (b) rates during the last 24 hours of SP

increased with HP growth rates. The results of approach A are shown in the main graph and the results of approach B are in the inset.

Figure 7. The relationship between P and C absolute and specific growth rates provides information about the P:C of growth and the relative P cost of new growth. Across all treatments and species, absolute P and C growth rates were tightly coupled with an intercept less than zero (a), indicating that the P:C of new growth increases with growth rate. Phosphorus and C specific growth rates were tightly coupled across all species and diets, with a negative intercept and a slope greater than one (b). This relationship indicates that the relative P cost of new growth ($\mu_P$:$\mu_C$) increases with growth rate. The negative intercept indicates that at low growth rates (i.e., in the LP treatment) growth is fueled by internal P sources. A species' degree of stoichiometric flexibility was positively related to the relative P cost of new growth in HP (c), indicating that the most stoichiometricaly flexible species also have the highest P demand when growing maximally.

Figure 1.

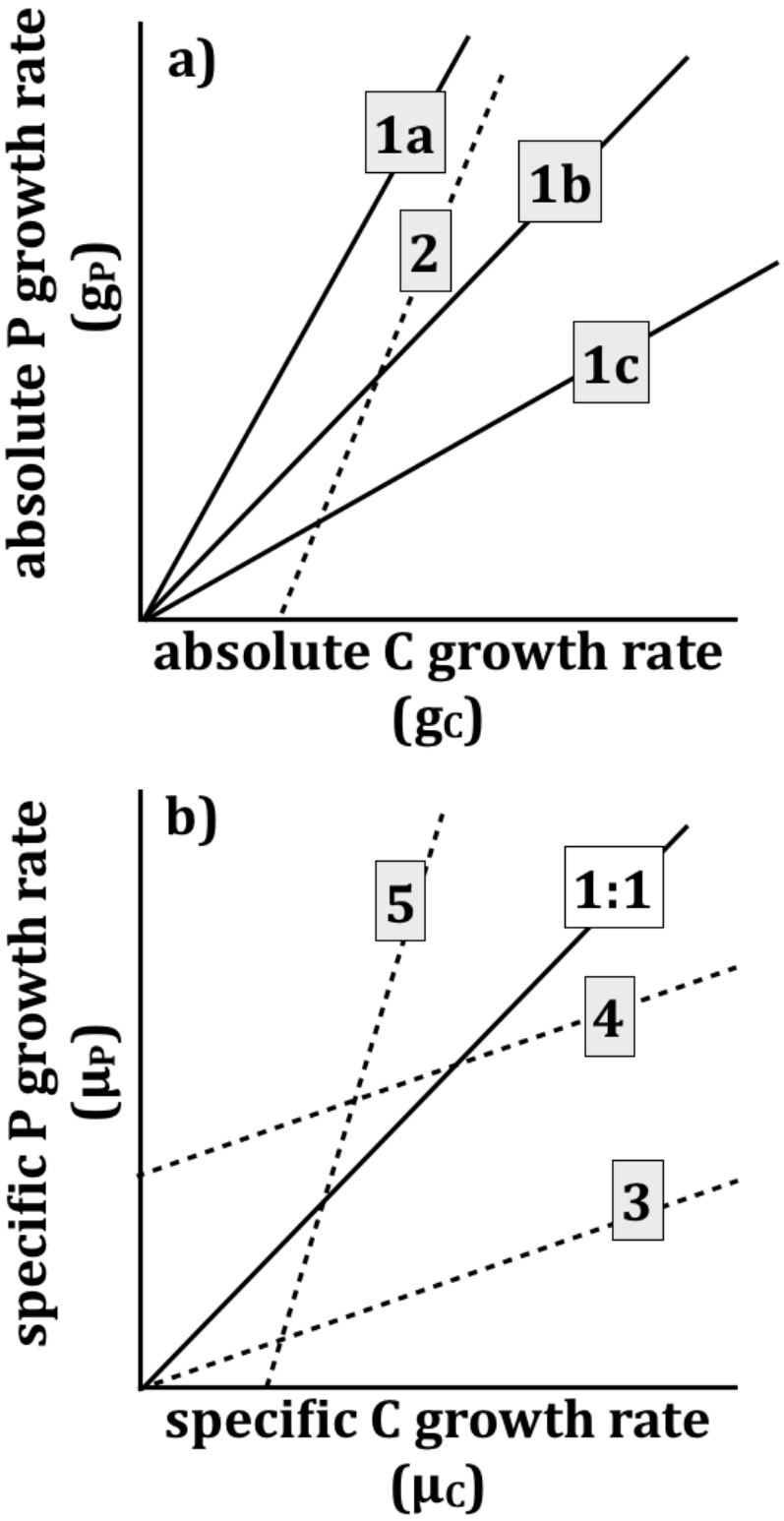

a)
1a
1b
2
1c
absolute P growth rate
(gP)
absolute C growth rate
(gC)
b)
5
1:1
4
3
specific P growth rate
(μP)
specific C growth rate
(μC)

Figure 2.

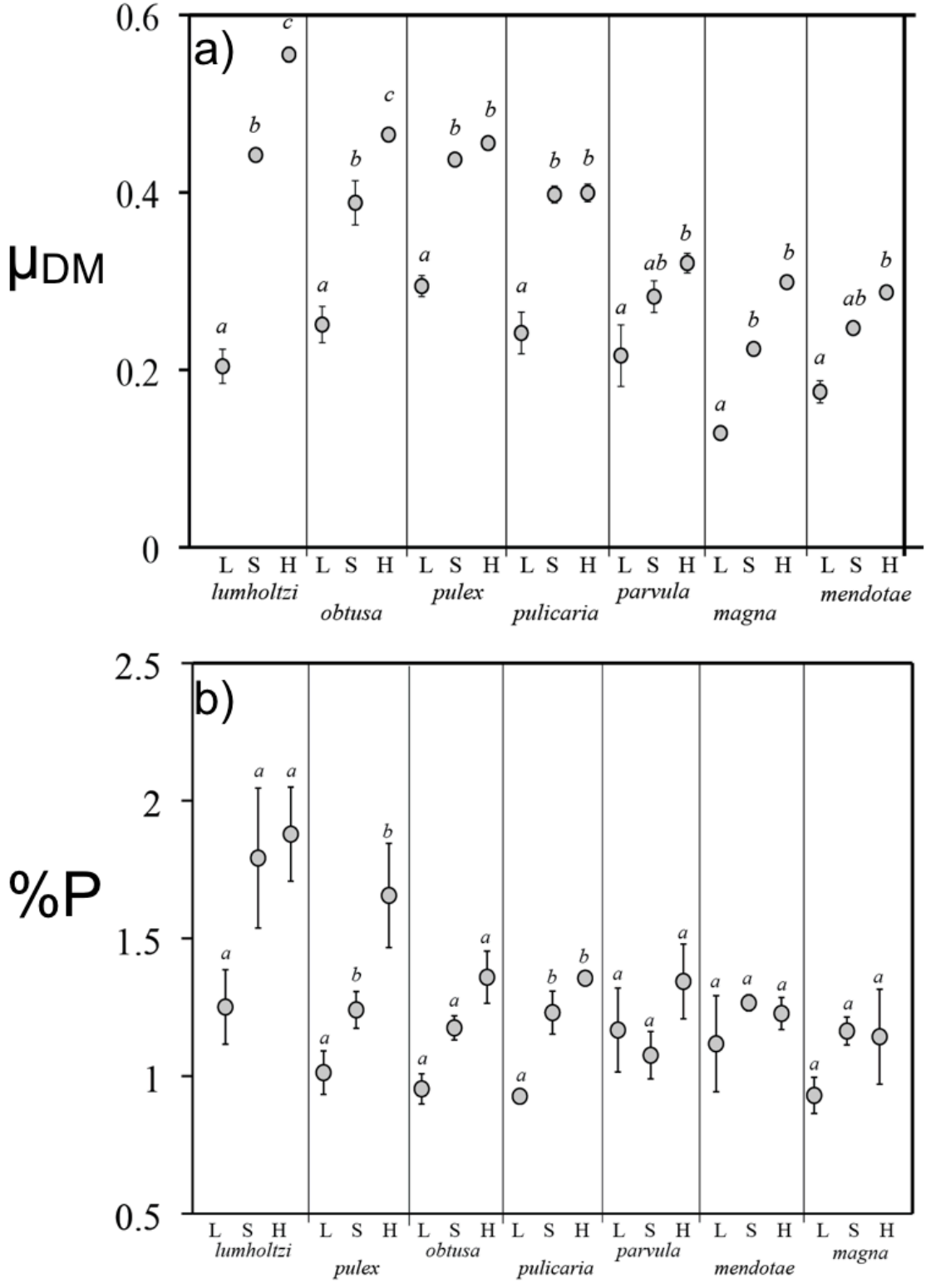

a)
μDM
0.6
0.4
0.2
0
L S H L S H L S H L S H L S H L S H L S H
lumholtzi
obtusa
pulex
pulicaria
parvula
magna
mendotae
b)
%P
2.5
2
1.5
1
0.5
L S H L S H L S H L S H L S H L S H L S H
lumholtzi
pulex
obtusa
pulicaria
parvula
mendotae
magna

Figure 3.

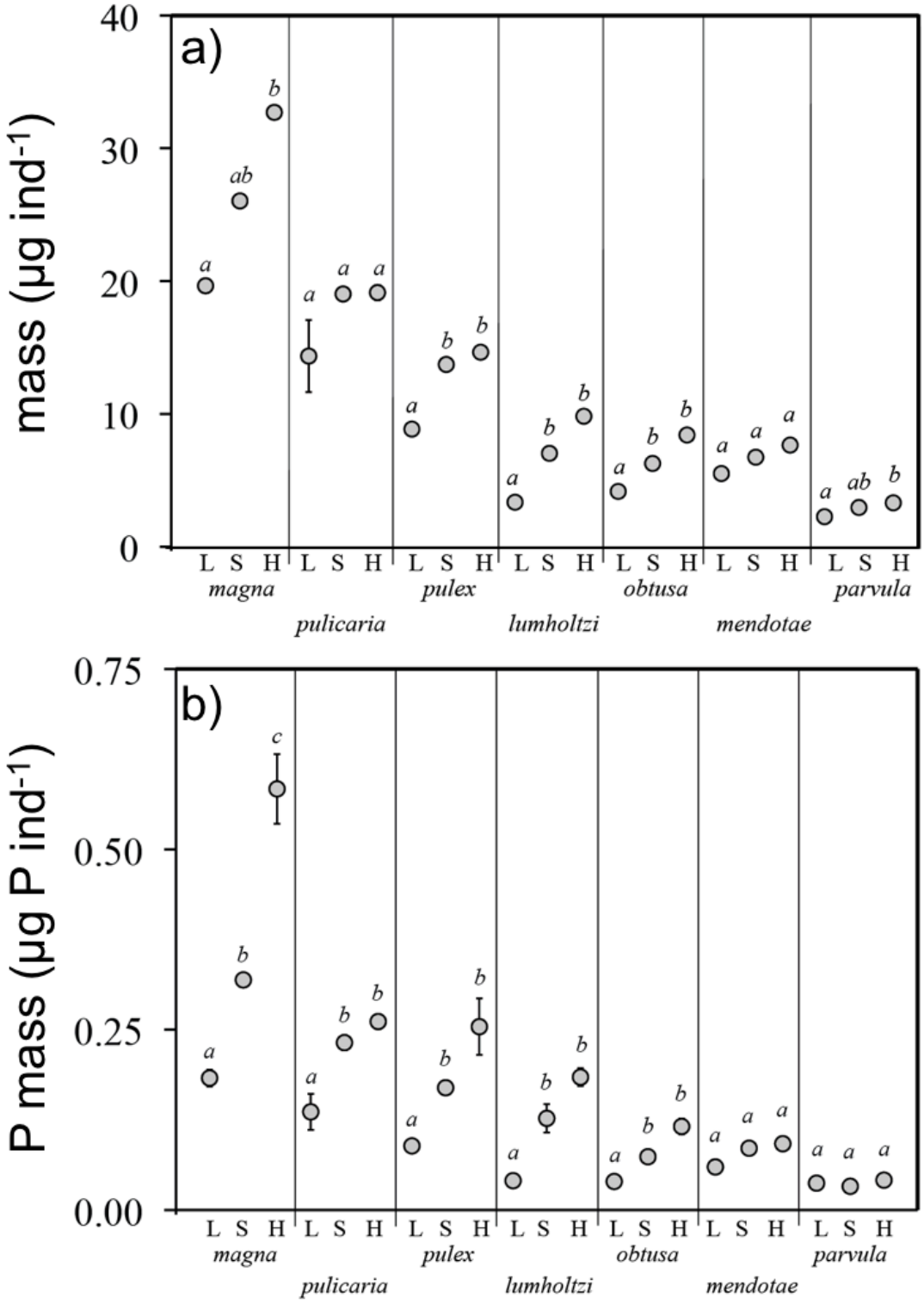

a)
mass (µg ind-1)
40
30
20
10
0
L S H L S H L S H L S H L S H L S H L S H
magna
pulicaria
pulex
lumholtzi
obtusa
mendotae
parvula
b)
P mass (µg P ind-1)
0.75
0.50
0.25
0.00
L S H L S H L S H L S H L S H L S H L S H
magna
pulicaria
pulex
lumholtzi
obtusa
mendotae
parvula

Figure 4.

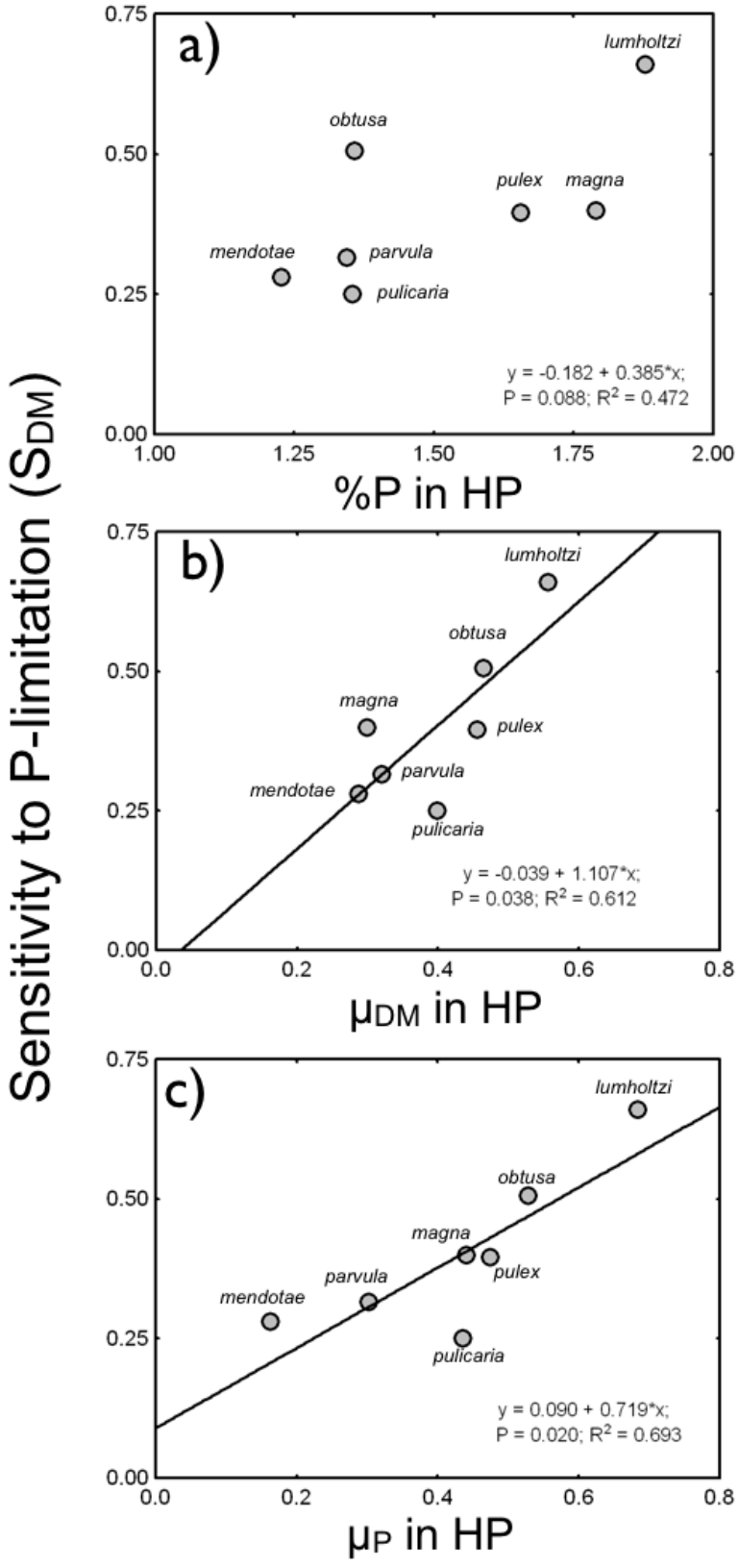

Sensitivity to P-limitation ($S_{DM}$)
a)
lumholtzi
obtusa
pulex
magna
mendotae
parvula
pulicaria
y = -0.182 + 0.385*x;
P = 0.088; $R^2$ = 0.472
%P in HP
b)
lumholtzi
obtusa
magna
pulex
parvula
mendotae
pulicaria
y = -0.039 + 1.107*x;
P = 0.038; $R^2$ = 0.612
$\mu_{DM}$ in HP
c)
lumholtzi
obtusa
magna
pulex
parvula
mendotae
pulicaria
y = 0.090 + 0.719*x;
P = 0.020; $R^2$ = 0.693
$\mu_P$ in HP

Figure 5.

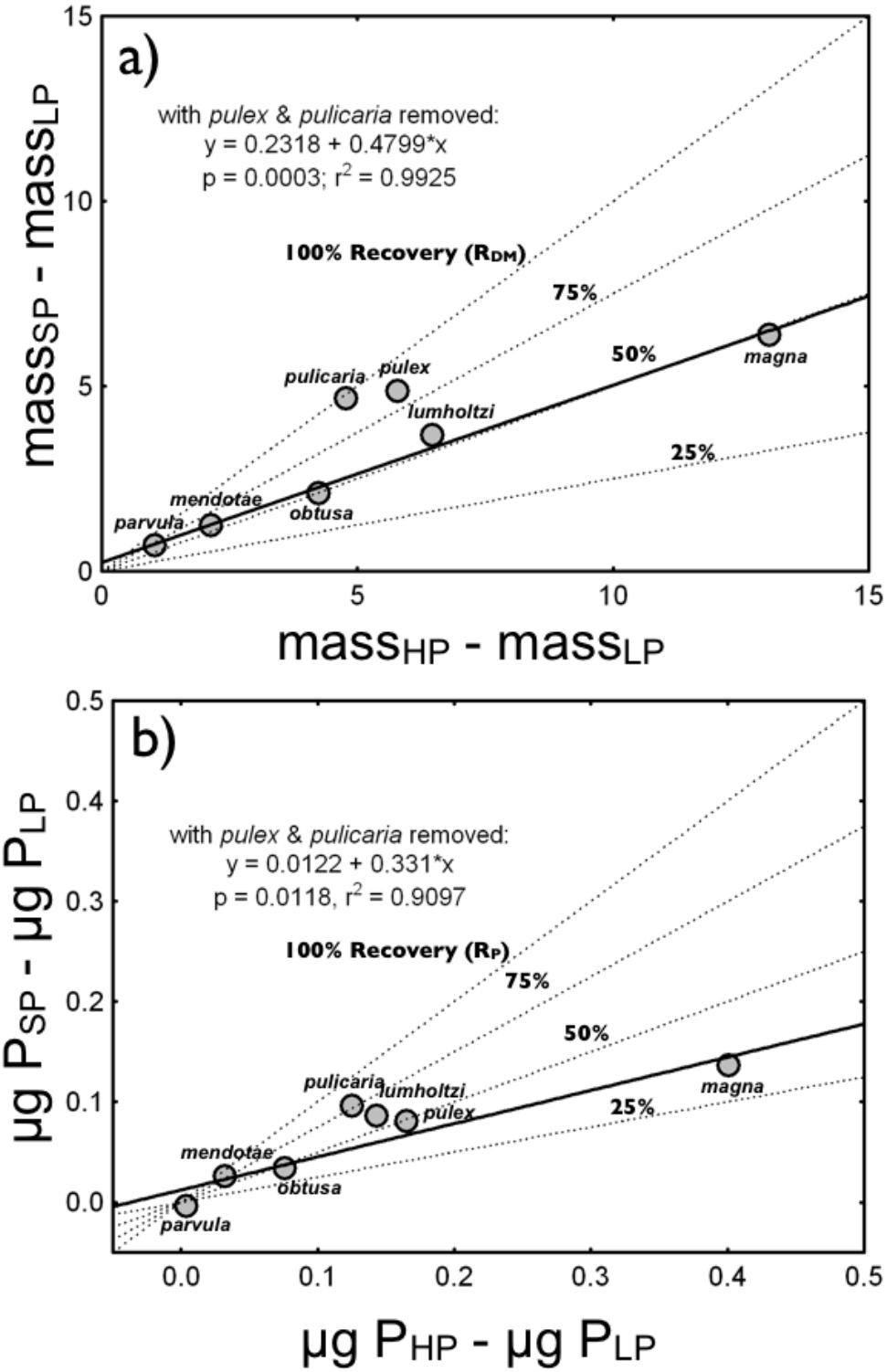

a)
with *pulex* & *pulicaria* removed:
y = 0.2318 + 0.4799*x
p = 0.0003; r² = 0.9925
100% Recovery (R_DM)
75%
50%
25%
pulicaria
pulex
lumholtzi
magna
mendotae
obtusa
parvula
mass_SP - mass_LP
mass_HP - mass_LP
b)
with *pulex* & *pulicaria* removed:
y = 0.0122 + 0.331*x
p = 0.0118, r² = 0.9097
100% Recovery (R_P)
75%
50%
25%
pulicaria
lumholtzi
pulex
magna
mendotae
obtusa
parvula
µg P_SP - µg P_LP
µg P_HP - µg P_LP

Figure 6.

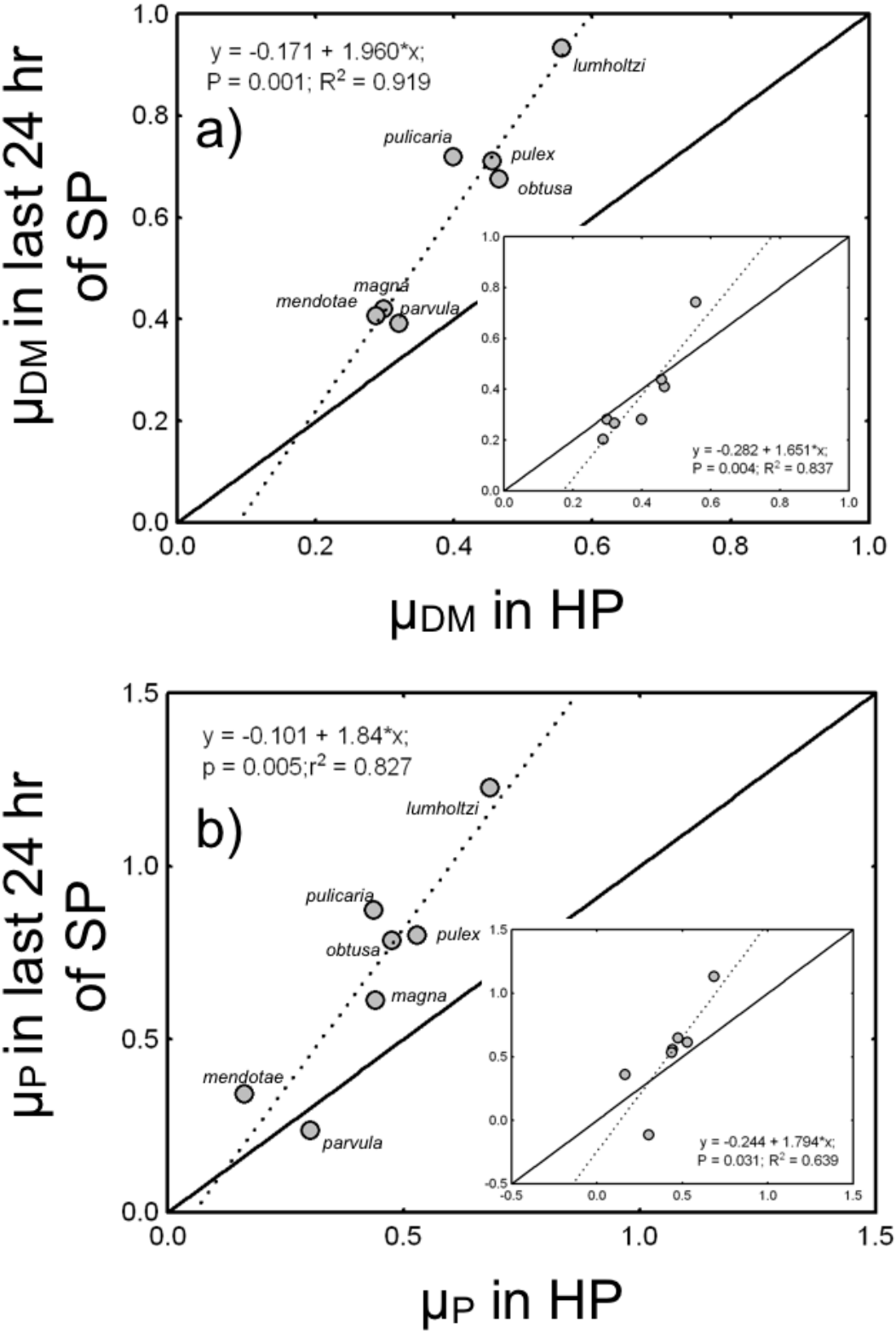
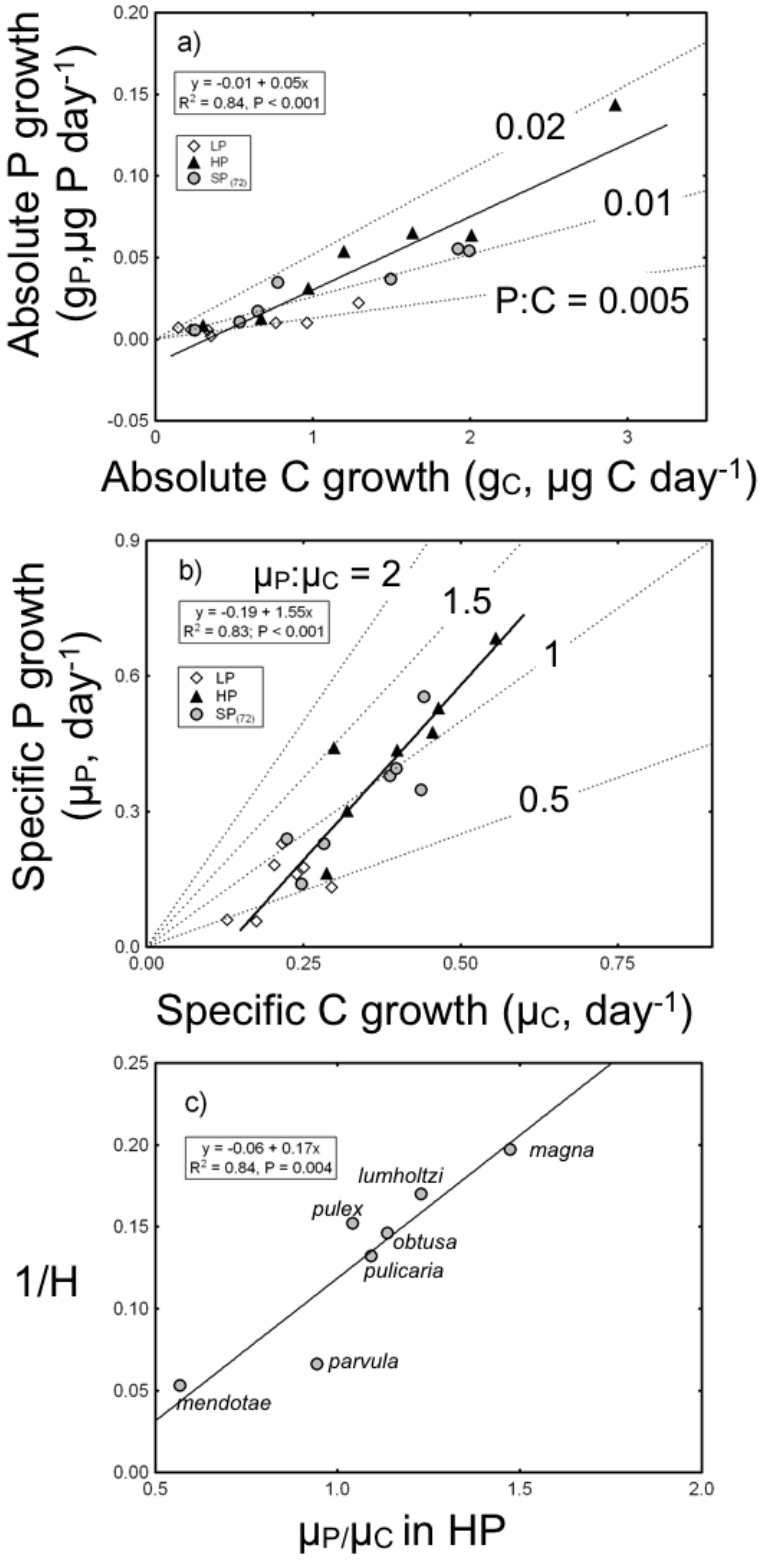

y = -0.171 + 1.960*x;
P = 0.001; R2 = 0.919
a)
lumholtzi
pulicaria
pulex
obtusa
magna
mendotae
parvula
y = -0.282 + 1.651*x;
P = 0.004; R2 = 0.837
μDM in last 24 hr of SP
μDM in HP
y = -0.101 + 1.84*x;
p = 0.005;r2 = 0.827
b)
lumholtzi
pulicaria
pulex
obtusa
magna
mendotae
parvula
y = -0.244 + 1.794*x;
P = 0.031; R2 = 0.639
μP in last 24 hr of SP
μP in HP

Figure 7


a)
y = -0.01 + 0.05x
R² = 0.84, P < 0.001
LP
HP
SP(72)
0.02
0.01
P:C = 0.005
Absolute P growth (gP,µg P day-1)
Absolute C growth (gC, µg C day-1)
b)
µP:µC = 2
y = -0.19 + 1.55x
R² = 0.83; P < 0.001
1.5
1
0.5
Specific P growth (µP, day-1)
Specific C growth (µC, day-1)
c)
y = -0.06 + 0.17x
R² = 0.84, P = 0.004
magna
lumholtzi
pulex
obtusa
pulicaria
parvula
mendotae
1/H
µP/µC in HP

**CHAPTER 3:**

# Variation in caddisfly C, N, and P within and among populations: importance of allometry and diet stoichiometry

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The carbon (C), nitrogen (N), and phosphorus (P) content of invertebrates is sometimes linked with their competitive ability and role in ecosystem function. Recent studies of freshwater invertebrates demonstrate considerable stoichiometric variability among species that might influence ecosystem function; however, these species to ecosystem linkages assume little intra-specific variation in animal stoichiometry. Few studies have examined spatial patterns of intra-specific variation in animal stoichiometry. We examined spatial variation in body %C, %N, %P and C:N of a generalist Trichopteran, *Psychoglypha* sp. In northern California streams (Mendocino County, CA), this caddisfly is abundant in streams spanning three orders of magnitude in watershed size. We measured caddisfly stoichiometry in ten streams and eleven sites within the South Fork Eel River watershed. Stable isotopes of N and C were used to identify diet sources. Across this gradient of stream size and productivity, *Psychoglypha* diets shift from fine particulate organic matter to algae resulting in an approximately 50% decline in diet C:N. Variation in *Psychoglypha* %C, %N, %P, and C:N was nearly as wide as the variation observed for all invertebrate taxa. Across sites, *Psychoglypha* %N and %P declined with body mass, indicating that the role of this species in nutrient cycles likely changes through ontogeny. Variation in *Psychoglypha* N-content within a given size class was negatively related to diet C:N, suggesting that *Psychoglypha* may be N-limited in small headwater streams within the South Fork Eel watershed. Our results indicate that the roles of animals in nutrient cycling may be as variable within a species, due to allometric scaling, as among species.

## INTRODUCTION

To gain a better understanding of the complexities of interactions among species, food webs, and ecosystems; ecologists seek to identify traits that simplify the complexity of the natural world. Ecological stoichiometry provides one approach for identifying traits. It uses elemental mass balances to understand competition, food webs, and nutrient cycles (Sterner and Elser 2002). The elemental stoichiometry of consumers is a key component of these mass balances. Consumer nitrogen (N) and phosphorus (P) content, often expressed per unit carbon (C), is a reflection of nutrient demand and, therefore, are traits that influence growth rate, sensitivity to nutrient limitation, and nutrient release rates and ratios (Vanni et al. 2002, Elser et al. 2003, Seidendorf et al. 2010). Thus, consumer stoichiometries can be used to predict the role that species play in consumer-resource dynamics, food webs, and nutrient cycles (Elser and Urabe 1999, Andersen et al. 2004, Cross et al. 2005).

These stoichiometric predictions utilize a simplifying assumption that the consumer tightly regulates its nutrient stoichiometry in response to changes in diet nutrient content. Variation in the stoichiometry of the consumer in response to changes in diet stoichiometry is described as the degree of stoichiometric homeostasis, when life stage and other factors are held constant (Sterner and Elser 2002). When the stoichiometry of the consumer does not change in response to variation in diet stoichiometry the consumer is strictly homeostatic. Not all invertebrates are strictly

homeostatic, as assumed in the mass balance models (Persson et al. 2010). For example, an approximately 10-fold increase in diet C:P decreased the P content of *Daphnia parvula* by approximately 0.5 %P and the P content of *D. dentifera* by approximately 0.1 %P (DeMott and Pape 2005). When a consumer is not strictly homeostatic, stoichiometric predictions using mass balance models may be misleading.

Diet stoichiometry is not the only factor that influences invertebrate stoichiometry. The nutrient content of invertebrates often declines through ontogeny (Frost and Elser 2002, Vrede et al. 2004, Elser et al. 2005b, Back et al. 2008), due to a decrease in nutrient demand as growth rate declines through development (Peters 1983, Elser et al. 1996). Invertebrate N and P stoichiometry has been shown to decline with temperature (Woods et al. 2003). In addition, chemical aspects of the diet other than N or P can influence the N or P content of the invertebrate. For example, crustacean carapaces contain high levels of P bound with calcium (hydroxyapatite: Vrede et al. 1999) and, therefore, both diet P and calcium concentrations influence *Daphnia* P content (Tan and Wang 2009). These factors influence invertebrate stoichiometry, but are not shaped by stoichiometric homeostasis. As a result, factors like temperature or ontogeny do not influence the accuracy of stoichiometric predictions though they may complicate comparisons across sites.

Factors influencing consumer stoichiometry vary widely through time and space (Sterner and Elser 2002). Understanding how this temporal and spatial heterogeneity shapes intra-specific patterns of stoichiometric variation is critical to interpreting

patterns among species, since the identity of the factor influences the accuracy of stoichiometric predictions. Yet, patterns of intra-specific variation across habitats have received little attention. Early surveys of pelagic zooplankton deemphasized intra-specific variation and emphasized inter-specific variation (Andersen and Hessen 1991). Though the variation observed by Andersen and Hessen (1991) is of the same degree as the variation later used to emphasize the potential for intra-specific variation (DeMott et al. 1998). Surveys of intra-specific stoichiometric variation do described considerable variation among populations in response to dietary P gradients. Schade et al. (2003) show that the P content of the terrestrial herbivore *Sabinia setosa* varies with leaf P, along a natural gradient of soil P. DeMott et al. (2004b) report a decline in *D. dentifera*'s P content across a gradient of algal C:P.

Yet consumer-resource mismatches are not the only factor which is both variable across habitats and potentially influences the stoichiometric composition of species. Temperature, diet identity, and food quantity also vary among habitats. Furthermore, at any point in time the life stage of a species may vary among sites in response to food quality, temperature, or the timing of hatching. The interaction of these factors and their summed influence on consumer stoichiometry will shape the nutrient demand of consumers and their role in nutrient cycles. Thus, it is important to understand how the stoichiometry of a consumer varies among habitats and if this variation can be attributed to ontogeny, dietary imbalances, or the environment.

Stream and river systems represent an excellent setting in which to examine intra-specific stoichiometric variation among and within invertebrate populations. Stream ecologists have recently become very interested in using stoichiometric approaches to understand stream food webs and nutrient cycling (Cross et al. 2005). This work shows that invertebrate N and P stoichiometry vary widely among species (Cross et al. 2003, Evans-White et al. 2005) and that these differences influence nutrient cycling (Evans-White and Lamberti 2006). We still know relatively little about the degree of intra-specific variation within and among these systems; however, the factors thought to influence animal stoichiometry vary both temporally and spatially in river networks. In temperate forested environments, gradients of stream size are associated with wide variation in stream physical, chemical, and biological characteristics (Vannote et al. 1980); many of which could influence the elemental content of individuals. For example, stream temperature increases with stream size**,** and food resources shift from terrestrial leaf litter in forested, headwater streams to algae in larger rivers.

The objectives of this study were to, first, examine the degree to which the stoichiometry of a stream consumer varied within and among streams and, second, to determine how variation in consumer stoichiometry was related to allometry and diet stoichiometry. We focus on a single species, *Psychoglypha* sp.*,* a generalist Trichopteran. We measured the stoichiometry of potential caddisfly diets and used stable isotopes to identify the putative diet at each site. Caddisfly and bulk diet stoichiometries are compared to assess the potential for consumer-resource

mismatches. By focusing on intra-specific variation in consumer stoichiometry across a landscape, this work will improve our understanding of how stoichiometric traits can be used to understand the role of organisms in ecosystem processes.

## METHODS

*Study site*

This study focused on 10 forested streams within the South Fork Eel River watershed (Mendocino County, CA; Figure 1). The majority of these streams are within the Angelo Coast Range Reserve. The region's climate is Mediterranean with warm, dry summers and cold, wet winters. Baseflow conditions occur between June and October, while winter floods routinely scour the stream bed, influencing midsummer abundances of algae and dominate grazers (Power et al. 2008). Watersheds within the Angelo Reserve are often deeply incised and contain a mixed conifer forest dominated by douglas fir (*Pseudotsuga menziessi*). The composition of the riparian community varies among streams; dominant species include bay (*Umbellularia californica*), madrone (*Arbutus menziesii*), douglas fir (*Pseudotsuga menziesii)*, alder (*Alnus rhombifolia*), and maple (*Acer macrophyllum*).

Our focal streams varied widely in watershed area (WA) from small, headwater streams (0.2 $km^2$) to the South Fork Eel River (135 $km^2$, Table 1). Variation in WA is coincident with variation in multiple physical and biological factors. Light and stream

temperature increase with WA (Finlay et al. in press). As a result, the contribution of autotrophs to ecosystem metabolism increases with WA, as does gross primary production (Finlay 2004). During the summer months, epilithic communities are dominated by a thin, heavily grazed layer of diatoms (McNeely and Power 2007). In the South Fork Eel River, *Cladophora glomerata* blooms are common during mid summer (Power et al. 2009). During the early summer, the grazer community is dominated by the caddisflies *Neophylax, Glossosoma,* and *Dicosmoecus*. These species pupate in mid July, depending upon conditions, after which the grazer community is dominated by *Psychoglypha* at most sites, with several other taxa also important in larger streams.

The watersheds and streams we studied are N poor. Terrestrial plants had very low N consistent with strong N limitation of the old growth forests. Similarly, stream water N concentrations were very low (Finlay et al. in press). Epilithic algae in this region (Hill and Knight 1988, Ambrose et al. 2004) and this system (Goodrich *unpublished data*) are commonly N-limited. Though stream water N concentrations and N:P increase sharply downstream (Finlay et al. in press).

*Focal species*

This work focuses on larvae of the caddisfly *Psychoglypha* sp. (Limnephilidae; hereafter *Psychoglypha*). We selected *Psychoglypha* because it is distributed widely throughout the South Fork Eel River watershed from small headwater streams to the

South Fork Eel River and is common during the summer months. *Psychoglypha* is commonly found in pools on or near cobbles.

*Sample collection and processing*

Organic matter and insect samples were collected from eleven sites in ten streams between 3 July and 16 July 2006 (Fig. 1). In Elder Creek, we sampled at a lower (WA = 17 $km^2$, L. Elder) and upper (WA = 13 $km^2$, U. Elder) site. Samples were collected over an approximately 100m reach, commonly in several different pool-run sequences. We collected three types of organic matter samples. CPOM (coarse particulate organic matter) was conditioned leaves without associated small woody debris or invertebrates. Fine particulate organic matter (FPOM) was considered the material removed from rocks with a gentle stream of water. "Epilithon" was defined as the biofilm remaining on these rocks after removing FPOM. All organic matter and caddisfly samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ as well as carbon (C), nitrogen (N), and phosphorus (P) content.

Within each reach, four sites were chosen for epilithon and FPOM sampling based on the distribution of *Psychoglypha* within each site, commonly shallow pools. We selected two cobbles per site (~ 7 to 20 cm diameter), removed the FPOM sample and then used a wire brush to remove biofilm surrounding a 4.2 $cm^2$ sample area on the top of each stone. A hard toothbrush was used to remove the epilithon sample. The two samples from each site were combined into a composite sample and filtered onto

three pre-weighed and ashed (550 ˚C) glass-fiber filters (Whatman GF/F) for stable isotopes, C:N, and P. Filters were dried at 60 ˚C and stored for analysis.

To collect the CPOM samples, we first separated the reach roughly into thirds. In each section, we collected one CPOM sample by haphazardly collecting litter from several locations associated with *Psychoglypha*. In the field, we rinsed the leaves to wash off FPOM and removed visible invertebrates. After drying (60 ˚C, > 48 hours), CPOM samples were further sorted to remove woody detritus (sticks and pine cones). CPOM samples were homogenized to a fine powder with a Wiley Mill.

We attempted to collect at least 30 individual *Psychoglypha* larvae at all eleven sites. The individuals we collected were widely distributed across each study reach. Each size class present at the site was well represented in the final sample. Caddisfly samples were transported back to the lab in cooled containers of stream water. In the lab, individuals were de-cased and allowed to clear their guts for approximately 24 hours in individual petri dishes. After 24 hours, the surviving individuals (survivorship > ~ 90%) were rinsed in deionized (DI) water and dried individually in glass vials at 60 ˚C. Dried individuals were weighed to the nearest 0.1 µg on a microbalance (Mettler UMT2) and then ground to a fine powder with a steel rod.

Once the CPOM and invertebrate stable isotope and stoichiometry (C, N, and P) samples were homogenized, samples were weighed out into tin capsules with a microbalance (Mettler UMT2). Invertebrates were analyzed for stable isotopes and

stoichiometry as individuals when possible (> 90% of all samples). In the smallest size classes, we pooled two similarly sized individuals into one sample.

Organic matter and invertebrate samples were analyzed for stable C and N isotopes with a Thermo Electron gas isotope-ratio mass spectrometer at the Colorado Plateau Stable Isotope Laboratory (Flagstaff, AZ). The mean standard deviation of 12 duplicates was 0.09‰ for $\delta^{13}C$ and 0.14‰ for $\delta^{14}N$. Stable isotope analysis also provided C and N values for a portion of the organic matter samples and the invertebrate C and N samples. The remaining organic matter and invertebrate C and N samples were analyzed with a Perkin Elmer 2400 CHNS analyzer. Epilithon, FPOM, and CPOM P samples were transferred to a borosilicate glass tube with a microbalance (Metler UMT2), ashed (550 ˚C) and hydrolyzed in HCl; then, $PO_4$ was measured colormetrically as molybdenum blue (DeMott et al. 1998). Invertebrate P samples were weighed out into a borosilicate glass tube with a microbalance, ashed (550˚C), and hydrolyzed in HCl. Subsamples of this solution were transferred to duplicate borosilicate glass tubes, diluted in an HCl solution and measured for $PO_4$ as described above. The mean standard deviation of duplicates was 0.07 %P.

*Determination of caddisfly resource use and diet stoichiometry*

To help interpret variation in *Psychoglypha* stoichiometry, we sought to identify the diet stoichiometry at each site. In the S.F. Eel watershed, *Psychoglypha* could potentially consume a diverse diet that could include CPOM, FPOM, epilithon, or

combinations of all three. We first identified the primary diet at each site (i.e., CPOM, FPOM, or epilithon), and assumed that the bulk stoichiometry of that material represented the diet stoichiometry.

Identifying the diet stoichiometry of stream consumers can be challenging because of the potential for selective feeding (Mulholland et al. 2000b) and factors which complicate the interpretation of consumer $\delta^{13}C$ and $\delta^{15}N$. Natural abundances of stable isotopes, particularly $\delta^{13}C$, are often useful when organic matter sources are isotopically unique and trophic fractionation is low and consistent among diets. In our study system, measuring the diet source of *Psychoglypha's* diet with $\delta^{13}C$ was complex because of spatial shifts in algal $\delta^{13}C$ and isotopic overlap among the sources that could contribute to *Psychoglypha's* diet (Finlay et al. 1999, Finlay 2001, Finlay 2004, McNeely et al. 2006, Finlay et al. 2010). As a result, our approach for identifying the diet at each site used a combination of organic matter and *Psychoglypha* $\delta^{13}C$ and $\delta^{15}N$ values, published $\delta^{13}C$ and $\delta^{15}N$ values for this system (Finlay et al. 1999, Finlay 2001, Finlay 2004, McNeely et al. 2006, Finlay et al. 2010), and knowledge of *Psychoglypha's* feeding behavior and distributions. We assume that $\delta^{15}N$ fractionation was between -1 and 1‰ (Jardine et al. 2005).

*Controls of* Psychoglypha *stoichiometry*

Using standardized major axis regression (SMA) and mixed effects models, we explored the factors related to *Psychoglypha* stoichiometry both among and within

sites. We focused on allometry (i.e., mass) and diet stoichiometry, two factors know to influence invertebrate stoichiometry (Frost and Elser 2002, Persson et al. 2010). Other factors such as water temperature might also influence *Psychoglypha*'s stoichiometry; however, the maximum variation in mean daily temperature among streams was only 3.6 ˚C (Table 1). The increase in temperature between McKinley, the smallest headwater stream, and the S.F. Eel is associated with much larger shifts in diet stoichiometry. For instance, within this dataset log body mass varied by 3 orders of magnitude, diet C:P quadrupled, and diet C:N nearly doubled.

Standardized major axis was used to examine the relationship between *Psychoglypha* stoichiometry (%C, %N, %P, C:N) and mass. While ordinary least squares (OLS) is useful for determining if Y is related to X, SMA regression provides the "best line" describing the bivariate relationship between Y and X (Warton et al. 2006). In this study, we are not just interested in whether *Psychoglypha* stoichiometry (Y) is related to mass (X). We wish to examine if the variation in Y around the "best fit" line is related to diet stoichiometry. For these regressions, we $\log_{10}$ transformed *Psychoglypha* mass to normalize this variable.

To examine the relationship between size-corrected *Psychoglypha* stoichiometry and diet stoichiometry, we use the Y residuals from the SMA fit (deviation in Y). The SMA line was fit in SMATR (Falster et al. 2003). The relationship between Y residuals and diet C:X (where X is either N or P) was evaluated with OLS because this regression technique is best suited for determining if Y is related to X (Warton et al.

2006). As an alternative approach, we also fit a mixed effects model with log mass and diet stoichiometry as the fixed effects and site as the random effect. For this model, we used the "lme" function from the nlme library (Pinehiro and Bates 2000) in the R-project statistical package (R Development Core Team 2010). Models were fit with the REML estimator. The proportion of variation explained by each model is the multiple $R^2$ of the relationship between the dependent variable and model fits. Differences in *Psychoglypha* and organic matter stoichiometry and stable isotopes among sites were examined with a one-way ANOVA. All tests were conducted in Statistica (StatSoft) with $\alpha = 0.05$.

## RESULTS

### *Organic matter stoichiometry,* $\delta^{13}C$, *and* $\delta^{15}N$

Epilithon C:N did not vary among the ten sites (ANOVA: $F_{9,23} = 0.658$, $P = 0.737$, Table 2). No epilithon samples were collected in Scully, a tiny headwater stream with few cobbles and a heavy layer of FPOM. Epilithon C:P was generally low, as expected for an N-limited system, but varied among streams (ANOVA: $F_{9,23} = 3.497$, $P = 0.007$, Table 2). Epilithon N:P varied widely among streams from values near the Redfield ratio (Redwood: 18.6) to values suggesting a scarcity of P relative to N (ANOVA: $F_{9.23} = 2.525$, $P = 0.035$, Table 2). Although the most P-depleted epilithon was found in the headwater streams McKinley and Skunk (Table 2), epilithic C:P and N:P were not linearly related to

log watershed area (C:P: $y = 378.95 - 76.69x$, $R^2 = 0.21$, $P = 0.18$; N:P: $y = 40.54 - 8.04x$, $R^2 = 0.20$, $P = 0.19$).

FPOM C:N did not vary significantly among the eleven sites (ANOVA: $F_{10,30} = 1.954$, $P = 0.076$), but did decrease with log watershed area ($y = 18.71 – 4.32x$, $R^2 = 0.83$, $P < 0.001$). FPOM C:P and N:P did not vary among the eleven sites (ANOVA: C:N: $F_{10,25}$, $P = 0.73$, N:P: $F_{10,25} = 0.96$, $P = 0.50$) or with watershed area ($P > 0.05$).

As expected, CPOM was nutrient poor compared to epilithon (Table 2). CPOM C:N and C:P varied widely among streams (ANOVA's: C:N: $F_{9,21} = 6.680$, $P < 0.001$, C:P: $F_{9,21} = 5.906$, $P < 0.001$). There was no variation in CPOM N:P among streams (ANOVA: $F_{9,21} = 2.147$, $P = 0.072$). In general, Redwood (WA = 9.0) and the smallest streams (Scully, McKinley, and Skunk) had the most nutrient depleted CPOM; however, there was no linear relationship between CPOM C:N, C:P, or N:P and log watershed area ($P > 0.05$).

Organic matter stable isotope results were consistent with previous measurements at the study site (Table 3, Finlay et al. 1999, Finlay et al. 2002, McNeely et al. 2006). In streams where we had both FPOM and epilithon samples, $\delta^{13}C$ and $\delta^{15}N$ did not differ between these organic matter sources (Factorial ANOVAs: $\delta^{13}C$: $F_{1,14} = 0.16$, $P = 0.69$; $\delta^{15}N$: $F_{1,14} = 1.53$, $P = 0.24$). CPOM $\delta^{13}C$ varied little among streams and was not related to watershed area (Table 3, $P > 0.05$). In small streams (< 10 WA), epilithon and FPOM $\delta^{13}C$ was similar to litter $\delta^{13}C$ (Table 3) and higher relative to algae and herbivores (McNeely et al. 2006), reflecting a high contribution of terrestrial carbon

to epilithic biomass. In the larger streams (> 10 WA), epilithic $\delta^{13}C$ was higher than CPOM by approximately 2 to 7‰, reflecting a greater contribution of algae to biomass. CPOM $\delta^{15}N$ was lower than epilithic $\delta^{15}N$ at all sites except JOH, where epilithic $\delta^{15}N$ was low and highly variable among samples (Table 3).

Psychoglypha *resource use*

*Psychoglypha* $\delta^{13}C$, $\delta^{15}N$, and diets varied with stream size (Table 4). In this system, small streams (< 10 WA) were heavily shaded and have low algal densities (McNeely et al. 2006). In these small streams, *Psychoglypha* $\delta^{13}C$ averaged approximately 27‰, similar to the $\delta^{13}C$ of CPOM, epilithon, and FPOM (Table 4). This $\delta^{13}C$ value is indicative of terrestrial derived C. *Psychoglypha* $\delta^{15}N$ was between 0.4 and 1.5‰ in these systems, which was similar to the $\delta^{15}N$ of epilithon and FPOM and 3 to 5‰ higher than CPOM $\delta^{15}N$. Assuming trophic fractionation of $\delta^{15}N$ was between -1 and 1‰ (Jardine et al. 2005), *Psychoglypha* $\delta^{15}N$ suggests that individuals consumed either epilithon or FPOM at these sites. Consistent with this interpretation, at these sites *Psychoglypha* was not found or collected from leaf packs of depositional areas with high concentrations of CPOM.

In small streams (< 10 WA), does *Psychoglypha* consume epilithon or FPOM? In this study, epilithon and FPOM were the tightly attached or loose fractions of the organic matter matrix covering cobbles, respectively. At the smallest headwater sites (Scully, McKinley, and Skunk), *Psychoglypha* was collected from pools where FPOM

accumulates and there is relatively little epilithon (McNeely and Power 2007). Based on the lack of available epilithon, we argue that it is reasonable to assume that *Psychoglypha* consumes FPOM in these smallest streams.

In the midsized headwater streams (Barnwell, Fox, Misery, and Redwood), the availability of epilithon was high enough to represent a potential diet source for *Psychoglypha* (McNeely et al. 2006); however, consumption of loosely attached organic matter is still more consistent with *Psychoglypha*'s feeding behavior. Stable isotopes suggest that *Psychoglypha* is a generalist consumer and does not have the capacity of scrappers to remove tightly attached algae. At these sites, scrappers such as *Neophylax* and *Glossosoma* show a clear algal signal where *Psychoglypha* does not track variation in algal $\delta^{13}C$ (Finlay 2004, McNeely et al. 2006). At these four midsized headwater streams, we also use the bulk FPOM stoichiometry as the diet stoichiometry; however, we evaluate this decision by removing these four streams from the analysis of the relationship between *Psychoglypha* stoichiometry and diet stoichiometry.

In the S.F. Eel watershed, streams with watersheds greater than approximately 10 $km^2$ had greater autotrophic production and more open canopies than smaller streams (Finlay 2004). In streams greater than 10 $km^2$ WA, *Psychoglypha* $\delta^{13}C$ averaged approximately 21‰. This is higher than our measurements of epilithon and FPOM $\delta^{13}C$ in these systems. Instead, *Psychoglypha* $\delta^{13}C$ is similar to the $\delta^{13}C$ of herbivores and epilithic algae in pools within these systems (Finlay et al. 1999, Finlay et al. 2002, Finlay 2004). These results suggest that *Psychoglypha* either selectively consumes algae from

the matrix of epilithon and FPOM. Our bulk epilithon sample, which does not contain loose FPOM, likely better reflects algal stoichiometry than bulk FPOM stoichiometry. Thus, we use epilithon stoichiometry as the diet stoichiometry in streams with watershed areas greater than 10 $km^2$.

*Caddisfly stoichiometry*

Values for *Psychoglypha* P, N and C content and C:N were within the range observed for aquatic insects in other studies (Cross et al. 2003). *Psychoglypha* stoichiometry (%P, %N, %C, and C:N) differed among sites ($P < 0.001$, Fig. 2). On average, individuals in the S.F. Eel had the lowest %P while those in Redwood had the highest %P. Individuals in Skunk had the lowest %N and highest C:N while those at U. Elder had the highest %N and lowest C:N.

*Psychoglypha* stoichiometry was dependant upon body size (Fig. 3). When all sites are combined, %P and %N declined with log mass whereas %C and C:N increased with log mass. We observed fewer relationships within sites (Table 5). Percent P decreased with log mass at only three out of the eleven sites (Table 5). Percent N and C:N varied with log mass at only two sites and a %C – log mass relationship was observed at only one site. The lack of significant relationships does not appear to be linked with the size range of individuals collected (Table 5).

*Psychoglypha* N stoichiometry (%N and C:N) was related to diet C:N. After accounting for the influence of mass on *Psychoglypha* stoichiometry (Y axis residuals),

*Psychoglypha* C, N, and C:N varied among sites (ANOVAs: C residuals: $F_{9,134} = 3.07$, P = 0.002; N residuals: $F_{9,138} = 8.59$, P < 0.001; C:N residuals: $F_{9,133} = 8.67$, P < 0.001) while P residuals did not vary among sties (P residuals: $F_{9,121} = 1.48$, P = 0.16). After accounting for size, P residuals were not related to diet C:P (Fig. 5a); however, residual variation in N decreased with diet C:N while its C:N increased with diet C:N, as predicted (Fig. 5b and 5c).

Our primary finding that *Psychoglypha* N stoichiometry was influenced by diet C:N was not influenced by the inclusion of midsized headwater streams or our statistical approach. Removing the midsized headwater streams, where the identity of the diet is difficult to discern, does not influence the relationships between these residuals and diet C:X (P residuals: P = 0.51, N residuals: P = 0.046, C:N residuals: P = 0.01). Furthermore, using OLS instead of SMA to fit the relationship between *Pyschoglypha* stoichiometry and log mass does not influence the nature of our findings (P OLS residuals: $y = 0.04 – 0.0002x$, $R^2 = 0.03$, P = 0.58; N OLS residuals: $y = 1.48 – 0.10x$, $R^2 = 0.42$, P = 0.04; C:N OLS residuals: $y = -1.30 + 0.07$, $R^2 = 0.61$, P = 0.008).

The mixed effects models also suggest similar relationships (Table 6). For instance, the C:N model indicates that *Psychoglypha* C:N increased with mass and diet C:N. This model explained 66% of the variation in *Psychoglypha* C:N. In contrast, the P model indicates that *Psychoglypha* P decreased with mass, but was not influenced by diet C:P. This model explained 38% of the variation in *Psychoglypha* P.

Caddisfly C:P and N:P varied widely with body mass, relative to the variation among invertebrate taxa. Analytical limitations precluded measurements of *Psychoglypha* C:P and N:P on the same individual. Instead, we estimated caddisfly C:P and N:P using species-level SMA fits of the relationship between stoichiometry and log mass. *Psychoglypha* C:P increased non-linearly with log mass and ranged from approximately 50 for the smallest individuals to 280 for the largest. *Psychoglypha* N:P also increased non-linearly with log mass from approximately 15 to 30.

## DISCUSSION

The nutrient stoichiometry of a consumer is a trait that reflects nutrient demand. As a result, this trait can be used to predict the role of the consumer in consumer-resource dynamics, food webs, and nutrient cycles (Elser and Urabe 1999, Andersen et al. 2004). Theory predicts that shifts in the consumer community from P to N rich species can change the balance of N and P cycling and potentially the nutrient limiting algal production (Elser and Urabe 1999). These predictions assume that consumers are strictly homeostatic (Elser and Urabe 1999, Andersen et al. 2004); however, not all species are strictly homeostatic (Persson et al. 2010). We observed wide variation in *Psychoglypha* C, N, P, and C:N stoichiometry, which is surprisingly similar in magnitude to the variation among invertebrate taxa (Elser et al. 2000, Sterner and Elser 2002, Cross et al. 2003, Evans-White et al. 2005, Liess and Hillebrand 2005). These results suggest

that intra-specific variation in consumer stoichiometry may be as important to food web and ecosystem dynamics as inter-specific variation.

*Breadth of intra-specific variation*

The range of *Psychoglypha* N and P contents we report (~ 0.6 – 1.8 %P and 6 – 12 %N) are similar to the range of N and P contents reported for invertebrate taxa (~ 0.1 – 2 %P and 6 – 13 %N: Fagan et al. 2002, Cross et al. 2003, Woods et al. 2003). In contrast to the wide variation in *Psychoglypha* nutrient content, variation in C:P and N:P ratios (C:P: 50 - 280 ; N:P: 15 - 30) was modest in comparison to variation among all invertebrates species (C:P: 100 - 1000; N:P: ~10 - 120: Cross et al. 2003, Evans-White et al. 2005, Liess and Hillebrand 2005). Our use of C, N, and P allometric relationships to estimate C:P and N:P ratios may underestimate the degree of variation. Nevertheless, the calculated range of *Psychoglypha* N:P was equivalent to the difference between *Daphnia* and calanoids (Elser and Urabe 1999); stoichiometric variation thought to be responsible for shifts in algal nutrient limitation due to consumer driven nutrient recycling (Sterner and Elser 2002). The factors responsible for this variation will influence the role of *Psychoglypha* in food web and ecosystem dynamics.

*Allometric patterns of* Psychoglypha *stoichiometry*

*Psycholgypha* %C, %N, %P, and C:N varied widely both among and within streams. Some of this variation could be attributed to allometry (Fig. 3). *Psychoglypha*

P and N scaled negatively with mass while its C content scaled positively with mass. Previous studies with mayflies and snails also indicate a strong P allometry (Frost and Elser 2002, Elser et al. 2005b), which is predicted by the growth rate hypothesis (Elser et al. 2003). Overall, scaling of carbon and nitrogen with size has received little attention and we can only speculate about the mechanisms. Negative N allometry may reflect the high protein and nucleic acid requirements of rapid growth (Larsen et al. 2009) or morphological changes such as an increase in the ratio of abdomen to thorax and head. The thorax and head of Trichoptera are plated in a hard chitinous exoskeleton, an N rich material. The increase in caddisfly C with size may result from increased lipid accumulation as the individual approaches pupation (Meier et al. 2000), which would also contribute to a decline in nutrient content. Irrespective of the mechanism, this work suggests that mass, a surrogate for ontogeny, is a structuring template for organismal stoichiometry on which diet and environmental factors operate.

*Psychoglypha*'s nutrient allometry suggests that nutrient demand declines through ontogeny. As a result, mass balance models predict that the role of *Psychoglypha* in food webs and nutrient cycles will shift as individuals grow, even when growing optimally. Small *Psychoglypha* are N and P rich and have a low body N:P ratio (N:P = 15), relative to other invertebrate taxa. Thus, small individuals should have a high N:P release ratio. The role of small *Psychoglypha* in food webs and nutrient cycles is likely stoichiometrically similar to *Daphnia* (Elser and Urabe 1999). In contrast, the role of large *Psychoglypha* in food webs and nutrient cycles is likely stoichiometrically similar

to calanoids. Large *Psychoglypha* are N and P poor relative to other invertebrate taxa and have a high body N:P (N:P = 30). Mass balance models predict that these individuals should have a low N:P release ratio. Thus, as *Psychoglypha* grows N:P release ratios should decrease as body N:P increases.

Taken together, these predictions suggest that in a closed system nutrient regeneration by this species should decrease future stoichiometric mismatches. Specifically, small *Psychoglypha* are predicted to have a high N:P release ratio. Thus, nutrient regeneration by *Psychoglypha* should increase soluble N availability relative to P, potentially leading to high N:P microbes and algae. High epilithic N:P would favor the growth of large *Psychoglypha* which are predicted to have a high N demand, relative to P. Although declines in N or P with mass are commonly observed for stream invertebrates (Frost and Elser 2002, Elser et al. 2005b, Back et al. 2008), we lack an adequate understanding of how the development of a single species might influence the stoichiometry of food webs and nutrient cycles.

*Influence of diet stoichiometry on* Psychoglypha *stoichiometry*

The increase in *Psychoglypha* C:N with diet C:N, after controlling for mass, suggests that *Psychoglypha* growth may be N-limited in the small headwater streams with in the S.F. Eel River watershed. The growth of many terrestrial herbivores is thought to be limited by leaf N content (White 1993); however, the potential for N-limitation of growth in freshwater invertebrates has received little attention (but see:

Acharya et al. 2004, Hessen et al. 2007). The high N content of individuals consuming a N-rich diet might reflect the high N requirements of rapid growth (Larsen et al. 2009) or N storage (Raubenheimer and Jones 2006).

Variation in *Psychoglypha* N stoichiometry with diet C:N at any given size suggests, with one important caveat, that *Psychoglypha* may not be strictly homeostatic for N. The caveat is that stoichiometric homeostasis (Sterner and Elser 2002) applies only to regulation of consumer stoichiometry in response to variation in diet stoichiometry, when all else is held constant. Here, we hold body mass constant; however, many biological and environmental factors varied among sites. As WA increases in this system, temperature increased slightly and the identity of the diet changed. In contrast to our results, the relatively limited information on invertebrate N homeostasis indicates strict homeostasis in 9 out of 13 cases (Persson et al. 2010). Trichoptera have not been examined. A species degree of stoichiometric homeostasis influences its fitness in variable environments (Chapter 1), quality as prey (Malzahn et al. 2007), and role in stream nutrient cycles (Small et al. 2009).

Why was there no relationship between *Psychoglypha* P and diet C:P? First, P residuals did not vary significantly among sites. Mass explained far more variation in *Psychoglypha* P contents than %C, %N, or C:N. Second, *Psychoglypha*'s diet was relatively P-rich at these sites. Diet C:P did not exceed 400 which is below the mean threshold elemental ratio for insects (Frost et al. 2006); the ratio at which the element limiting growth transitions from C to P. When diet C:P is below the threshold elemental

ratio the P content of an individual should not change with diet C:P unless P storage occurs. To our knowledge, there is no evidence that Trichoptera can store P, although the Lepidopteran *Manduca sexta* can store P as a-glycerophosphate in the hemolymph (Woods et al. 2002).

We examined the relationship between diet and *Psychoglypha* stoichiometries with caution for two reasons. First, it was difficult to determine whether *Psychoglypha* consumed epilithon or FPOM in the mid-sized headwater streams (Barnwell, Fox, Misery, and Redwood) because of their isotopic similarities. Removing these mid-sized streams from our analyses does not influence our primary finding that *Psychoglypha* N content decreased with diet C:N. Second, in large streams (WA > 10 $km^2$) *Psychoglypha* $\delta^{13}C$ was higher than epilithic $\delta^{13}C$ and more similar to the $\delta^{13}C$ reported for epilithic algae and herbivore in pools (Finlay et al. 1999). As a result, we used the epilithon stoichiometry as the diet stoichiometry. The use of bulk epilithic stoichiometry may be misleading if *Psychoglypha* selectively ingests algal cells from the epilithic matrix of algae, FPOM, and microbes. We do not know the stoichiometry of pure algae at these sites; however, algal cells are likely more N rich than the bulk epilithon due to the increase in soluble N concentrations at these sites, relative to upstream sites. If this were the case, we would expect an even stronger relationship between *Psychoglypha* and diet stoichiometries. Alternatively, epilithic bulk stoichiometry would not be misleading if *Psychoglypha* $\delta^{13}C$ was high because it consumed bulk epilithon, but

selectively assimilated algal C. Taken together, this suggests that these caveats will likely have little influence on our primary findings.

*Significance*

Here, we show that variation in *Psychoglypha* N and P was nearly as wide as the variation observed for invertebrate taxa, suggesting that this single species could play a wide variety of roles in nutrient cycles within our study system. We attribute this variation to two factors: allometry and diet. *Psychoglypha* N and P both decline through ontogeny. Variation in N content at any given size was related to diet C:N, suggesting that *Psychoglypha* may not be strictly homeostatic and that it likely experiences N-limitation of growth in small headwater streams. Taken together, our results suggest that the stoichiometry of a single consumer can vary widely through time (i.e., ontogeny) and space. As a result, the growth of a species and dietary mismatches could have the same effect on nutrient cycling as a shift in community composition.

## TABLES

Table 1. Study sites, watershed area, and stream temperatures. Mean daily temperature (27 June – 15 July 2006) using raw data when available or a value estimated (*) from the relationship between temperature and log watershed area (daily mean temp = 14.83 + 1.29*log W.A., $R^2 = 0.76$, $p = 0.011$).

| Site | W.A. ($km^2$) | Temperature (˚ C) |
|---|---|---|
| Scully | 0.2 | 14.3* |
| McKinley | 0.6 | 14.5 (0.6) |
| Skunk | 0.8 | 14.7* |
| Barnwell | 2.3 | 15.5 (0.6) |
| Fox | 2.7 | 15.9 (0.7) |
| Misery | 4.0 | 15.6* |
| Redwood | 9.0 | 16.1* |
| J.O.H. | 11.0 | 15.8 (1.0) |
| U. Elder | 12.0 | 15.2 (0.6) |
| L. Elder | 17.0 | 16.7 (0.9) |
| S.F. Eel | 135.0 | 17.9 (0.6) |

Table 2. Epilithon, FPOM, and litter C:N, C:P, and N:P (molar, ± 1 standard deviation). Scully had no measurable epilithon. Streams and sites are listed by watershed area, from smallest to largest.

| Sites | Epilithon C:N | Epilithon C:P | Epilithon N:P | FPOM C:N | FPOM C:P | FPOM N:P | Litter C:N | Litter C:P | Litter N:P |
|---|---|---|---|---|---|---|---|---|---|
| Scully | n.a. | n.a. | n.a. | 11.8 (2.7) | 127.1 (7.9) | 9.2 (2.1) | 75.8 (4.8) | 2688.7 (906.4) | 35.2 (9.7) |
| McKinley | 10.1 (1.4) | 604.2 (33.3) | 60.6 (11.5) | 20.8 (3.1) | 31.6 (2.8) | 1.6 (0.4) | 77.9 (18.9) | 2151.7 (668.6) | 28.3 (8.0) |
| Skunk | 8.1 (0.9) | 388.7 (135.6) | 49.2 (19.3) | 19.1 (4.0) | 205.4 (96.6) | 14.4 (9.0) | 89.2 (8.2) | 3329.6 (941.4) | 38.2 (14.6) |
| Barnwell | 9.1 (0.7) | 270.0 (61.6) | 30.0 (9.1) | 16.5 (0.3) | 265.3 (26.1) | 16.1 (1.4) | 72.5 (5.3) | 2145.4 (290.8) | 29.5 (2.0) |
| Fox | 10.3 (2.5) | 228.4 (80.7) | 22.5 (7.6) | 17.2 (0.4) | 220.6 (60.6) | 13.1 (3.7) | 51.3 (9.9) | 1901.8 (564.3) | 36.7 (4.1) |
| Misery | 12.3 (5.9) | 343.9 (128.0) | 29.6 (11.7) | 17.1 (0.7) | 150.7 (33.7) | 9.0 (2.2) | 62.6 (13.7) | 2078.4 (177.4) | 33.9 (4.8) |
| Redwood | 10.3 (2.0) | 187.1 (57.3) | 18.6 (6.6) | 15.4 (1.9) | 76.8 (11.4) | 5.5 (1.4) | 82.3 (2.6) | 4218.4 (1036.6) | 51.4 (13.1) |
| J.O.H. | 10.7 (4.6) | 388.7 (118.2) | 41.9 (21.4) | 11.5 (0.9) | 189.0 (51.0) | 17.3 (6.1) | 38.1 (3.7) | 1550.1 (231.0) | 40.9 (7.2) |
| U. Elder | 8.5 (0.3) | 309.7 (60.5) | 36.6 (7.9) | 13.8 (0.3) | 307.5 (111.0) | 22.1 (7.6) | 54.2 (9.1) | 1949.3 (646.5) | 35.5 (7.0) |
| L. Elder | 8.9 (0.2) | 191.9 (110.7) | 21.7 (12.4) | 12.0 (0.8) | 244.5 (48.6) | 21.4 (6.0) | 51.4 (10.3) | 1492.9 (379.8) | 29.4 (6.5) |
| S.F. Eel | 9.2 (1.2) | 323.8 (161.3) | 36.7 (19.3) | 11.3 (0.2) | 223.7 (44.4) | 19.6 (3.7) | 72.9 (22.3) | 2978.3 (368.0) | 42.0 (7.8) |

Table 3. Epilithon, FPOM, and litter $\delta^{13}C$ and $\delta^{15}N$ (± 1 S.E.). Scully had no measurable epilithon. Sites are listed by watershed area, from smallest to largest.

| Site | Epilithon $\delta^{13}C$ | Epilithon $\delta^{15}N$ | FPOM $\delta^{13}C$ | FPOM $\delta^{15}N$ | Litter $\delta^{13}C$ | Litter $\delta^{15}N$ |
|---|---|---|---|---|---|---|
| Scully | n.a. | n.a. | -23.49 (3.40) | 1.58 (1.12) | -28.93 (1.18) | -3.77(0.22) |
| McKinley | -28.03 (0.95) | 4.26 (3.76) | -27.53 (0.09) | 3.08 (0.96) | -29.40 (0.28) | -4.30 (0.23) |
| Skunk | -28.83 (n.a.) | 1.44 (n.a.) | -28.78 (0.71) | 5.11 (0.54) | -29.14 (0.83) | -4.69 (0.72) |
| Barnwell | n.a. | n.a. | n.a. | n.a. | -28.79 (0.34) | -4.78 (0.15) |
| Fox | -27.68 (0.09) | 1.83 (1.13) | -27.93 (0.18) | 2.37 (0.87) | -28.59 (0.16) | -3.80 (0.66) |
| Misery | -28.12 (0.23) | 1.18 (0.57) | n.a. | n.a. | n.a. | n.a. |
| Redwood | -27.07 (5.56) | 2.14 (2.93) | -29.28 (0.32) | 1.71 (0.71) | -29.71 (0.35) | -5.03 (0.48) |
| J.O.H. | -26.90 (3.68) | -8.74 (13.25) | -25.7 (1.60) | 1.80 (0.17) | -28.16 (0.25) | -2.31 (1.12) |
| U. Elder | -25.345 (0.63) | -0.18 (0.06) | n.a. | n.a. | -28.27 (0.76) | -3.84 (0.54) |
| L. Elder | -27.81 (3.71) | 0.66 (2.16) | -25.75 (0.15) | 1.03 (0.68) | -28.35(0.35) | -3.07 (0.08) |
| S.F. Eel | -21.42 (2.39) | 0.11 (0.88) | -24.26 (0.44) | 0.73 (0.28) | -28.46(0.38) | -3.82 (0.03) |

Table 4. *Psychoglypha* $\delta^{13}C$, $\delta^{15}N$ (± 1 S.E.), and diet at each site. Sites are listed by watershed area, from smallest to largest.

| Site | $\delta^{13}C$ | $\delta^{15}N$ | diet source |
|---|---|---|---|
| Scully | -26.63 (1.08) | 0.44 (0.72) | FPOM |
| McKinley | -27.26 (3.77) | 0.87 (0.84) | FPOM |
| Skunk | -26.78 (0.82) | 0.70 (0.34) | FPOM |
| Barnwell | -27.94 (0.39) | 0.53 (0.24) | FPOM |
| Fox | -26.35 (n.a.) | 1.16 (n.a.) | FPOM |
| Misery | -27.88 (1.50) | 1.02 (0.51) | FPOM |
| Redwood | -30.96 (0.80) | 1.52 (0.57) | FPOM |
| J.O.H. | -20.24 (1.40) | 0.33 (0.43) | epilithon |
| U. Elder | -23.50 (2.44) | 1.04 (0.40) | epilithon |
| L. Elder | -20.51 (2.12) | 0.13 (0.38) | epilithon |
| S.F. Eel | -19.87 (2.55) | -0.06 (0.33) | epilithon |

Table 5. Standardized major axis regression statistics for the relationship between *Psychoglypha* stoichiometry (%P, %N, %C, C:N) and log mass. The range of log mass for each analysis is also provided. This relationship was significant (*P < 0.05) at very few sites.

| Sites | log mass range | n | $R^2$ | p-value | slope | intercept |
|---|---|---|---|---|---|---|
| Phosphorus (%) | | | | | | |
| McKinley* | 1.76 | 16 | 0.61 | 0.00 | -0.50 | 1.43 |
| Skunk* | 1.41 | 17 | 0.39 | 0.01 | -0.43 | 1.40 |
| Barnwell | 0.59 | 13 | 0.02 | 0.65 | -0.58 | 1.43 |
| Fox | 0.81 | 12 | 0.01 | 0.78 | 0.34 | 1.07 |
| Misery | 1.35 | 15 | 0.09 | 0.27 | -0.28 | 1.28 |
| Redwood | 1.29 | 12 | 0.00 | 0.87 | -0.29 | 1.47 |
| J.O.H. | 1.29 | 10 | 0.03 | 0.62 | 0.39 | 1.05 |
| L. Elder* | 1.17 | 11 | 0.46 | 0.02 | -0.46 | 1.36 |
| U. Elder | 0.90 | 15 | 0.00 | 0.89 | 0.44 | 0.88 |
| S.F. Eel | 0.92 | 11 | 0.17 | 0.21 | -0.97 | 1.73 |
| Nitrogen (%) | | | | | | |
| McKinley* | 1.67 | 19 | 0.35 | 0.01 | -1.75 | 10.51 |
| Skunk | 1.90 | 21 | 0.01 | 0.67 | -1.49 | 9.45 |
| Barnwell | 0.76 | 18 | 0.01 | 0.65 | -3.26 | 11.06 |
| Fox | 0.79 | 11 | 0.15 | 0.25 | -5.63 | 11.23 |
| Misery | 1.16 | 13 | 0.20 | 0.13 | -2.73 | 11.53 |
| Redwood | 1.06 | 11 | 0.16 | 0.22 | -1.40 | 11.11 |
| J.O.H. | 2.02 | 25 | 0.04 | 0.33 | -0.85 | 11.09 |
| L. Elder* | 0.69 | 11 | 0.35 | 0.06 | -3.34 | 12.16 |
| U. Elder | 0.63 | 13 | 0.01 | 0.78 | -2.05 | 12.13 |
| S.F. Eel | 0.56 | 9 | 0.27 | 0.15 | -4.79 | 13.33 |
| Carbon (%) | | | | | | |
| McKinley* | 1.67 | 19 | 0.59 | 0.00 | 4.00 | 43.13 |
| Skunk | 1.90 | 21 | 0.17 | 0.06 | 4.52 | 42.37 |
| Barnwell | 0.76 | 18 | 0.03 | 0.53 | 6.81 | 40.21 |
| Fox | 0.79 | 11 | 0.01 | 0.82 | -5.60 | 46.09 |
| Misery | 1.16 | 13 | 0.10 | 0.30 | -2.85 | 46.37 |
| Redwood | 1.06 | 11 | 0.00 | 0.97 | -3.80 | 46.06 |
| J.O.H. | 2.02 | 25 | 0.03 | 0.38 | 2.96 | 43.65 |
| L. Elder | 0.69 | 11 | 0.17 | 0.20 | -6.76 | 47.15 |
| U. Elder | 0.63 | 13 | 0.16 | 0.18 | 4.26 | 42.38 |
| S.F. Eel | 0.56 | 9 | 0.35 | 0.10 | -14.65 | 53.95 |
| C:N (molar) | | | | | | |
| McKinley* | 1.67 | 19 | 0.59 | 0.00 | 1.32 | 4.87 |
| Skunk | 1.90 | 21 | 0.08 | 0.22 | 1.35 | 5.41 |
| Barnwell | 0.76 | 18 | 0.04 | 0.45 | 1.51 | 4.67 |
| Fox | 0.79 | 11 | 0.06 | 0.46 | 4.41 | 4.28 |
| Misery | 1.16 | 13 | 0.15 | 0.19 | 1.40 | 4.49 |
| Redwood | 1.06 | 11 | 0.17 | 0.20 | 0.56 | 4.72 |
| J.O.H. | 2.02 | 25 | 0.05 | 0.26 | 0.62 | 4.66 |
| L. Elder* | 0.69 | 11 | 0.38 | 0.04 | 1.03 | 4.34 |
| U. Elder | 0.63 | 13 | 0.07 | 0.40 | 0.99 | 4.21 |
| S.F. Eel | 0.56 | 9 | 0.03 | 0.69 | 1.54 | 4.00 |

Table 6. Slope estimates (± 1 S.E.) and percent of variation explained by the mixed effects models predicting *Psychoglypha* %P, %N, and C:N. The fixed effects were log mass and diet C:X (where X is phosphorus in the P model and N in the N and C:N models) while site was the random effect. *P < 0.05, **P<0.01, ***P<0.001

| model | intercept | log mass | diet C:X | % of variation explained |
|---|---|---|---|---|
| Phosphorus | 1.29 (0.07)*** | -0.17 (0.04)*** | -0.001 (0.0002) | 38 |
| Nitrogen | 11.72 (0.64)*** | -0.68 (0.16)*** | -0.10 (0.04)* | 60 |
| C:N | 3.91 (0.34)*** | 0.54 (0.09)*** | 0.07 (0.02)** | 66 |

## FIGURE LEGENDS

Figure 1. Study sites in or near the Angelo Coast Range reserve.

Figure 2. Variation in mean (± 1 S.E.) *Psychoglypha* %P (a), %N (b), %C (c), and C:N (d) among sites.

Figure 3. *Psychoglypha* %P (a), %N (b), %C (c), and C:N (d) was strongly dependant upon log mass. All sites are show; however, one SMA line was fit to the entire dataset. See Table 5 for site-specific SMA fits.

Figure 4. Relationship between %P (a), %N (b), and C:N (c) residuals (variation in Y) and diet C:X (where X is either P or N). Scully was eliminated from this analysis due to low sample size.

Figure 1.

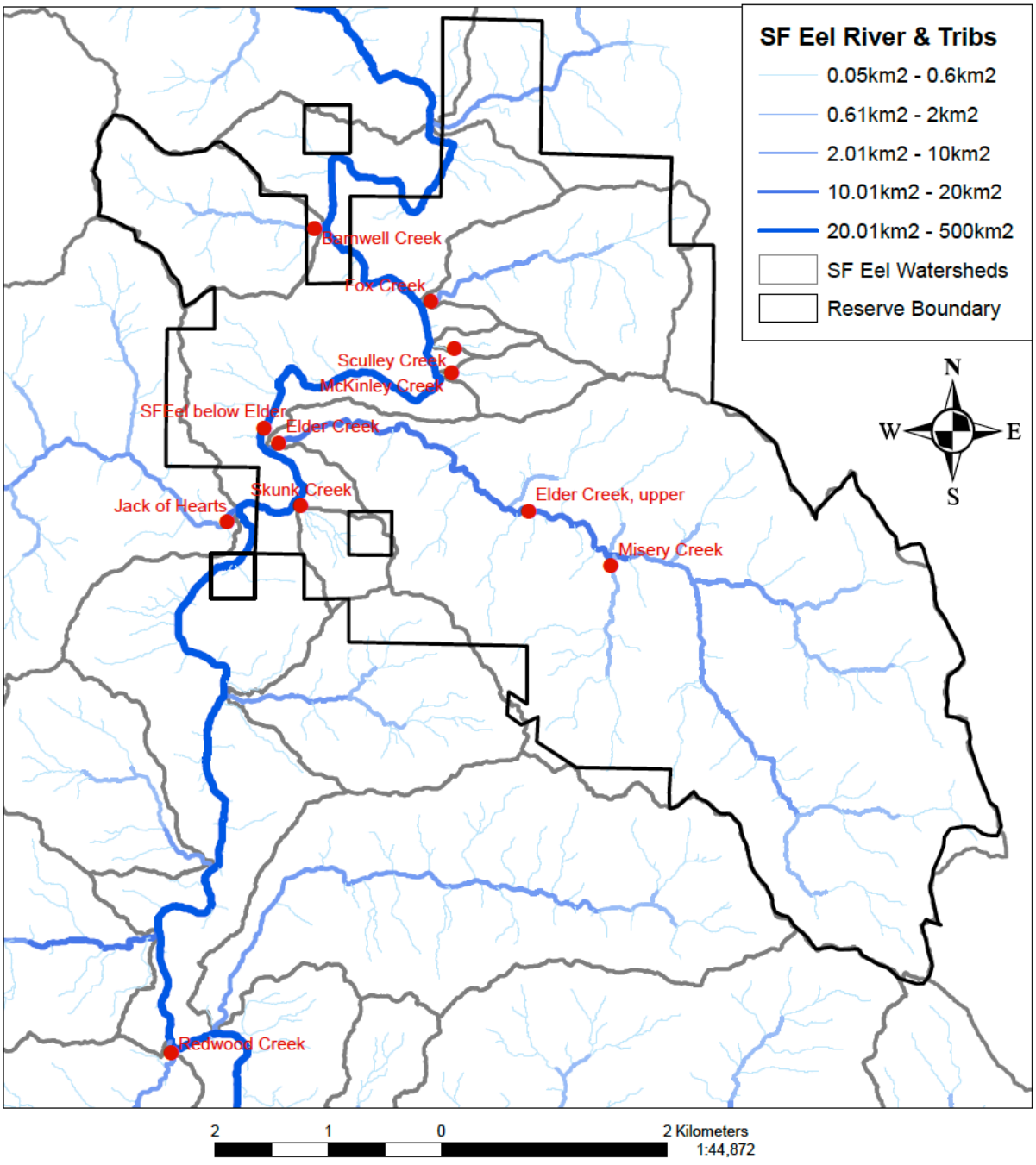

SF Eel River & Tribs
0.05km2 - 0.6km2
0.61km2 - 2km2
2.01km2 - 10km2
10.01km2 - 20km2
20.01km2 - 500km2
SF Eel Watersheds
Reserve Boundary
N
W
E
S
Barnwell Creek
Fox Creek
Sculley Creek
McKinley Creek
SFEel below Elder
Elder Creek
Skunk Creek
Jack of Hearts
Elder Creek, upper
Misery Creek
Redwood Creek
2
1
0
2 Kilometers
1:44,872

Figure 2.

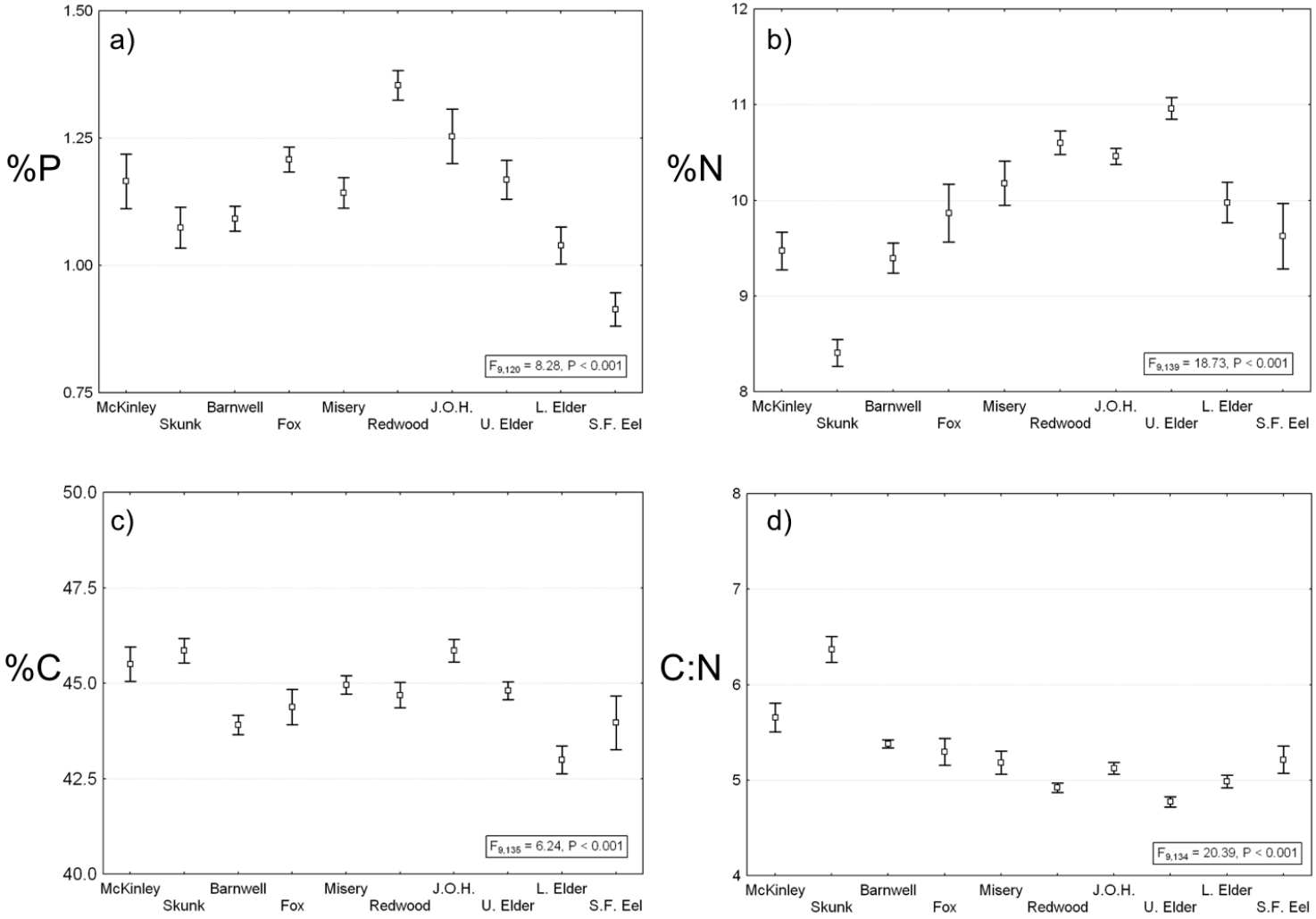

a)
%P
1.50
1.25
1.00
0.75
$F_{9,120}$ = 8.28, P < 0.001
b)
%N
12
11
10
9
8
$F_{9,139}$ = 18.73, P < 0.001
c)
%C
50.0
47.5
45.0
42.5
40.0
$F_{9,135}$ = 6.24, P < 0.001
d)
C:N
8
7
6
5
4
$F_{9,134}$ = 20.39, P < 0.001
McKinley
Skunk
Barnwell
Fox
Misery
Redwood
J.O.H.
U. Elder
L. Elder
S.F. Eel

Figure 3.

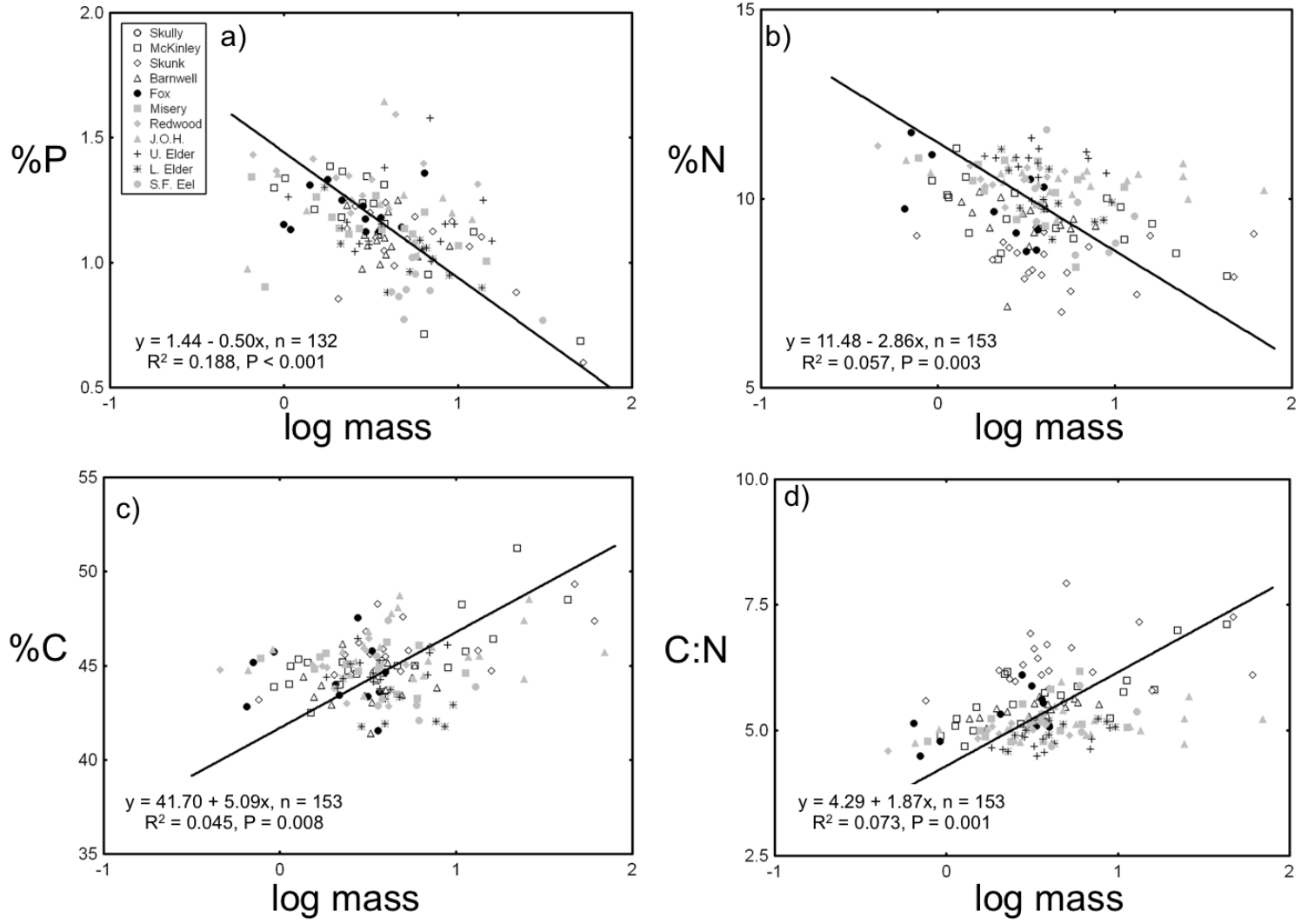
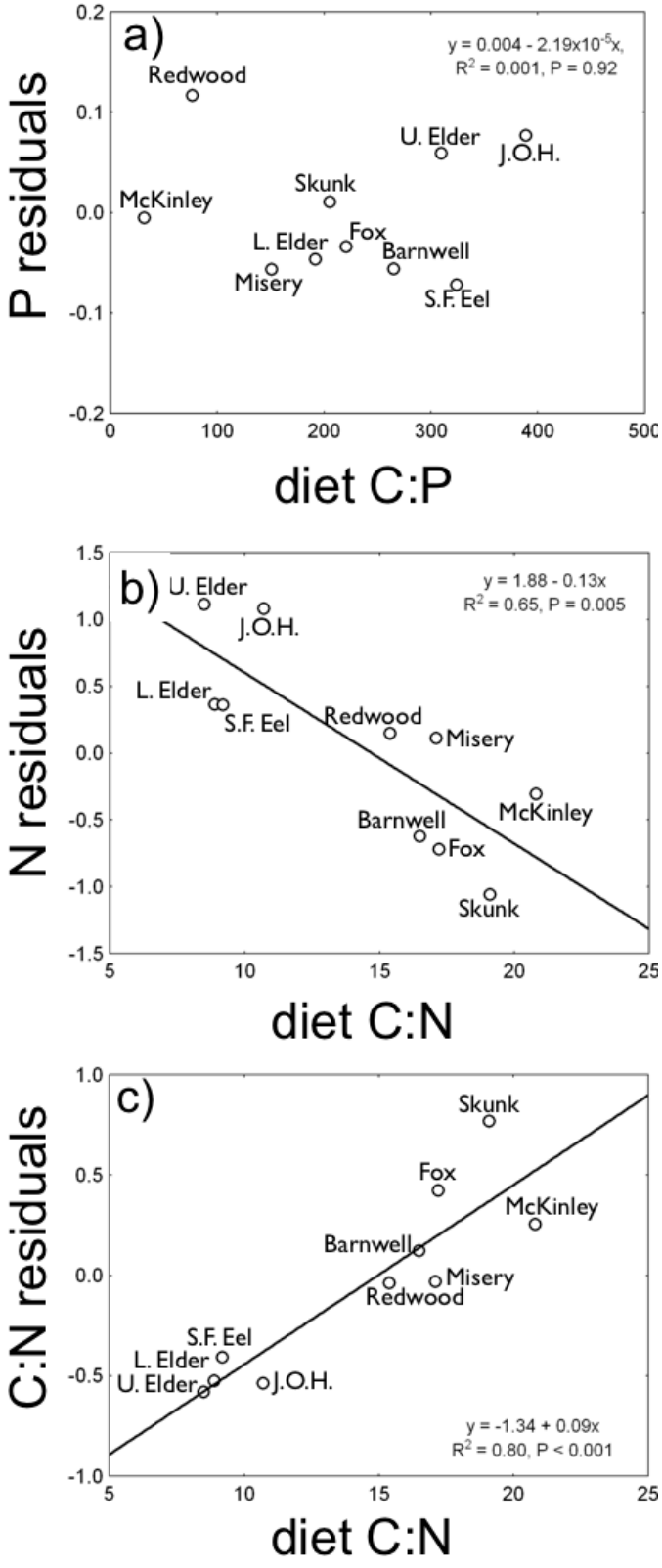

a)
Skully
McKinley
Skunk
Barnwell
Fox
Misery
Redwood
J.O.H.
U. Elder
L. Elder
S.F. Eel
%P
y = 1.44 - 0.50x, n = 132
R² = 0.188, P < 0.001
log mass
b)
%N
y = 11.48 - 2.86x, n = 153
R² = 0.057, P = 0.003
log mass
c)
%C
y = 41.70 + 5.09x, n = 153
R² = 0.045, P = 0.008
log mass
d)
C:N
y = 4.29 + 1.87x, n = 153
R² = 0.073, P = 0.001
log mass

Figure 4.


a)
y = 0.004 - 2.19x10-5x,
R2 = 0.001, P = 0.92
Redwood
U. Elder
J.O.H.
Skunk
McKinley
Fox
L. Elder
Barnwell
Misery
S.F. Eel
P residuals
diet C:P
b)
y = 1.88 - 0.13x
R2 = 0.65, P = 0.005
U. Elder
J.O.H.
L. Elder
S.F. Eel
Redwood
Misery
McKinley
Barnwell
Fox
Skunk
N residuals
diet C:N
c)
Skunk
Fox
McKinley
Barnwell
Misery
Redwood
S.F. Eel
L. Elder
U. Elder
J.O.H.
y = -1.34 + 0.09x
R2 = 0.80, P < 0.001
C:N residuals
diet C:N

**CHAPTER 4:**

# Influence of diet stoichiometry on nutrient release rates and ratios of selectively feeding detritivores.

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Nutrient excretion and egestion by consumers can play an important role in stream nutrient cycles. Thus, predicting the role of consumers in nutrient cycles is an important goal. Stoichiometric theory predicts that consumers will preferential assimilate limiting elements and release excess elements to maintain homeostasis, yielding a positive relationship between nutrient release and diet nutrient content with a slope above unity. Thus, nutrient release by invertebrates should magnify variation in organic matter stoichiometry in ecosystems. Here, we examined the influence of diet stoichiometry on the rates and ratios of excretion and egestion. We focus on four generalist invertebrates that consume both leaf litter and epilithon in streams located in Northern California and Minnesota. These invertebrates were fed diets consisting of cultured algae, stream epilithon, and several species of conditioned litter. Nutrient release was measured in short-term incubations after a two-day feeding period. In general, nutrient release rates and ratios did not vary with diet stoichiometry, contrary to our predictions. Nutrient release by these invertebrates dampens, instead of magnifies, the stoichiometric variation among diet sources. These results do not reflect a failure of stoichiometric principles. Instead, by analyzing the stoichiometry of foregut material we show that two of the four invertebrates selectively feed on a nutrient-rich portion of leaf litter. We argue that the other two species, for which we were not able to collect foregut material, also selectively feed. Selective feeding on leaf litter greatly reduced the imbalance between consumer and diet stoichiometries. Our results suggest

that if selective feeding is common among stream detritivores, these consumers may be an important source of nutrient regeneration in detrital-based systems.

## INTRODUCTION

There is increasing recognition that animals play an important role in freshwater nutrient cycles (Elser and Urabe 1999, Vanni 2003). This research primarily focuses on nitrogen (N) and phosphorus (P) because these nutrients are thought to most commonly limit the growth of autotrophs and heterotrophic microbes (Rosemond et al. 2000, Elser et al. 2009). Animals influence nutrient cycles indirectly through ingestion (Mulholland et al. 1991, Rosemond et al. 1993, Wallace and Webster 1996, Shurin and al. 2002). Ingested nutrients are either egested as feces or assimilated and allocated to growth or excreted. These processes have a direct effect on nutrient cycles (*sensu*: Vanni 2003).

Nitrogen and P excretion by invertebrates and fish can be an important source of nutrient regeneration in benthic systems (Grimm 1988, Hall et al. 2003, McIntyre et al. 2008, Liess and Kahlert 2009). Freshwater consumers primarily excrete N and P in forms easily assimilated by algae and microbes (Vanni 2003), which can stimulate the growth of these producers. By preferentially retaining one nutrient over another, consumers influence the ratio of available nutrients (Vanni et al. 2002, Evans-White and Lamberti 2005), epilithic nutrient content (Evans-White and Lamberti 2006), as well as nature of algal nutrient limitation (Elser and Urabe 1999).

Unassimilated nutrients are egested as fecal pellets. These particles can constitute a large fraction of the fine particulate organic matter (FPOM) pool in streams (Mulholland et al. 1985, Grimm 1988, Wallace et al. 1991). Experimental removal of

invertebrates decreased FPOM export by 56% in one forested headwater stream (Wallace et al. 1991). Fecal pellets are also an important substrate for microbes and invertebrates (Grimm 1988, Wallace and Webster 1996, Jonsson and Malmqvist 2005). Nutrient assimilation by the consumer determines the nutrient content of fecal pellets, which may influence the nutrient demand and uptake rates of microbes colonizing this material. Fecal nutrient content may also determine the quality of this material for other invertebrate and vertebrate consumers. In streams, transport of these particles between habitats creates longitudinal linkages among both food webs and nutrient cycles (Short and Maslin 1977).

Thus, predicting consumer release rates and ratios, across a gradient of streams, is a major goal of this field. Nutrient release rates and ratios vary widely among systems in response to changes in community structure and diet nutrient content (Vanni et al. 2002, James et al. 2007, Rothlisberger et al. 2008). The nutrient stoichiometry of invertebrate diets varies widely among streams (Cross et al. 2005). Allochthonous leaf litter is commonly nutrient poor and widely variable among species (Cross et al. 2003, Hladyz et al. 2009). FPOM and epilithon are, in contrast, more nutrient rich; though also widely variable in time and space (Cross et al. 2003, Cross et al. 2005, Evans-White et al. 2005). Solute nutrient concentrations influence the nutrient content of all of these resources (Cross et al. 2003, Rothlisberger et al. 2008, Small and Pringle 2010).

Consumer nutrient recycling theory (CNR) can be used to predict the nutrient release rates and ratios of consumers (Elser and Urabe 1999). To predict relative

release rates and ratios, CNR uses a mass balance approach and assumes that animals tightly regulate body nutrient stoichiometry against wide variation in diet stoichiometry, described as strict stoichiometric homeostasis (Sterner and Elser 2002). This assumption makes nutrient release a function of the stoichiometry of the consumer, an indicator of nutrient demand, and diet nutrient content. If the consumer maintains homeostasis, nutrient release must increase more rapidly than diet nutrient content (Figure 1). The accuracy and ramifications of these predictions have been widely examined in pelagic (Elser and Urabe 1999, Andersen et al. 2004) and benthic systems (Vanni et al. 2002, Frost and Tuchman 2005, Evans-White and Lamberti 2006, James et al. 2007, Rothlisberger et al. 2008).

Many tests of CNR were conducted in pelagic systems where consumer diets are stoichiometrically variable, but not structurally complex like benthic epilithon, leaves, or detritus. In pelagic systems, composite or bulk seston stoichiometry is a reasonable predictor of gazer diet stoichiometry (Sterner and Elser 2002). In other systems, the correspondence between the bulk stoichiometry of a resource and consumer diets may not be as strong. Benthic epilithon is a three-dimensional matrix of algae, bacteria, detritus, and inorganic particles (Lock et al. 1984). Although epilithic grazers (scrapers) sometimes consume the bulk epilithon (Mulholland et al. 2000b), scrapers commonly selectively feed within this matrix. Scrapers have been shown to ingest algae from within a highly heterotrophic biofilm (McNeely et al. 2006) as well as select a high turnover component of the biofilm (Mulholland et al. 2000b). Species consuming

allochthonous leaf litter (shredders) often selectively ingest or assimilate the microbes growing on leaf litter (Arsuffi and Suberkropp 1989, Chung and Suberkropp 2009a). When the nutrient content of the bulk diet does not correspond to that of the ingested material, application of stoichiometric mass balance models may be misleading (Cross et al. 2005, Evans-White and Lamberti 2005, Evans-White and Lamberti 2006).

Most freshwater tests of CNR theory have examined excretion and have not measure egestion. Egestion and excretion are two independent stages (i.e., assimilation v. excretion) at which a consumer may regulate homeostasis. When homeostasis is regulated at both stages nutrient egestion and excretion would have similar relationships with diet nutrient content (Fig. 1). Fish do not appear to maintain homeostasis at the assimilation stage (Sterner and George 2000, Hood et al. 2005). Studies with invertebrates show mixed results (Pandian and Marian 1986, Balseiro and Albarino 2006, Fink and Von Elert 2006).

The central role of fecal pellets in ecosystems makes predicting nutrient egestion rates and ratios an important goal. Here, we describe experiments in which four common shredder species were fed a suite of diets varying in identity (algae v. litter species) and stoichiometry. Following the feeding, we measured both egestion and excretion rates and ratios. Our predictions are based on CNR theory (Elser and Urabe 1999) and are described in Figure 1. This work will improve our understanding of how diet stoichiometry influences nutrient egestion rates and ratios, an important aspect of benthic food webs and nutrient cycles.

## METHODS

*Study sites*

Feeding studies were conducted with animals from Elder Creek (Mendocino County, CA) and Valley Creek (Washington County, MN). Both streams are cool water, moderately shaded, and have stable baseflow during the summer. Elder Creek is a third order steam in the Angelo Coast Range Reserve (Watershed area = 13 $km^2$). The region's climate is Mediterranean with warm, dry summers and cold, wet winters. The composition of the riparian community includes bay (*Umbellularia californica*), madrone (*Arbutus menziesii*), douglas fir (*Pseudotsuga menziesii)*, alder (*Alnus rhombifolia*), and maple (*Acer macrophyllum*). During the summer months, epilithic communities are dominated by a thin, heavily grazed layer of diatoms (McNeely and Power 2007).

The Elder Creek experiments focused on *Lepidostoma* and *Psychoglypha sp.* (hereafter *Psychoglypha*), common benthic consumers in this system. *Psychoglypha* primarily consumes epilithon in Elder Creek, though it consumes allochthonous detritus at other locations in this system (Chapter 3). There are several species of *Lepidostoma* (hereafter Elder *Lepidostoma*) within the Angelo Reserve (F. McNeely *unpublished data*) that cannot be keyed to species as larvae. Elder *Lepidostoma's* primary diet shifts ontogenetically in Elder Creek from epilithon to allochthonous detritus (Hood *unpublished data*).

Valley Creek is a first order, ground water fed stream (Watershed area = 161 $km^2$, drainage area = 45 $km^2$; Zimmerman and Vondracek 2007). Samples were collected within the Belwin Reserve. At this site, the stream was surrounded by a narrow riparian zone and a grass lawn. Riparian trees are primarily willow (*Salix* sp) and eastern cottonwood (*Populus deltoides*), though there is only moderate shading. *Gammarus pseudolimnaeus* (hereafter *Gammarus*) and *Lepidostoma* (hereafter VC *Lepidostoma*) are common shredders in Valley Creek (Ruetz et al. 2002).

*Elder Creek experiments*

Diet sources for Elder Creek experiments included Elder Creek epilithon, South Fork Eel River epilithon (*Psychoglypha* experiment only), as well as bay (*Umbellularia californica*), madrone (*Arbutus menziesii*), maple (*Acer macrophyllum*), and oak (*Quercus wislizenii*) leaves. Freshly fallen litter was collected from common forest species and conditioned in flow-through chambers for at least one month before experiments. Epilithion was collected just before initiation of feeding experiments from Elder *Lepidostoma* and *Psychoglypha* habitats.

Experiments were done for *Psychoglypha* and Elder *Lepidostoma* in July and September, respectively. We collected mid-sized *Psychoglypha* (case length = 1.5 ± 0.16 mm, mean ± 1 S.D.) and Elder *Lepidostoma* (0.9 ± 0.1 mm) from Elder Creek, sorted these animals to a single size class (± 2 mm case length), and distributed them to 24 flow-through chambers (1.9 L), containing one of the five (Elder *Lepidostoma*) or six

(*Psychoglypha*) diets. Ten Elder *Lepidostoma* and eight *Psychoglypha* were placed in each container. Epilithon treatments were two 5-10 cm diameter rocks per chamber. Flow-through chambers were placed in Elder Creek for a 48-hour acclimation period prior to nutrient release measurements.

All Elder Creek nutrient release measurements followed the same protocol (Vanni et al. 2002). After the 48-hour acclimation period, animals in flow-through containers were distributed as follows. Six *Psychoglypha* or eight Elder *Lepidostoma* were rinsed in stream water and transferred to containers with 60 or 80 mL of filtered (Whatman GF/F) Elder Creek water. Two other animals were rinsed, placed in a glass vial on ice, and frozen upon return to the lab (~ 2 - 3 hours later). These animals were used for foregut and hindgut analysis. We made control containers with filtered stream water and empty caddisfly cases, which account for non-excretory changes in nutrient concentrations. Nutrient release incubations were approximately 30 minutes for *Psychoglypha* and 60 minutes for Elder *Lepidostoma*. Following the incubation, container contents were filtered (Whatman GF/F) and immediately analyzed for SRP and $NH_4$ in duplicate. The filter was dried at 60 ˚C and retained to estimate particulate C, N and P egestion.

Following the experiment, leaf diets were consolidated into two replicate samples, dried (60 ˚C), and stored for C, N, and P analysis. We used a wire brush to remove biofilm surrounding a 4.2 $cm^2$ diameter sample area on the top of each stone. A hard toothbrush was used to collect the epilithon sample, which was filtered onto two

pre-weighed and ashed (550˚C) glass fiber filters (Whatman GF/F) for C, N, and P analysis. Filters were dried at 60 ˚C and stored for analysis.

*Valley Creek experiments*

*Gammarus* and VC *Lepidostoma* were collected from Valley Creek in October. Animals were returned to the lab, sorted into size classes, and placed inter-specifically into 10 aerated aquaria containing 10 L of Valley Creek water and one of five diet treatments: Oak (*Quercus* sp.), Willow (*Salix* sp.), Maple (*Acer* sp.), Pine (*Pinus strobus*), or *Scenedesmus obliquus*, a planktonic green alga. *Scenedesmus obliquus* was cultured in chemostats under N-limited conditions, as described in Chapter 1. Aquaria were kept in an environmental chamber at 10 ˚C during a 48 (*Gammarus*) or 72 hr (VC *Lepidostoma*) acclimation period. Following the acclimation periods, nutrient release measurements were conducted as described above. Nutrient release incubations were approximately 60 minutes. In the *Gammarus* experiments, controls contained only filtered water. Leaf diets were treated as described above. *Scenedesmus obliquus* was siphoned out of the aquaria and filtered onto duplicate pre-weighed and ashed (550 ˚C) glass fiber filters for C, N, and P analysis.

*Foregut and hindgut material*

We dissected animals from all experiments to extract foregut material samples. Adequate mass for analysis could only be collected from the Elder Creek experiments.

Samples were composited across replicates, resulting in approximately two foregut samples per treatment. Trichopteran larvae have simple, cylindrical guts. We classified the first third as the foregut. Material was removed from this section, placed in a pre-weighed tin capsule and dried at 60 ˚C. The material was weighed to the nearest 0.1 µg then analyzed for C and N as described below.

*Chemical and statistical analyses*

Filtrate samples were analyzed for SRP using the acid-molybdate method on a spectrophotometer and for $NH_4$ using the fluorometric method (Holmes et al. 1999, Taylor et al. 2007). $NH_4$ standard curves were created with stream water (Elder Creek Experiments) or sample water (Valley Creek Experiments). Excretory products create matrix effects which lead to an underestimate of $NH_4$ when stream and not sample water is used for standard curves (Whiles et al. 2009). In 2008, we conducted Elder *Lepidostoma* and *Psychoglypha* $NH_4$ excretion experiments using both approaches (Hood and McNeely *unpublished data*). $NH_4$ concentrations calculated through these two approaches are correlated ($\mu g\ NH_4/L_{sample} = 1.554 * \mu g\ NH_4/L_{stream} - 13.62$, $R^2 = 0.982$, $P < 0.001$). The relationship does not differ between species (SMA test for similar slopes: n = 12, 2 species, P = 0.723); therefore, we applied this correction to all Elder Creek $NH_4$ samples.

Fecal samples on filters were dried (60 ˚C), weighed, and cut in half for nutrient analysis (i.e., N or P); then, each half was reweighed. Leaf litter samples were dried (60

˚C), homogenized with a Wiley Mill, and sub-sampled for C, N, and P analysis. Particulate P samples were ashed (550 ˚C) and hydrolyzed in HCl; then, $PO_4$ was measured colormetrically as molybdenum blue (DeMott 1998). Particulate C and N samples were analyzed with a Perkin Elmer 2400 CHNS analyzer.

Mass-specific N and P excretion rates were calculated as the change in $NH_4$-N or SRP per unit time divided by the dry mass of animals. Mass-specific C, N, and P egestion rates were calculated as the mass of C, N, or P on the filter per unit time divided by the dry mass of animals. Total nutrient release was calculated as the sum of nutrient excretion and nutrient egestion. We compared bulk diets and foregut material stoichiometry, expressed in molar ratios, with a factorial ANOVA and Tukey HSD posthoc tests. The influence of diet on release rates and ratios was examined with one-way ANOVAs. All tests were conducted in Statistica (StatSoft).

## RESULTS

*Elder* Lepidostoma

The diets fed to Elder *Lepidostoma* differed in terms of %C, %N, and %P (ANOVA, $P < 0.05$). All diets were N poor compared to foregut material (Factorial ANOVA: $F_{1,16} = 591.0$, $P < 0.001$, , Fig. 2b). Diets and foregut material also differed in terms of %C (Factorial ANOVA: $F_{1,16} = 4.919$, $P = 0.041$, Fig. 2a), though post hoc tests indicate differences for only maple.

Diet influenced many aspects of nutrient release by Elder *Lepidostoma*, though not always as predicted. Diet influenced total P release but not total N release (Table 1). As predicted, total release N:P increased with diet N:P with a slope greater than one (Table 1, Figure 3a). Excretion rates of Elder *Lepidostoma* were nearly three times higher than egestion rates (Table 1). The balance between excretion and egestion rates was influenced by diet (Table 1).

Diet influenced the rate and ratio of excretion by Elder *Lepidostoma* (Table 1). As predicted, excretion N:P increased with diet N:P with a slope greater than one (Table 2, Fig 3b). Nitrogen and P excretion rates were not related to diet C:N or C:P, respectively (Table 2). Diet also influenced N and C egestion rates, but not P egestion. Egestion C:N and C:P increased with diet C:N and C:P, respectively; though slopes were far less than one (Table 2, Fig 4a and 4b). Egestion N:P was not related to diet N:P (Table 2, Fig 4c). Egestion C:N was greater than the C:N of foregut material (Figure 5), though these parameters were not related ($P > 0.05$).

Psychoglypha

The diets fed to *Psychoglypha* differed in terms of %C, %N, and %P (ANOVA, $P < 0.05$). *Psychoglypha* also appears to selectively feed on leaf litter. All diets were N poor compared to foregut material (Factorial ANOVA: $F_{1,17} = 202.052$, $P < 0.001$, Tukey HSD, $P < 0.05$, Fig. 1c), except for Elder epilithon. Diets and foregut material also differed in

terms of %C (Factorial ANOVA: $F_{1,17}$ =4.553, P = 0.048, Fig. 1d), though post hoc tests indicate that these differences were not significant (Tukey HSD, P > 0.05).

Diet did not influence the rate or ratio of total nutrient release by *Psychoglypha* (Table 1). Nitrogen excretion by this species was 55% (± 15%) of N egestion. Phosphorus excretion rates were 30% (± 8%) of P egestion rates. In contrast to Elder *Lepidostoma*, the balance between excretion and egestion rates was not influenced by diet (Table 1). Diet influenced P egestion by *Psychoglypha*, but not N egestion rate or egestion N:P (Table 1). Egestion C:N increased with diet C:N, though the slope of this relationship was far less than unity (Table 2, Fig. 4a). Egestion C:P and N:P was not related to diet C:P or N:P, respectively (Table 2, Fig. 4b and 4c). Similar to Elder *Lepidostoma*, egestion C:N was greater than the C:N of foregut material (Figure 5), though these parameters were not related (P > 0.05).

Gammarus

For *Gammarus,* diet did not influence total nutrient release or excretion rates or ratios; however, diet did influence *Gammarus'* N and C egestion rates (Tables 1 and 2). The C:P of *Gammarus* egestion increased with diet C:P, with a slope far shallower than predicted (Table 2, Fig. 4b). Egestion C:N and N:P was not related to diet C:N or N:P (Table 2, Figs. 4a and 4b). Nitrogen excretion rates of *Gammarus* were 2.1 (± 0.2) times greater than N egestion rates, while P excretion rates were 41% of P egestion rates. Diet did not influence the balance of excretion and egestion rates (Table 1).

*VC* Lepidostoma

In contrast to Elder *Lepidostoma*, total nutrient release, excretion, and egestion rates and ratios did not differ among diets for VC *Lepidostoma* (Table 1 and 2). However, the excretion N:P increased with diet N:P, as predicted, with a slope greater than unity (Table 2). Nitrogen excretion rates were 80% (± 12%) of N egestion rates, while P excretion rates were 30% (± 6%) of P egestion rates. The balance between N excretion and egestion rates was influenced by diet (Table 1). Individuals consuming litter egested more N than they excreted, while those consuming algae excreted more N than egested. The balance between P excretion and egestion was not influenced by diet (Table 1).

## DISCUSSION

Consumer nutrient regeneration theory predicts that the requirements of strict homeostasis forces consumers to retain rare nutrients and release excess nutrients (Elser and Urabe 1999). The requirements of strict homeostasis results in an amplification of regeneration stoichiometry relative to diet stoichiometry (Fig. 1), potentially leading to shifts in algal community composition, changes in nutrient limitation, and a negative feedback on the consumer's fitness (Elser and Urabe 1999). In contrast to theory, the stoichiometry of material released by stream shredders was

rarely related to bulk diet stoichiometry in the manner we predicted (Figs. 1, 3, and 4). Our results suggest that consumer nutrient regeneration can dampen stoichiometric variation of excreted and egested material relative to diet (Figs. 3 and 4). We argue that selective feeding of a nutrient rich fraction of available food (Fig. 5) led to the dampening of stoichiometric release, relative to the ratios predicted based on bulk diet stoichiometry.

*Influence of selective feeding on stoichiometric imbalances*

Our results show that selective feeding can balance diets that appear to be highly stoichiometrically imbalanced. Elder *Lepidostoma* and *Psychoglypha* were presented with diets ranging in bulk C:N from approximately 10 to 60, while the material ingested had a mean C:N of 8.6 and varied little among diets (Fig. 2). It is highly likely that *Gammarus* and VC *Lepidostoma* also selectively ingested a nutrient rich diet, given their relationships between egestion C:X (where, X is N or P) and diet C:X (Fig. 4). The mean C:N of Elder *Lepidostoma* and *Psychoglypha* was 6.3 and 5.4, respectively (Hood chap 3, *unpublished data*). Thus, selective feeding by Elder *Lepidostoma* consuming oak leaves reduced an apparent 8.4:1 stoichiometric imbalance to an imbalance of 1.5:1. When consumers selectively feed, using bulk diet stoichiometry to assess consumer-resource imbalances can yield misleading results. Though previously recognized (Cross et al. 2005, Frost et al. 2005), we believe this is the first time the

influence of selective feeding on stoichiometric imbalances has been demonstrated for benthic invertebrates.

Our results suggest that Elder *Lepidostoma* and *Psychoglypha* selectively ingest a portion of litter high in nitrogen, and presumably rich in microbes. The bacteria and fungi that colonize leaf litter are far richer in N and P than the litter itself (Graca 2001, Sterner and Elser 2002). We used a two compartment (microbes + leaf) mixing model to estimate the microbial contribution required to go from bulk diet N content to ingested N content. Fungal N content is approximately 5 % (Ooijkaas et al. 2000), though it can vary widely in response to N availability (Levi and Cowling 1969). The mixing model suggests that if microbial N content was 5%, this species' diet would have to be 75 to 90 % microbial to achieve the N content observed in its foregut. If microbes colonizing this leaf litter were 10% N, perhaps the highest percentage possible (Levi and Cowling 1969), the material ingested by this species would only have to be 40% microbial.

While these estimates bracket the likely contributions of microbes to consumer diets, their accuracy is limited by lack of information for three areas. First, we do not know the N content of microbes growing on this litter. The N content of fungal and bacterial communities varies in response to N availability (Persson et al. 2010). Microbial N content may be relatively low in Elder Creek, which has low $NH_4$ and $NO_3$ concentrations (Finlay et al. in press). Second, we used the N content of newly collected oak leaves in the mixing model. This may under-estimate actual leaf N because rapid leaching occurs when litter enters the stream (Webster and Benfield 1986). Third, while

care was taken in dissection, some of the N in the foregut material may have come from the caddisflies (i.e., tissue or digestive enzymes). Inclusion of caddisfly tissue (mean = 6.3 %N) could lead to an over-estimate of ingested N. Nevertheless, there is no other plausible explanation for these results other than selective feeding.

Our finding, that selective feeding balances apparently stoichiometrically imbalanced diets, could be common in benthic systems. In one detrital stream, some shredder, collector-gatherer, and scrapper species selectively consumed a diet with high turnover rates relative to bulk diets (Mulholland et al. 2000b). Rapid growth requires large nutrient expenditures (Sterner and Elser 2002), so selective feeding in this system might diminish stoichiometric imbalances. In addition, there is widespread agreement that many shredders selectively consume or assimilate the microbial cells growing on leaf litter (Arsuffi and Suberkropp 1989, Graca 2001, Chung and Suberkropp 2009a). Many shredder taxa, including Trichoptera and Amphipoda, seek out and selectively consume litter conditioned by microbes over unconditioned litter (see review in: Graca 2001). Shredders also preferentially ingest certain fungal species (Arsuffi and Suberkropp 1988, 1989). Indeed, microbial C can be responsible for up to 100% of shredder growth (Chung and Suberkropp 2009a). Yet, it is not clear whether shredders (1) selectively ingest microbial cells or (2) consume the entire leaf and only assimilate microbial nutrients and C (Graca 2001, Chung and Suberkropp 2009b). Elder *Lepidostoma* and *Psychoglypha* clearly do the former; they selectively ingest a diet dominated by microbial cells.

The difference may not be important to the nutrition of the consumer; however, it does influence the use of ecological stoichiometry in detrital systems. If shredders consume the entire leaf, but only assimilate the microbial fraction; then, we would expect release stoichiometry to increase with diet stoichiometry, as predicted by CNR (Fig. 1) and shown for the shredder *Klapopteryx kuscheli* (Balseiro and Albarino 2006). In contrast, when a shredder selectively ingests a nutrient rich fraction of the leaves; there may be no relationship between release stoichiometry and bulk diet stoichiometry, as we see here. When ingested stoichiometry is different from bulk stoichiometry, predictions based on bulk stoichiometry and mass balance models will be misleading. There is wide-spread recognition that benthic shredders and even herbivores selectively feed or assimilate (Arsuffi and Suberkropp 1989, Mulholland et al. 2000b, McNeely et al. 2007, Chung and Suberkropp 2009b). Thus, predictions about stoichiometric imbalances or consumer nutrient cycling using ecological stoichiometry should be made with caution.

*Role of selectively feeding consumers in stream nutrient cycles*

Our results show the role of consumer nutrient regeneration is some benthic systems, particularly detrital systems, differs from the role predicted from bulk diet stoichiometries. We fed four species diets varying in bulk C:N from 10 to 60 and C:P from 140 to over 5000, reflecting a diet shift from algae to leaf litter. Theory predicts a steep decline in N and P release rates across this gradient. Yet, we observed no

relationship between total nutrient release and diet C:X. No relationship between N or P egestion and diet C:X and only a shallow relationship between P excretion and diet C:P for VC *Lepidostoma*. These findings can be attributed to selective feeding which greatly reduced stoichiometric imbalances in the litter treatments. Our results challenge the assumption that shredders consume an energy rich and nutrient poor diet and changes stoichiometric predictions regarding the role of these consumers in nutrient cycles.

In general, nutrient excretion rates did not differ between the litter and epilithon or algae treatments, suggesting consumers may be a source of nutrient regeneration in both algal-based and detrital-based systems. Diet did influence N and P excretion rates of Elder *Lepidostoma*, but the highest N and P excretion rates were for oak and madrone, respectively. These results are surprising given the large stoichiometric differences between bulk epilithon and leaf litter. CNR theory predicts that, given the relatively stoichiometrically balanced diet of benthic herbivores, these consumers would be a source of nutrient regeneration. In an algal-based desert stream, invertebrates were responsible for 70% of N regeneration during baseflow (Grimm 1988). In contrast, we would predict that benthic detritivores would be a nutrient sink, since these consumers are far more nutrient rich than their bulk diet. Our results suggest the opposite. Shredder ingestion and excretion of microbial N and P may produce rapid nutrient cycles in nutrient-poor detritus-based systems. Recent evidence suggests that many stream consumers selectively consume a high turnover fraction of their bulk diet (Mulholland et al. 2000b); suggesting that, if consumer densities are high enough, some

detrital streams may be similar to autotrophic streams where invertebrate consumers are responsible for a large fraction of nutrient regeneration (Grimm 1988).

Our results help clarify one of the fundamental links in stream consumer processing chains (Heard 1994), a critical component of stream food webs. These chains begin with shredders that consume allochthonous leaf litter and egest fecal pellets. Fecal pellets subsequently become food for collector-gatherers or filter feeders. Shredder activity increases the growth rate of these consumers (Dieterich et al. 1997, Jonsson and Malmqvist 2005) and mediates nutrient transfer to them (Short and Maslin 1977, Grimm 1988). It is widely argued that shredder feces are a nutrient rich, high quality food (Covich et al. 1999, Crowl et al. 2001, Jonsson and Malmqvist 2005); whereas, the stoichiometric imbalance between shredders and bulk leaf litter suggests that shredder feces should be even more nutrient poor than the leaf litter. The high nutrient content of fecal particles may be due to microbial colonization; however, our results offer an alternative explanation. When shredders selectively feed on a nutrient rich diet, similar to Elder *Lepidostoma* and *Psychoglypha*, then these consumers would also be expected to egest relatively nutrient rich fecal pellets.

The four species we examined created stoichiometrically homogeneous fecal pellets, relative to the wide variation in bulk diet stoichiometry. Fecal pellets were nutrient poor relative to epilithon and nutrient rich relative to bulk leaf litter. Egestion by stream consumers often makes a substantial contribution to the FPOM pool (Grafius and Anderson 1979, Grimm 1988, Wallace et al. 1997). Bacterial densities are highest

on FPOM and these microbial communities play a large role in shaping stream nutrient cycles and metabolism (Mulholland et al. 1985, Mulholland et al. 2000a, Tank et al. 2000, Dodds et al. 2002). Particulate nutrient content likely influences microbial metabolism and nutrient uptake; therefore, consumers shape microbial metabolism and nutrient uptake by creating FPOM and determining its nutrient content. Our results show that when invertebrates selectively feed, they dampen the stoichiometric variation of particles entering the FPOM pool, relative to the parent material of this organic matter; thereby stabilizing the stoichiometric quality of microbial substrates.

When selectively feeding, these consumers leave behind material more depleted in nutrients than the bulk diet. This material reflects the imbalance between the consumer and its bulk diet, which is large when the diet is leaf litter. We do not know if this material remains a part of the leaf litter or enters the FPOM pool. Yet, this material is nutrient depleted, striped of microbes, and perhaps more recalcitrant then the original bulk diet. It likely is a poor substrate for both microbes and invertebrates.

*Significance*

Here, we examined the relationship between nutrient release and bulk diet stoichiometry. Nutrient release by these selectively feeding consumers dampens stoichiometric variation relative to the bulk diet, contrary to stoichiometric theory. We found little support for stoichiometric predictions, not because the homeostasis assumptions were violated; instead, all four invertebrates we examined appear to

selectively ingest a nutrient rich fraction of leaf litter. Selective feeding greatly reduced the stoichiometric imbalance between these consumers and leaf litter. Taken together, this work suggests that where selective feeding is common, consumers may play a very different role in benthic nutrient cycles then predicted using bulk diets.

Table 1. Mean release rates and ratios (± 1 standard error) and the influence of diet on release rates. *P < 0.05; **P < 0.01; ***P < 0.001

| | *Gammarus* | Elder *Lepidostoma* | VC *Lepidostoma* | *Psychoglypha* |
|---|---|---|---|---|
| Total release | | | | |
| N (µg N mg $DM^{-1}$ $hr^{-1}$) | 1.09 (0.06) | 2.69 (0.10) | 0.70 (0.07) | 0.70 (0.08) |
| P (µg P mg $DM^{-1}$ $hr^{-1}$) | 0.05 (0.00) | 0.31 (0.02)** | 0.04 (0.00) | 0.09 (0.02) |
| N:P (molar) | 24.02 (1.61) | 21.78 (1.70)* | 19.05 (1.40) | 23.34 (3.77) |
| Excretion | | | | |
| N (µg N mg $DM^{-1}$ $hr^{-1}$) | 0.67 (0.04) | 1.94 (0.10)* | 0.28 (0.03) | 0.21 (0.05) |
| P (µg P mg $DM^{-1}$ $hr^{-1}$) | 0.01 (0.00) | 0.24 (0.02)** | 0.01 (0.00) | 0.02 (0.00) |
| N:P (molar) | 140.61 (11.46) | 23.05 (2.48)* | 95.44 (11.43) | 52.40 (13.54) |
| Egestion | | | | |
| C (µg C mg $DM^{-1}$ $hr^{-1}$) | 3.77 (0.30)*** | 14.09 (0.66)** | 7.88 (0.96) | 10.89 (1.34)** |
| N (µg N mg $DM^{-1}$ $hr^{-1}$) | 0.42 (0.04)* | 0.74 (0.03)* | 0.42 (0.05) | 0.54 (0.08) |
| P (µg P mg $DM^{-1}$ $hr^{-1}$) | 0.04 (0.00) | 0.08 (0.00) | 0.03 (0.00) | 0.09 (0.02)* |
| C:N (molar) | 11.21 (0.62) | 22.78 (0.84)*** | 22.86 (1.62) | 24.92 (1.50)** |
| C:P (molar) | 295.88 (29.06)*** | 459.24 (22.27)*** | 714.59 (75.32) | 473.14 (72.88)* |
| N:P (molar) | 27.93 (3.00)* | 20.31 (0.78) | 32.90 (3.13) | 18.08 (2.09) |
| Excretion:Egestion | | | | |
| N | 2.08 (0.22) | 2.94 (0.29)** | 0.82 (0.13)* | 0.55 (0.15) |
| P | 0.41 (0.06) | 2.88 (0.21)** | 0.32 (0.06) | 0.28 (0.07) |

Table 2. Least-squared slopes from the relationship between nutrient release and diet stoichiometry are shown when significantly different from zero ($P < 0.05$).

| | *Gammarus* | Elder *Lepidostoma* | VC *Lepidostoma* | *Psychoglypha* |
|---|---|---|---|---|
| Total release | | | | |
| N v. diet C:N | | | | |
| P v. diet C:P | | | | |
| N:P v. diet N:P | | 1.663 | | |
| Excretion | | | | |
| N v. diet C:N | | | | |
| P v. diet C:P | | | $-2.1 \times 10^{-6}$ | |
| N:P v. diet N:P | | 2.620 | 2.420 | |
| Egestion | | | | |
| N v. diet C:N | | | | |
| P v. diet C:P | | | | |
| C:N v. diet C:N | | 0.177 | | 0.195 |
| C:P v. diet C:P | 0.078 | 0.122 | | |
| N:P v. diet N:P | | | | |

## FIGURE LEGENDS

Figure 1. Conceptual plot illustrating the influence of diet stoichiometry on release stoichiometry. Here, the consumer is assumed strictly homeostatic. To maintain homeostasis, consumers must retain scare elements and release those in excess. If homeostasis is maintained, the relationship between X:Y release and X:Y ingested must be greater than isometry. X and Y are any element. Figure adapted from Sterner and George (2000).

Figure 2. Material from the foregut of Elder *Lepidostoma* (left panels) and *Pyschoglypha* (right panels) was more N rich than their diets, suggesting that these species selectively feed. Foregut material differed from diet in terms of %C, %N, and C:N ($P < 0.05$). Asterix indicate significant differences between foregut material and diet (Tukey HSD, $P < 0.05$).

Figure 3. The influence of diet N:P on total release N:P (a) and excretion N:P (b). Significant least-squares fits are shown.

Figure 4. The influence of diet stoichiometry on egestion C:N (a), C:P (b), and N:P (c). Significant least-squares fits are shown.

Figure 5. Elder *Lepidostoma* (a) and *Psychoglypha* (b) retained N relative to C. Egestion C:N was not related to foregut C:N. Error bars are one standard error. Filled circles have an n of one.

Figure 1.

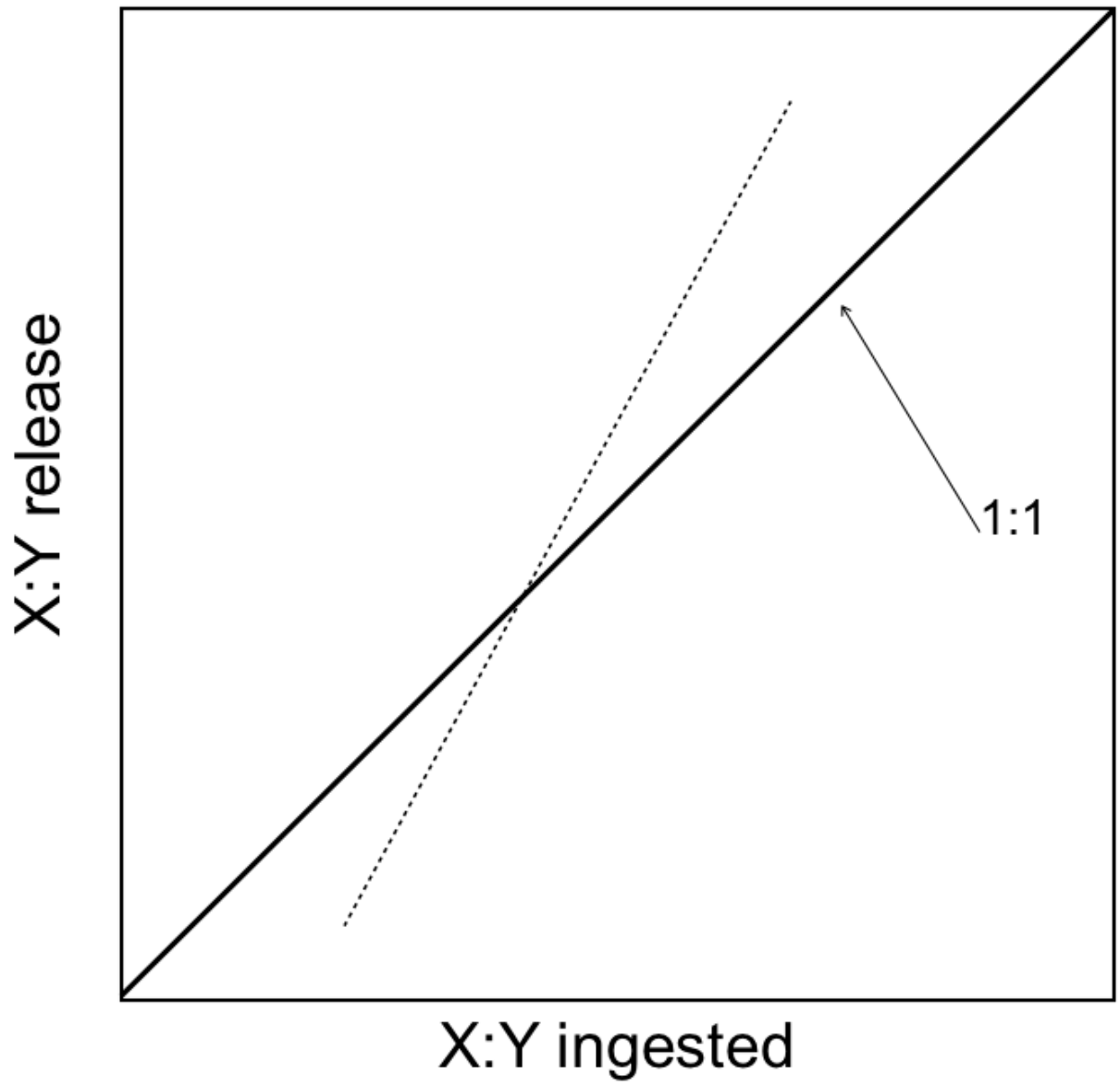


Figure 2.

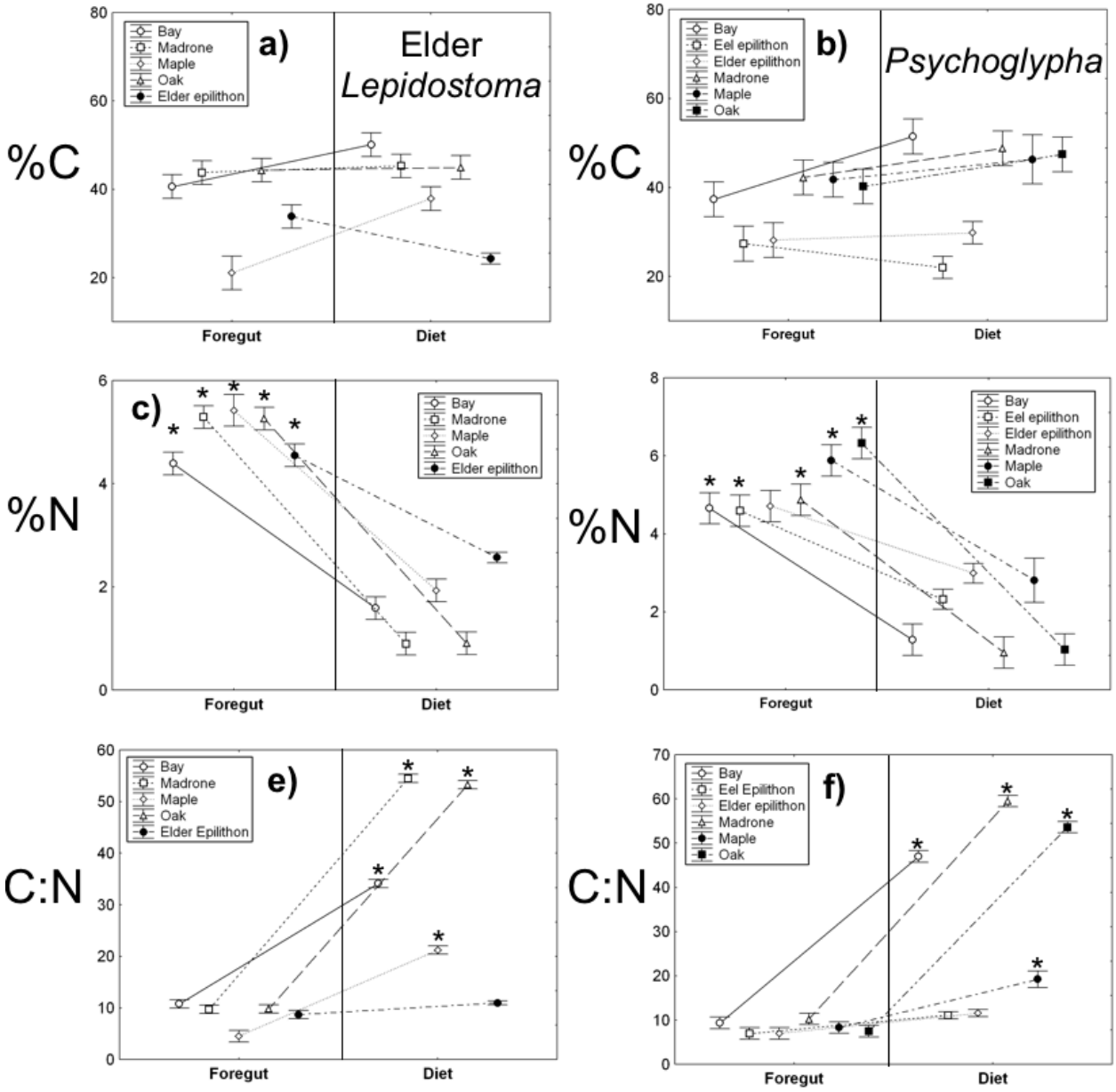

a)
Elder
Lepidostoma
b)
Psychoglylpha
%C
%N
C:N
c)
e)
f)
Bay
Madrone
Maple
Oak
Elder epilithon
Eel epilithon
Elder epilithon
Elder Epilithon
Eel Epilithon
Foregut
Diet

Figure 3.

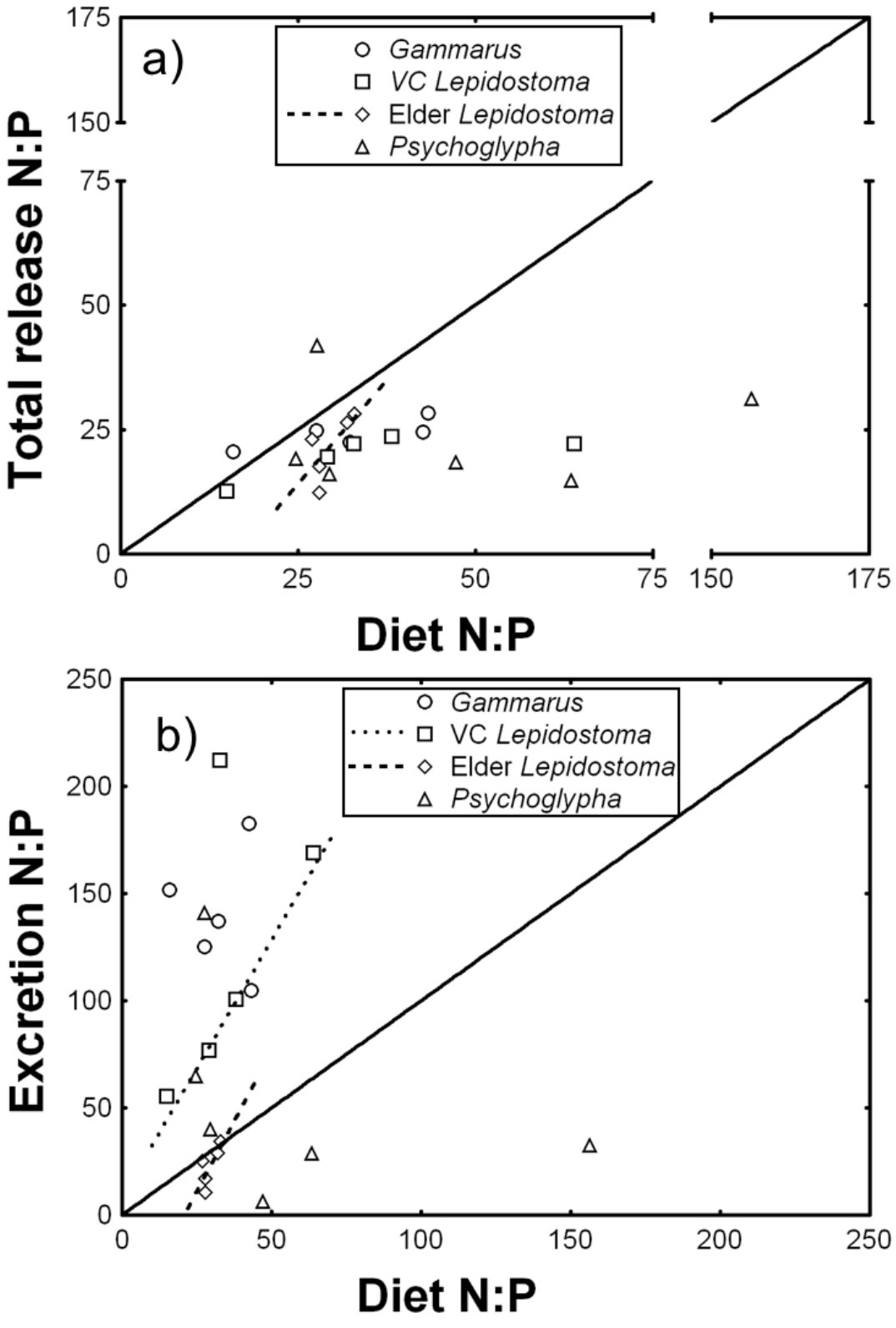

a)
Gammarus
VC Lepidostoma
Elder Lepidostoma
Psychoglypha
Total release N:P
Diet N:P
0
25
50
75
150
175
b)
Gammarus
VC Lepidostoma
Elder Lepidostoma
Psychoglypha
Excretion N:P
Diet N:P
0
50
100
150
200
250

Figure 4.

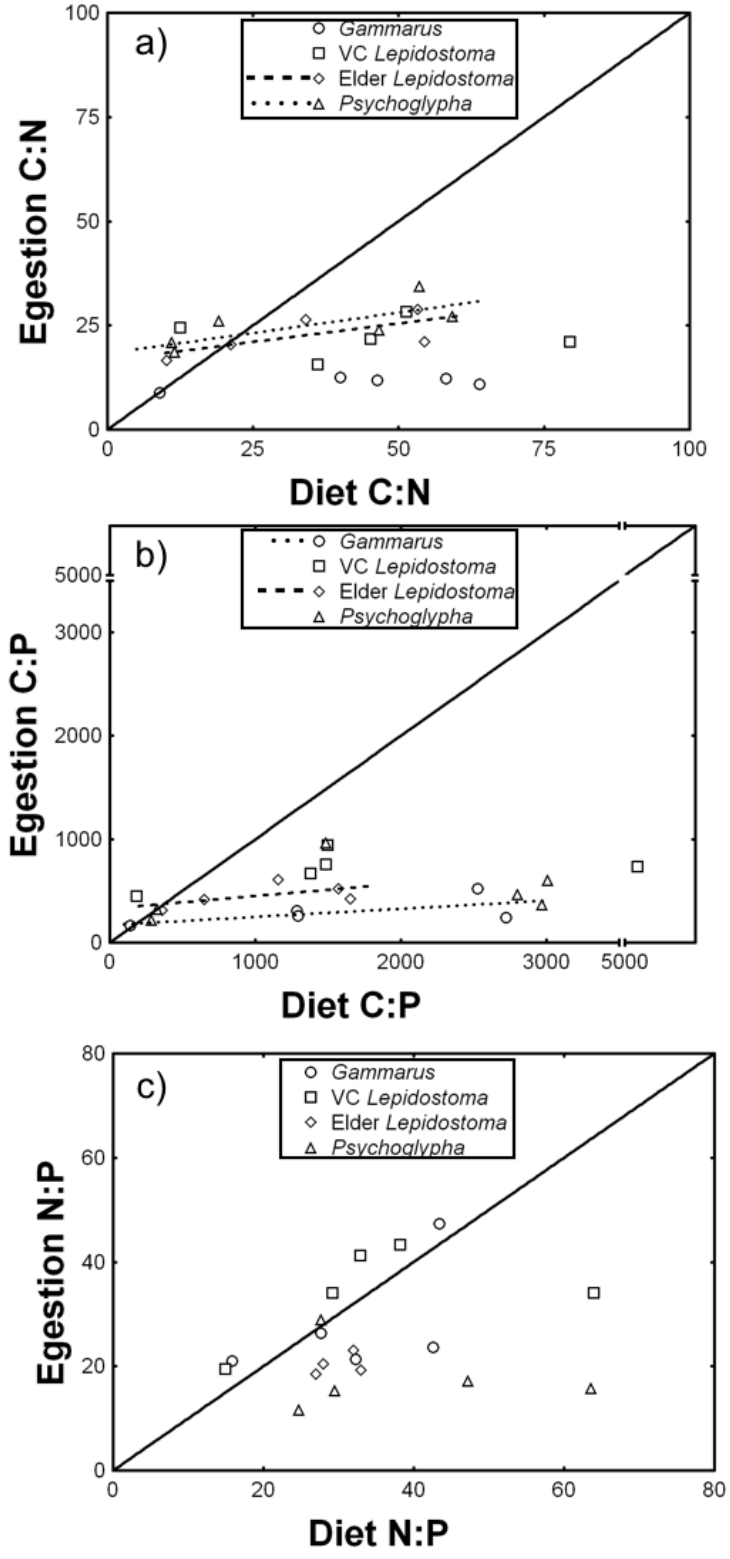
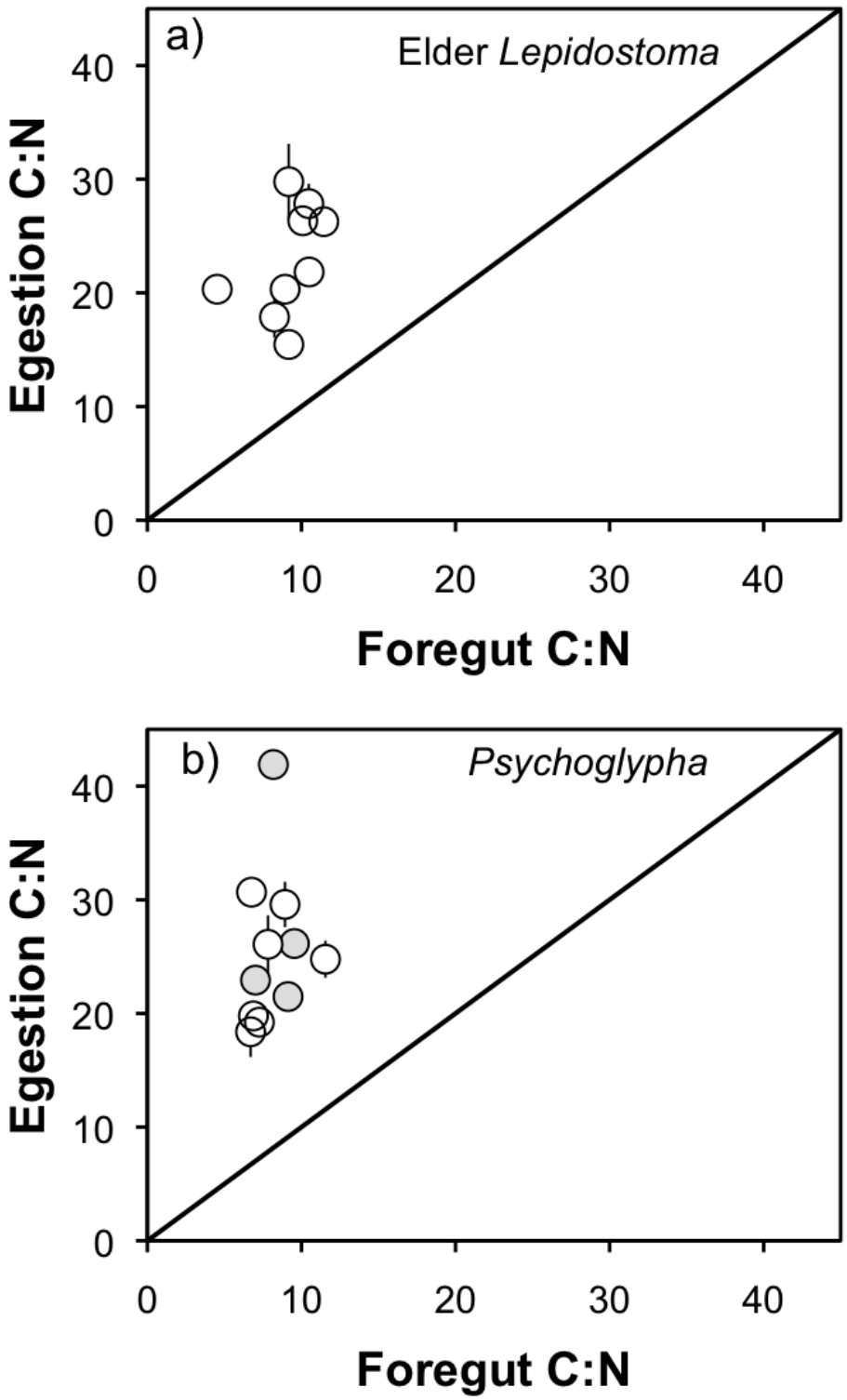

a)
Gammarus
VC Lepidostoma
Elder Lepidostoma
Psychoglypha
Egestion C:N
Diet C:N
0
25
50
75
100
b)
Egestion C:P
Diet C:P
0
1000
2000
3000
5000
c)
Egestion N:P
Diet N:P
0
20
40
60
80

Figure 5.


a)
Elder *Lepidostoma*
Egestion C:N
Foregut C:N
0
10
20
30
40
b)
*Psychoglypha*
Egestion C:N
Foregut C:N
0
10
20
30
40

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