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LETTER

Threshold elemental ratios of carbon and phosphorus in aquatic consumers

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Abstract

Inadequate supply of one or more mineral elements can slow the growth of animal consumers and alter their physiology, life history and behaviour. A key concept for understanding nutrient deficiency in animals is the threshold elemental ratio (TER), at which growth limitation switches from one element to another. We used a stoichiometric model that coupled animal bioenergetics and body elemental composition to estimate TER of carbon and phosphorus ($\mathrm{TER}_{C:P}$) for 41 aquatic consumer taxa. We found a wide range in $\mathrm{TER}_{C:P}$ (77–3086, ratio by atoms), which was generated by interspecific differences in body C : P ratios and gross growth efficiencies of C. $\mathrm{TER}_{C:P}$ also varied among aquatic invertebrates having different feeding strategies, such that detritivores had significantly higher threshold ratios than grazers and predators. The higher $\mathrm{TER}_{C:P}$ in detritivores resulted not only from lower gross growth efficiencies of carbon but also reflected lower body P content in these consumers. Supporting previous stoichiometric theory, we found $\mathrm{TER}_{C:P}$ to be negatively correlated with the maximum growth rate of invertebrate consumers. By coupling bioenergetics and stoichiometry, this analysis revealed strong linkages among the physiology, ecology and evolution of nutritional demands for animal growth.

Keywords

Bioenergetics, carbon efficiency, ecological stoichiometry, metabolism, phosphorus.

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INTRODUCTION

Animals require a mixture of energy, vitamins, biochemicals and minerals to grow and reproduce. Inadequate supply of one or more mineral elements slows the growth of animals (Urabe *et al.* 1997; Elser *et al.* 2000a; Pimentel-Rodrigues & Oliva-Teles 2001; Frost & Elser 2002) and alters their physiology, life history and behaviour (Sterner & Elser 2002; Frost *et al.* 2005). The dietary mixture where growth limitation switches from one element to another is known as the threshold elemental ratio (TER; Sterner & Hessen 1994; Sterner 1997). Quantitative estimates of TERs for individual animals can be provided by stoichiometric models that couple animal bioenergetics and body elemental composition (Sterner 1997; Frost & Elser 2002; Frost *et al.* 2004; Logan *et al.* 2004a,b; Anderson *et al.* 2005). TERs calculated in this manner thus provide a more meaningful index of the elemental imbalance between a consumer and its food resource (at given quantity) than simple arithmetic differences between body and food elemental composition. Despite this, there has been limited use (restricted to only a few taxa) of stoichiometric models to calculate the TER of aquatic consumers.

Much of the theoretical analysis of animal stoichiometry and TERs has been for a single genus of lake crustacean, *Daphnia* (Sterner 1997; Frost *et al.* 2004; Anderson *et al.* 2005). These studies and others (Logan *et al.* 2004a,b) predict animal TER to be a function of an animal's physiological attributes and of ambient food quantity. Food quantity is predicted to be important because, under low food supply rates, inadequate carbon (C) supply constrains an animal's potential to grow and thus reduces its P requirements to some minimal level (Sterner 1997; Frost & Elser 2002). To date no studies have considered how and why TER estimates vary among taxa (i.e. physiological, trophic and phylogenetic causes). At a proximate level, stoichiometric models predict that interspecific variation in animal TERs for C and phosphorus (P) would reflect both animal body C : P ratios and the proportion of ingested C used for growth (i.e. gross growth efficiency of C, $\mathrm{GGE_C}$). However, whether animal body C : P ratios and $\mathrm{GGE_C}$ covary and how they contribute to interspecific differences in $\mathrm{TER_{C:P}}$ among consumers has not been assessed. Moreover, little is known about whether interspecific variation in $\mathrm{TER_{C:P}}$ is related to broad differences in feeding strategy, phylogeny, or mass-specific growth rates (Sterner & Elser 2002).

Here, we couple stoichiometry with bioenergetics to estimate, for the first time, interspecific variation in $\mathrm{TER_{C:P}}$ and to assess the potential physiological, taxonomic, trophic and evolutionary determinants of this variation. We examined the relative importance of interspecific differences in body C : P ratios and bioenergetics on $\mathrm{TER_{C:P}}$ among aquatic consumers. We subsequently examined the effects of higher taxonomic groupings and feeding strategy on animal body C : P ratios and $\mathrm{GGE_C}$. Finally, we related $\mathrm{TER_{C:P}}$ to the maximum growth rate of invertebrate consumers. This analysis shows how the physiology, ecology and evolution of nutritional demands are interrelated in a diverse array of animal consumers from aquatic ecosystems.

METHODS

Derivation and parameterization of TER model

We used a stoichiometric model of animal growth to calculate the $\mathrm{TER_{C:P}}$ of 41 aquatic animal taxa from different habitats and of different higher taxonomic identity and maximum growth rates [Table S1 in Supplementary Material; for additional details of the model rationale and development see Sterner (1997), Frost & Elser (2002), Frost *et al.* (2004) and Logan *et al.* (2004a)]. While previous models calculated a threshold isocline that varied as a function of food quantity and C : P ratio (Sterner 1997), we restricted our model estimates of $\mathrm{TER_{C:P}}$ in consumers to a situation in which food was present above a saturating level. Under these conditions (i.e. food present in excess amounts), $\mathrm{TER_{C:P}}$ can be calculated as the product of physiological nutrient efficiencies and body elemental composition:

$$\mathrm{TER_{C:P}} = \frac{A_\mathrm{P}}{\frac{I_\mathrm{C}A_\mathrm{C} - R_\mathrm{C}}{I_\mathrm{C}}} \times \frac{Q_\mathrm{C}}{Q_\mathrm{P}} \tag{1}$$

where A_P and A_C are the assimilation efficiencies of P and C, respectively (dimensionless), I_C (mg C mg $\mathrm{C^{-1}}$ $\mathrm{day^{-1}}$) is the mass-specific ingestion rate above a saturating food level, R_C (mg C mg $\mathrm{C^{-1}}$ $\mathrm{day^{-1}}$) is the mass-specific respiration rate and Q_C (mg C mg $\mathrm{DM^{-1}}$) and Q_P (mg P mg $\mathrm{DM^{-1}}$) are the proportion of animal dry mass in C and P.

All parameters were obtained from literature sources for growing animals and carefully screened to ensure that they met the above criteria. Additional details of data extraction are presented in the Supplementary Material. We restricted this analysis to two elements (C and P), in part, for simplicity and also due to the important role that P plays in animal growth metabolism (Elser *et al.* 2000b; Sterner & Elser 2002). For some taxa, A_P was not available and in those instances a value of 0.8 was assumed. Given that many aquatic taxa are homeostatic (Sterner & Elser 2002), we additionally assumed that body C and P content was constant and equivalent to the elemental content of new growth (Frost & Elser 2002). In addition, our model partitioned C metabolism into either respiration or, when in excess of these demands, into growth. This partitioning was assumed to be constant and not to vary as a function of food quality (Nisbet *et al.* 2000; Kooijman 2000). Estimates of TER do not appear to be influenced by this assumption given their general consistency with measured TERs (Sterner & Elser 2002). High $\mathrm{TER_{C:P}}$ in this analysis indicates a low likelihood of P-limited growth and results from a high P assimilation efficiency, low P content in body tissues and/or inefficient C use (i.e. low $\mathrm{GGE_C}$). $\mathrm{GGE_C}$ was calculated for each taxon using the bioenergetics data that were collected from the literature. We calculated $\mathrm{GGE_C}$ as the percentage of ingested C that was assimilated into new growth:

$$\mathrm{GGE_C} = \frac{(I_\mathrm{C} \times A_\mathrm{C}) - R_\mathrm{C}}{I_C} \tag{2}$$

using the parameters as defined above.

Statistical analysis

The proportion of interspecific variation in $\mathrm{TER_{C:P}}$ associated with variation in body C : P ratios and $\mathrm{GGE_C}$ was assessed with multiple factor regression using SAS (SAS Institute 2001). This multiple factor regression was used only to determine the approximate partitioning of variance in $TER_{C:P}$ among the body C : P ratios and estimates of GGE_C (see Tables S2 and S3 in Supplementary Material for details of parameter statistics and covariance). We further assessed the correlations between these two components and maximal growth rate, phylogeny and feeding strategy. Differences in body C : P ratios, GGE_C and $TER_{C:P}$ among taxonomic groups and feeding strategies were assessed with one-way ANOVA. If the one-way ANOVA showed significant differences among taxonomic groups or feeding strategies, between-category differences were assessed using *post hoc* Tukey's HSD tests.

RESULTS AND DISCUSSION

We found a wide range (77–3086, all ratios by atom) of $TER_{C:P}$ calculated for the 41 aquatic animals included in this study (Fig. 1). The mean $TER_{C:P}$ for all animals was *c.* 2.4 times higher than the mean body C : P ratio (346 vs. 143, respectively). Multiple regression analysis showed that animal body C : P ratio (partial $r^2 = 0.71$) was *c.* 2.4 times more important than GGE_C (partial $r^2 = 0.29$) in explaining variation in $TER_{C:P}$ among this diverse group of aquatic animals. Consequently, the wide range of $TER_{C:P}$ among aquatic consumers calculated here was produced by inter-specific differences in both body C : P ratios and animal GGE_C.

As both body C : P ratios and GGE_C contributed to the documented variability of $TER_{C:P}$ estimates in aquatic organisms, we further asked what accounts for the large range in these two key variables. Variability in body C : P ratios may be partly related to animal phylogeny given that animal taxa differ considerably in their body construction, which affects their elemental composition (Sterner & Elser 2002). We found significant differences in body C : P ratios among animals sorted by higher taxonomic groupings (Fig. 2). Fish and tunicates were the most P-rich (lowest body C : P ratios), while crustaceans, mollusks and especially insects had higher body C : P ratios. The higher mean body C : P ratios in insects reflected, in part, the influence of three particularly P-poor species. No taxonomic differences were found in GGE_C among these five higher phylogenetic groups (Fig. 2). Consequently, intertaxonomic differences in $TER_{C:P}$ (Fig. 2) reflect significant deviations in body C : P ratios but not GGE_C among phylogenetic groups.

While the GGE_C was not related to higher-order phylogenetic groupings, it was significantly different among aquatic invertebrates when grouped by their primary nutritional strategy (Fig. 3). Consistent with other studies, detritivores were found to have significantly lower values of GGE_C than grazers and particularly predators (Cummins &

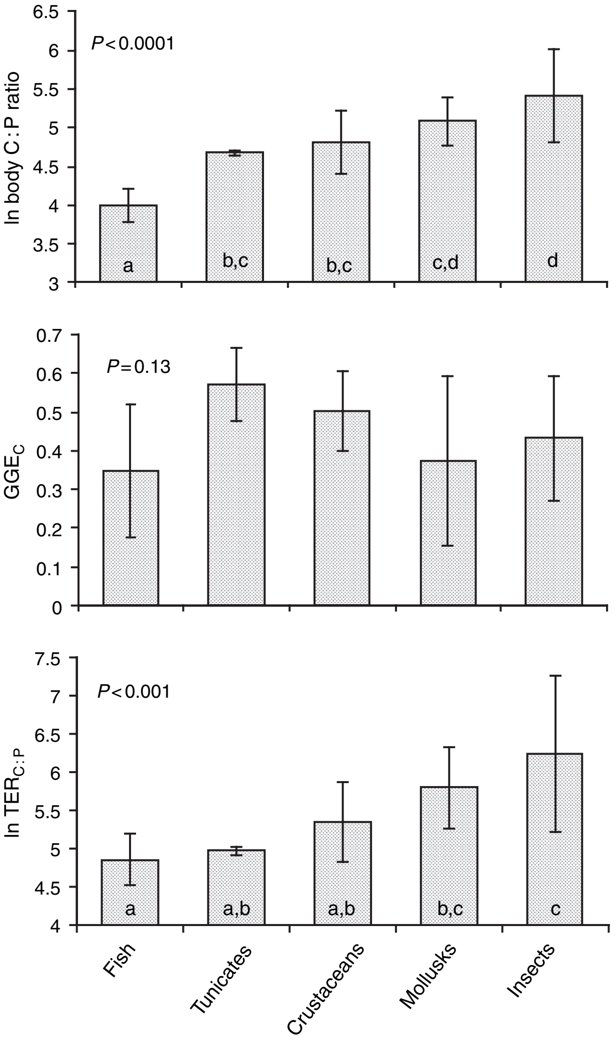

Figure 2 Body C : P ratios, gross growth efficiency of C (GGE_C) and threshold elemental ratio $(TER)_{C:P}$ (mean ± 1 SD) of five taxonomic groups of aquatic animals.

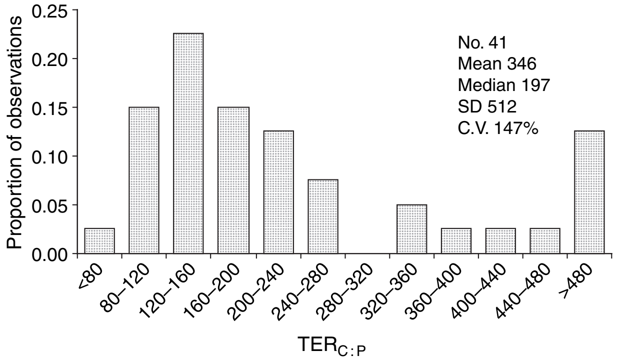

Figure 1 Frequency histogram of threshold elemental ratio $(TER)_{C:P}$ among 41 taxa of aquatic animals.

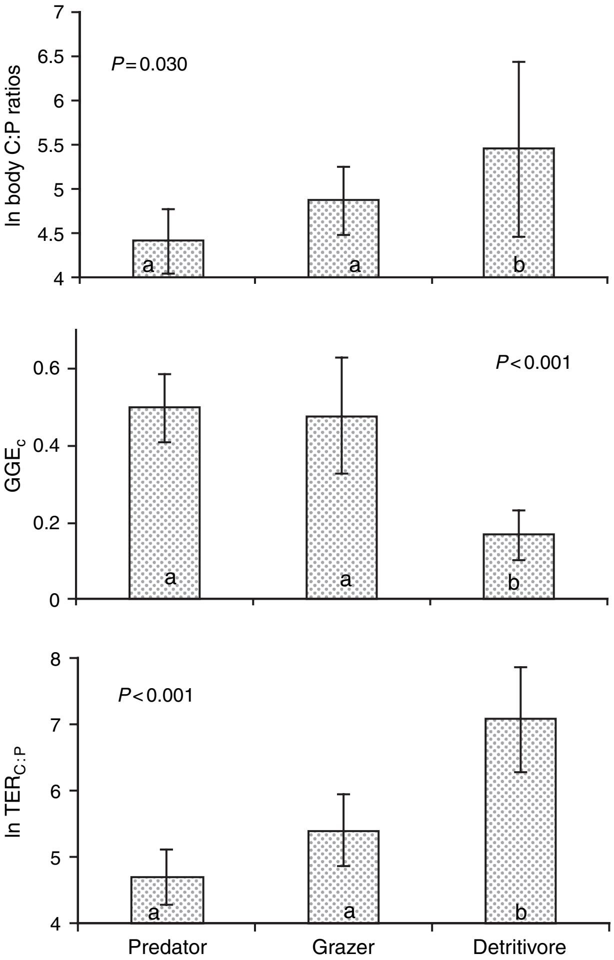

Figure 3 Body C : P ratios, gross growth efficiency of C (GGE_C) and threshold elemental ratio $(TER)_{C:P}$ (mean ± 1 SD) of aquatic invertebrates grouped by feeding strategy.

Klug 1979; Sterner & Hessen 1994; Sterner & Elser 2002), presumably due to lower C assimilation efficiencies and increased mass-specific respiratory rates in detritivores. We also found that detritivorous invertebrates had higher body C : P ratios than their grazing and predatory counterparts (Fig. 3). Consequently, detritivores are predicted to have relatively lower P requirements (i.e. higher $TER_{C:P}$) for growth metabolism compared with grazers and predators (Fig. 3).

At a proximate level, phylogeny and nutritional strategy account for significant interspecific variation in $TER_{C:P}$ among aquatic consumers. However, we should ask why the $TER_{C:P}$ ultimately varies among the taxa in this study. One key component of animal fitness that would presumably have strong effects on $TER_{C:P}$ is somatic growth rate. Indeed, recent study has shown strong links among animal ribosomal RNA content, body P content, and maximum growth rates (Elser *et al.* 2000b; Acharya *et al.* 2004). In support of these connections, we found a negative relationship between $TER_{C:P}$ and maximum growth rate of aquatic invertebrates (Fig. 4). Fast growth in organisms thus appears to be costly, in one sense, due to the greater likelihood of P-limited somatic growth (i.e. lower $TER_{C:P}$). The benefits of fast growth (e.g. more immediate or more frequent reproduction) must ultimately outweigh these stoichiometric costs. Relative to the invertebrates and regardless of their growth rate, fish were found to have relatively high P demands (Fig. 4), undoubtedly due to the P-rich composition of their bones (Sterner & Elser 2002; Vanni *et al.* 2002).

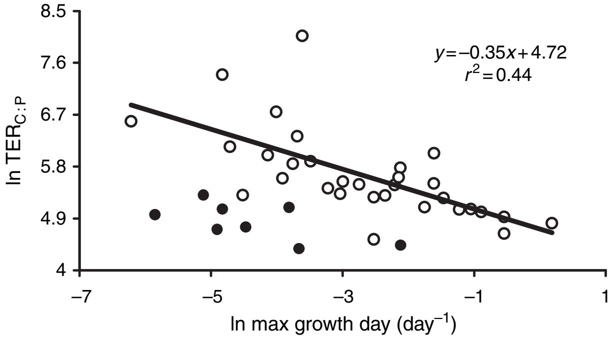

Figure 4 Relationship between threshold elemental ratio $(TER)_{C:P}$ and maximum growth rates among 41 taxa of aquatic animals. Open circles denote invertebrates and closed circles show fish. (a) Maximum growth rates were obtained from published studies of each species included in the database. (b) Fish have been excluded from the linear regression analysis given that their fundamentally different body construction (i.e. high P content of bony skeleton) displaces their $TER_{C:P}$–growth relationship relative to invertebrates.

A high $TER_{C:P}$, on the other hand, likely reflects physiological adjustments used by animals to process low P food. From an evolutionary perspective, this physiological flexibility may reflect adaptation to environments saturated with low P resources. For example, termites, a common terrestrial detritivore, employ various behavioural and physiological mechanisms to process and assimilate extremely C-rich wood resources (Higashi *et al.* 1992). In this study, aquatic invertebrate detritivores had relatively higher $TER_{C:P}$, which presumably allows them to exploit similarly nutrient-poor resources (e.g. leaf detritus; Cross *et al.* 2003). In both cases (i.e. aquatic detritivores and termites), the greater extraction of nutrients and diminished use of C by altered digestive and assimilative processes allows nearly exclusive access to a nutrient-poor food source, while also placing strong constraints on potential growth rates of these animals. Although this slow growth strategy presumably results in slower and/or less reproduction, it nonetheless appears to be a successful strategy for detritivorous organisms that feed on C-rich resources, for which competition would largely be absent from faster-growing animals adapted to higher quality food.

Fast-growing animals can also adjust their energetic metabolism and body elemental composition when eating P-deficient food to compensate for poor quality of this food (Frost *et al.* 2005). These adjustments include altering the intake (i.e. increased feeding rate) and/or reducing the demand (i.e. reduced body P content) for the limiting element (Frost & Elser 2002). The extent to which these physiological measures can compensate for reduced quantities of P in high C : P ratio food consumed by aquatic consumers is not yet clear. In the planktonic crustacean, *Daphnia*, low food P content results in modest reductions in animal body P content (DeMott *et al.* 1998), increased P assimilation efficiency (DeMott *et al.* 1998) and increased rates of respiration (Darchambeau *et al.* 2003). Despite these physiological changes, *Daphnia* growth and reproduction is strongly P-limited when food C : P ratios are elevated above the $\mathrm{TER}_{\mathrm{C:P}}$ (Urabe *et al.* 1997; DeMott *et al.* 1998; Boersma 2000; Elser *et al.* 2001). Short-term, physiological adjustments in this animal thus appear unable to compensate fully for the reduced supply of one element and to eliminate ensuing changes to animal growth, reproduction and mortality. Our results provide a quantitative assessment of which aquatic taxa (i.e. animals with comparatively low $\mathrm{TER}_{\mathrm{C:P}}$) would likely employ compensation mechanisms and/or experience the greatest negative effects on growth due to a low P content in their food.

Another proximate solution that animals could use to avoid P limitation of somatic growth is to select foods having a greater content of P. Predators appear to have adopted this strategy as their food sources (i.e. other animals) are generally relatively P-rich compared with plant-derived food sources (Sterner & Elser 2002). Consequently, despite the low $\mathrm{TER}_{\mathrm{C:P}}$ documented here, fish, many of which are predatory, may not often be limited by P in nature (Schindler & Eby 1997). This food choice strategy may come with a price: fish (and other predators) frequently face food shortages due to the rarity of their relatively nutrient-rich prey (Schindler & Eby 1997).

We used information on body elemental composition and energetics to show how the relative growth requirements for C and P relate to phylogeny and feeding strategy. This coupling of bioenergetic and stoichiometric approaches is an improvement over the alternative of using body C : P ratios alone to predict sensitivity of animal growth to P-deficient food. Our results show the extent that body C : P ratios can overestimate the elemental imbalance between consumers and their resources as the average $\mathrm{TER}_{\mathrm{C:P}}$ was 2.4 times higher than the average body C : P ratio. While recent study with *Daphnia* has shown a good correspondence between theoretical and empirical estimates of $\mathrm{TER}_{\mathrm{C:P}}$ (Sterner & Elser 2002), future efforts should focus on assessing the growth responses to food P content in a diverse assemblage of aquatic and terrestrial consumers with contrasting $\mathrm{TER}_{\mathrm{C:P}}$. Such analyses will provide an independent test of the ability of stoichiometric models to predict elemental limitation of animal growth.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available online from http://www.Blackwell-Synergy.com:

Table S1 Taxa included in C : P threshold analysis with their calculated $\mathrm{TER}_{C:P}$ and taxonomic group listed by increasing body size.

Table S2 Summary statistics [range, mean, standard deviation and coefficient of variation (CV)] of $\mathrm{TER}_{C:P}$, body C : P, GGE_C and A_P.

Table S3 Correlations among $\mathrm{TER}_{C:P}$, body C : P, GGE_C and A_P.

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