ORIGINAL ARTICLE

Katie Schneider · Adam D. Kay · William F. Fagan

Adaptation to a limiting environment: the phosphorus content of terrestrial cave arthropods

Abstract Stoichiometric imbalances (mismatches between elemental ratios of consumers and their food) are expected to be especially important in detritus-based systems because poor resource quality may impose severe growth constraints. Such imbalances have been highlighted in producer-based food webs and detritus-based aquatic systems, but similar investigations of detritus-based terrestrial ecosystems are absent. Cave animals are dependent on detrital subsidies from the surface and display adaptations to caves (e.g., decreased growth and metabolic rates). Here we examined how nutrient quality may constrain consumer strategies in caves. We found that the quality of cave resources was comparable to resources on the surface, but there was some evidence that cave animals may face nutritional constraints for at least a part of the year. Such constraints may be especially important for millipedes, whose C:P was particularly low (i.e., nutrient-demanding) relative to cave detritus. Based on the growth rate hypothesis, we predicted that cave-adapted species would have lower %P, lower %RNA, and a lower RNA/DNA ratio relative to surface-dwelling counterparts; however, the differences we discovered between congeneric millipedes may not necessarily be due to P scarcity. Consistent with stoichiometric theory, we found significant negative %P allometry across phylogenetic groupings of 17 cave arthropods. We did not see this allometric relationship in millipedes, perhaps because of the lowered P content of the cave-obligate species. Our results highlight the potential for stoichiometric challenges of caves to influence the adaptations of terrestrial cave animals. This novel explanation for cave adaptation may yield insights into cave biodiversity and biogeography.

Keywords Detritus · Ecological stoichiometry · Millipedes · Nutritional constraints · RNA/DNA

K. Schneider (✉)
Graduate Program in Behavior, Ecology, Evolution,
and Systematics, University of Maryland,
College Park, MD 20742, USA
E-mail: katie2@umd.edu

A. D. Kay
Department of Biology, University of St. Thomas,
St. Paul, MN 55105, USA

W. F. Fagan
Department of Biology, University of Maryland,
College Park, MD 20742, USA

Introduction

The impact of nutrition on consumer success often hinges on imbalances between the supply and demand of nutrients (Urabe and Watanabe 1992; Elser and Urabe 1999; Frost et al. 2005; Schade et al. 2005). When supply of a nutrient decreases, an organism must either increase intake of that nutrient or minimize nonessential usage to maintain nutritional homeostasis. Mobile consumers facing nutrient shortages can increase intake through dispersal or migration (Denno et al. 1980, 2002; Lee et al. 2004; Moe et al. 2005; Huberty and Denno 2006; McGlynn et al. 2007). In addition, consumers faced with short-term resource shortages may compensate by increasing feeding rates (Simpson and Simpson 1990; Slansky 1993; Fink and Von Elert 1996; Huberty and Denno 2006) or by supplementing their diet through exudate-feeding (Mira 2000; Cook and Davidson 2006) or cannibalism (Denno and Fagan 2003). Alternatively, chronic resource constraints may select for modified life history strategies that are compatible with reduced resource availability. Indeed, several authors have hypothesized that scarcity of a specific nutrient [such as nitrogen (N) or phosphorus (P)] in available food can favor physiological, morphological, and behavioral adaptations that reduce consumer demand for that nutrient (Elser et al. 2000b; Cross et al. 2003; Denno and Fagan 2003; Kay et al. 2005; Elser 2006). This hypothesis predicts that the magnitude of the stoichiometric imbalance between consumer demand and environmental availability influences nutrient acquisition costs

associated with growth (Elser et al. 1996; Urabe et al. 1997; Sterner and Elser 2002), reproduction (Faerovig and Hessen 2003), and development (Villar-Argaiz and Sterner 2002).

Stoichiometric imbalances are expected to be especially important in detritus-based systems (Moe et al. 2005), where nutrient limitation may impose severe constraints on the species that reside there (Cross et al. 2003; Tibbets and Molles 2005), and thus may favor consumers with low nutrient-demands (Schulz and Sterner 1999; Elser et al. 2000b; Martinson et al. 2008; Hambäck et al. 2009). Caves exemplify nutrient-poor, detritus-based terrestrial ecosystems, and offer an ecosystem in which to test this thesis. In the absence of photosynthetic primary production underground, almost all caves are detritus-based systems that are supported entirely by food resources that passively fall, wash or are blown in, or by resources that are actively deposited via animal vectors (cave crickets, bats, wayward animals; Barr 1967; Culver 1982; Poulson and Lavoie 2000; Fagan et al. 2007). Although some of these resources are nutrient-rich (e.g., animal carcasses, eggs, or feces), most of the food resources that regularly enter cave environments is nutrient-poor leaf and wood debris. These nutrient-poor plant materials are colonized by bacteria and fungus. Detritivorous arthropods, such as millipedes, some mites and collembola, either feed directly on this leaf material or on the microbial/fungal colonists. Predatory arthropods, such as spiders, pseudoscorpions and beetles, feed on the detritivores (Barr 1967). Terrestrial species that are found in caves can be divided into four categories (Barr 1967): *troglobionts* (organisms restricted to caves), *troglophiles* (organisms that are found in caves but can also be found in moist, dark habitats on the surface), *trogloxenes* (organisms that use caves for shelter but routinely exit) and *accidentals*. Of these, troglobionts, which spend their entire-life cycles in caves, are highly adapted to subterranean life. To maximize clarity, we use this specialized terminology throughout the paper. Previous cave researchers have hypothesized that these cave adaptations are a result of low energy (the energy economy hypothesis: Poulson 1963; Culver 1982; Hüppop 2005). However, if materials at the base of the cave food web are particularly low in nutrients, the life-history strategies of cave animals may be constrained more by nutrient availability than by energy scarcity.

Nutrient constraints on growth rate may be particularly important because an increase in P-rich ribosomal RNA is needed to meet the protein synthesis demands of rapid growth (Elser et al. 1996; Sterner and Elser 2002). Cave animals have slow metabolic and reproductive rates, and long ontogenetic development and life spans (Barr 1968; Mitchell 1969; Poulson and White 1969; Culver 1982). For example, estimates of growth rates of cave crickets are a magnitude slower than surface insects (Studier 1996), and, among spiders, those species that are adapted to caves have the slowest metabolic rates (Kunter et al. 1999). Explanations for these physiological adaptations include (but are not limited to) the ecological stability of caves, lack of predators, and food scarcity (Kunter et al. 1999; Hüppop 2005). Although growth rates of cave animals are known to be low relative to surface counterparts (Barr 1968; Mitchell 1969; Poulson and White 1969), no previous study has investigated whether the unique stoichiometric challenges of cave environments may contribute to this pattern.

In this paper we draw several links between the availability of a key nutrient, P, and previously reported adaptations of terrestrial cave invertebrates. Though we focus on P, it is also possible that protein synthesis may be limited by other nutrients, such as N, whereby it is not transcription, but translation that is inhibited (Hessen et al. 2007). Indeed, previous research showed low concentrations of N and potassium in cave orthopterans and their egg-predator, an obligate cave carabid (Studier 1996). However, to our knowledge, no research on the P content of cave arthropods has yet been reported. We also examine the RNA content, DNA content, and RNA/DNA ratio of cave animals. The RNA/DNA ratio, an index for protein synthetic capacity, measures the concentration of protein-making machinery per cell (Buckley 1984) and is a known correlate of growth rate (Buckley 1984; Vrede et al. 2002, and references therein; Kyle et al. 2003; Makino et al. 2003; Weider et al. 2005). High food quality leads to an increased RNA/DNA ratio (Vrede et al. 2002), and generally reflects elevated protein production in response to beneficial conditions (Buckley and Szmant 2004).

In general, our expectation was that variation in resource quality and interspecific stoichiometric condition would covary with previously established variation in above- versus below-ground life histories. Here, we analyze cave resources and invertebrates to test the predictions that: (1) resources found in caves are low quality (low P and high C: P ratio) compared to surface resources; (2) arthropods found in caves, especially those that are cave-obligates (troglobionts), will have low nutrient demands (low body %P) compared to related species that are not restricted to caves (troglophiles); (3) predatory species will have similar %P to the primary consumers (detritivores), as seen in other systems (Martinson et al. 2008); (4) imbalances between resource C:P and consumer C:P will reflect those seen in other detritus-based systems (Cross et al. 2003); (5) previously established allometric patterns, wherein %P content decreases with body size (Woods et al. 2004; Hambäck et al. 2009), will also be seen for cave species; and (6) troglobiotic cave animals will have less P, less RNA, and decreased RNA/DNA ratios than closely related troglophiles (not restricted to caves), reflecting the slower growth rates of cave animals (Mitchell 1969; Hüppop 2005). Investigating the stoichiometry of cave resources and the animals that inhabit these nutrient-poor environments will test these predictions of ecological stoichiometry in a novel system and may help to explain some of the well-known physiological adaptations of these unique species.

Methods

Study site

The study site was a cave-rich region located within a 20 km² area just north of Lewisburg, WV, within the Buckeye Creek Drainage System (USGS HUC 05050003). Pits (vertical caves) chosen for the intensive analysis of resource quality were all located on private land interspersed in a karstic area (a limestone area characterized by dissolution rather than erosion) typical for West Virginia. Dominant trees in this area include elm (*Ulmus* sp.), hickory (*Carya* sp.), oak (*Quercus* sp.), and maple (*Acer* sp.), the leaves of which constitute the major source of detritus into these caves.

Collection methods and sample preparation

We first compared the stoichiometric quality of surface leaves to the quality of resources removed from 12 caves in West Virginia. To provide baseline measures of in-cave resource quality, all macroscopic organic material and the top 6 cm soil were removed from 12 pits. Vertical caves (commonly called "pits") were chosen, as opposed to caves with horizontal entrances, because the resources that fall into pits can be quantified easily and are localized primarily within the drop zone (the area directly below the opening to the surface). The chosen pits ranged in depth from 4.5 m to 19 m. Organic material (leaves, dead animals, fungi, fecal material, and organic-rich soil) was removed from each pit using garbage bags and a pulley system in July 2005. A total of 1.5 metric tons of material (wet-weight) was removed to create a detritus-free baseline condition for a related project that will be reported elsewhere.

Representative subsets from each cave were lightly rinsed over a 250 μm sieve to separate dirt and other inert materials from organic material. This rinsing may have disrupted any bacterial films coating the decomposing organic materials and potentially removed bacteria, arthropod fecal material, and other nutrient-rich components. Additional representative subsets, which were not rinsed and thus still contained soil and other particles, were also assembled. Though these samples retained all the nutrient-rich components potentially affected by rinsing, carbon could not be reliably quantified in these samples because of an excess of inert, inorganic material. However, taken together, the two subsets from each cave allowed us to calculate both carbon and phosphorus of cave detrital resources, respectively. Both subsets were dried at 60°C for a minimum of 5 days, ground to a fine powder using a coffee grinder and a mortar and pestle, and prepared for chemical analysis.

To assess how the detrital resources found in caves differ from those available on the surface, detritus was collected monthly for 1 year via flower pots (dimensions: height 20 cm, top circumference 0.04 m) embedded at the surface next to the entrance to each pit. Surface detritus consisted of leaves shed in autumn as well as year-round materials or organisms that fell, blew or crawled into the flower pots. We emptied these flower pots monthly because we expected seasonal differences in the quality of surface detrital resources. The contents were prepared for chemical analysis as above.

To explore the sources of variation in body % P among cave invertebrate species, we hand-collected representatives of 17 morphospecies from within Buckeye Creek Cave. We supplemented these collections with additional arthropods collected over 24-h periods in empty pitfall traps smeared with Limburger cheese, which is the standard protocol for baiting cave arthropods (Schneider and Culver 2004). The collection consisted of troglobiotic and troglophilic cave hexapods (Collembola, Coleoptera, Orthoptera), diplopods (millipedes), and arachnids (mites, spiders, pseudoscorpions). Collections were sorted to major groupings and included a representative subset of the core terrestrial cave community. Two pairs of species (millipedes and rhagidiid mites) contained both a troglobiotic and a troglophilic member. Cave-obligate species (troglobionts) exhibit characteristic adaptations to cave life (e.g., absence of pigmentation, elongated appendages, loss of vision) whereas troglophiles are essentially surface-dwelling species that are also found in caves. For each morphospecies in these pairs, at least two individuals were collected; due to the rarity and conservation status of cave organisms, more individuals could not be collected. Specimens were then stored in a refrigerator for 1 day to clear their digestive systems and subsequently frozen until preparation for chemical analysis. We designated each morphospecies as either predatory or detritivorous based on the classifications typical for that order/family and literature on cave animals.

To examine further the long-term impacts of prolonged exposure to nutrient constraints on cave species, we compared the C and P content of paired samples of troglobionts and their closest available surface-dwelling relatives. First, we examined *Pseudanophthalmus* beetles (Coleoptera: Carabidae), a clade of 157 predaceous species and subspecies wholly restricted to caves (Christman and Culver 2001). Here, we focus on two species, one of which (*P. fuscus* Valentine 1931) is smaller than the other (*P. grandis* Valentine 1931; range of size of *P. fuscus* = 4.4–5.6 mm vs *P. grandis* = 4.9–6.8 mm; Valentine 1932). While the majority of arthropods for this study were collected from the largest cave in the area (Buckeye Creek Cave), the beetles were collected from four neighboring caves within 1.2 km of the entrance to Buckeye. To compare this exclusively cave-dwelling genus to surface dwelling relatives, we searched the literature and recovered previously published P levels for carabid beetles (data from Woods et al. 2004).

The next species pair we examined included two detritivorous millipedes, *Pseudotremia hobbsi* Hoffman 1950 and *Pseudotremia fulgida* Loomis 1943 (Chordeumatida: Cleidogonidae). While *P. hobbsi* can be found in

caves, it is not a cave-obligate species, and does not show the morphological adaptations typical of cave-obligate species. *Pseudotremia fulgida*, on the other hand, is a blind, depigmented, troglobiont. These two millipedes co-occur in many caves, and representatives of both species, (including subadult individuals) were hand-collected from Buckeye Creek Cave. Subadult individuals were not identifiable to the species level because identification is based on mature male gonopods (Shear 1969), but were known to be either of the two *Pseudotremia* species of interest. Specimens were stored in a refrigerator for 1 day and subsequently frozen.

To investigate the potential molecular mechanism underpinning the differences observed in P content, we measured the RNA content, DNA content, and the RNA/DNA ratio of the millipedes. Because P is differentially allocated to RNA to meet the demands of protein synthesis (Sterner and Elser 2002), and cave animals typically show reduced growth rates, we predicted that troglobiotic millipedes would have less P and lower RNA/DNA ratios than their surface counterparts. This is a key prediction of the molecular mechanisms underlying the growth rate hypothesis of ecological stoichiometry (see Kay et al. 2005 and references therein). Animals that were set aside for these analyses were collected in the field and brought home alive in a cooler prior to storage in a −80°C freezer.

Chemical analyses

Analysis of C content was performed on dried samples of detritus (surface and cave) and prepared animal specimens using a LECO CHN analyzer. For analysis of P content, animal specimens (at least two individuals per morphospecies) were removed from the freezer and dried at 60°C for 3 days. Animals smaller than 2 mg were assayed whole, whereas animals greater than 2 mg were homogenized into a fine powder, subsamples of which (0.5–2 mg) were then analyzed via colorimetric analysis after persulfate digestion using the ascorbate-molybdate method (APHA 1992; Woods et al. 2004). Percent recovery in P and CN assays was determined by comparison to either apple leaves or bovine muscle standards.

DNA and RNA concentrations were measured in whole organisms stored in a −80°C freezer until analysis. DNA and RNA were measured using the assay described in Kyle et al. (2003); this involves sample homogenization (with mortar and pestle) in an extraction buffer containing *N*-lauroylsarcosine, followed by sonication, and then staining with Ribogreen (Molecular Probes, Eugene, OR). DNA and RNA content was estimated from comparisons of fluorescence in replicate subsamples that were treated with RNase, RNase and DNase, or were left untreated. DNA and RNA estimates per wet mass were quantified from comparisons to fluorescence in standards; standards were baker's yeast RNA and calf thymus DNA (Sigma-Aldrich, St. Louis, MO). DNA and RNA estimates per wet mass were converted to estimates per dry mass using the parameters of the relationship between wet mass and dry mass [previously determined using separate *P. hobbsi* ($n = 11$) and *P. fulgida* ($n = 8$) individuals].

Data analyses

Surface resource quality was obtained by calculating the average %P per month using the flower pot samples. We chose to examine averages over time to account for temporal variation in input rates (e.g., leaf fall) and litter quality, and to assess the seasonality of the resources that are most likely to fall into a cave. Because chemical analyses for C and P were each independently replicated on different resource subsets from a given cave, we calculated the average C content and average P content for each pit and used these values to calculate the average molar C:P ratio for each pit. We then compared the average C:P across all pits to the quality of the surface detritus (as reflected by monthly molar C:P). After log transforming the C:P values and removing one outlier (from $n = 135$ samples) to account for non-normal data, we performed a *t*-test with unequal variances to test if cave resources and surface litter differed in average C:P ratio. We also performed a Wilcoxon rank-sum test on the means between the cave samples and the July surface samples to investigate if cave resources differed from surface resources during the same season as when the cave resources were removed.

Designating the cave animals as either predators or detritivores, we then tested whether trophic and phylogenetic constraints could explain variation in the stoichiometry of cave arthropods. Based on the findings of Woods et al. (2004), Hambäck et al. (2009) and Martinson et al. (2008), we predicted that there would be no distinguishable difference in P between predators and detritivores. This prediction follows from Woods et al. (2004) who suggested that while herbivores eat lower quality food, they eat more of it, whereas predators eat higher quality food, but consume smaller quantities. To examine the validity of this prediction, we first averaged the body mass and %P concentrations for all individuals within a morphospecies (excluding subadult millipedes unidentifiable to species). We included body mass in the model because of the previously observed pattern of negative P allometry (Woods et al. 2004; Hambäck et al. 2009). This pattern, which was based upon the theory that small, fast-growing organisms should have high P content (Elser et al. 1996) builds on the observed negative relationship of mass-specific growth rates across groups (Woods et al. 2004). After log-transforming average P values and dry mass, we performed an ANCOVA, with the model: log (body P) ∼ trophic level × log(body mass), with each species as an observation. To account for variation driven by phylogeny, we compared log-transformed P content across the major groups (Diplopoda, Hexapoda, and Arachnida) with an ANCOVA model also including log(body mass) as a covariate. We tested

for paired differences between groups using the same linear model with planned contrasts.

Using average %C and %P, we calculated the degree to which the (molar) C:P of resources differed from the C:P of the consumers by looking at the ratio of these two numbers (Fagan and Denno 2004). This ratio provides one measure of how mismatched the consumer is from its resources (i.e., the stoichiometric constraint faced by the consumer). We examined the ratio between cave resources and one type of cave detritivore (the troglobiotic millipede) as well as the ratio between one type of cave predator (the troglobiotic beetle) and a potential prey species (either the troglobiotic millipede or a collembola). Because we did not explicitly measure carbon content of the collembola, we used data from the literature to acquire the average carbon content of three species of entomobryid collembolans (=47.5 %C, data from Teuben and Verhoef 1992), and used this to generate an approximate C:P of the collembola (incorporating our quantification of P; see Elser et al. 2000a for similar methods). We assume that these potential prey species are representative of the types of prey that the beetles may consume. We compare these mismatches in caves to published values provided in Table 1 of Cross et al. (2003). Though the authors in that paper used the arithmetic difference between ratios as "elemental imbalance", we calculated the ratio of their C:P values for a more direct comparison with our results.

Because troglobionts are completely dependent on allochthonous detritus (detritus that originated on the surface), we predicted that they would have lower body P content than surface-dwelling animals. Within each of the two groups of species (beetles, millipedes), we used linear models to test whether %P differed across species based on habitat. We constructed a model of body P content with the categorical predictor species, the continuous variable size, and their interaction. If the interaction term was not significant, that covariate was removed from the analysis. Both models required the exclusion of one outlier to correct for non-normal residuals. The same model ($y \sim$ species $\times$ size) was also used to test if the %RNA and the RNA/DNA concentration (log transformed) differed between congeneric troglobionts and troglophilic millipedes. To account for non-normal residuals as determined by the normal-quantile plot and the residual-fit spread plot, the model for the RNA/DNA ratio necessitated the removal of three outliers.

All analyses were performed in R (version 2.7.0; R Development Core Team 2008).

Results

Characterizing the elemental stoichiometry of cave resources

Across the 12 caves, the resources removed varied greatly in %P, ranging from 0.05% to 0.96%, with an average of $0.22 \pm 0.05\%$. The resources on the surface varied seasonally, ranging from 0.06 ± 0.01 %P in November/December to 0.12 ± 0.01 %P in April. The C:P of the cave resources was significantly different from the annual surface litter (t-test with unequal variances $t = -2.36$, $df = 11.74$, $P = 0.036$), with the cave samples having a lower average C:P than the surface samples. The average C:P of the cave resources was higher (of lower quality) than the surface litter during the same time of year as when the caves were originally "emptied", although this difference was not significant (Cave resource C:P = 913.1 vs July surface detritus C:P 698.7, $W = 40$, $P = 0.3451$). Surface leaves collected in the fall (October and November/December) were of the lowest quality, with high C:P ratios (average C:P 2,598 and 2,697, respectively) compared to the other months (Fig. 1).

Phosphorus content of terrestrial cave invertebrates

Average values for the %P in arthropods found in Buckeye Creek Cave ranged from 0.71 %P (oribatid mites) to 3.11 %P (immature millipedes). Overall, the millipedes and collembola were the groups highest in P (Fig. 2). The species that we identified as cave-obligate (denoted with asterisks in Fig. 2) included the two carabids (*P. grandis* and *P. fuscus*), the troglobiotic millipede (*P. fulgida*), and a troglobiotic rhagidiid mite. The troglobiotic rhagidiid mite and chordeumatid millipede both contained less P than their respective surface-dwelling counterparts (Fig. 2).

As predicted, we found no significant difference in body %P between detritivores (including the millipedes, collembola, oribatid mites, and crickets) and predators (rhagidiid mites, carabid beetles, spiders, and pseudoscorpions; $F_{1,14} = 0.938$, $P = 0.394$, Fig. 2, inset). When species were classified into broad phylogenetic groupings, the interaction of body size and phylogenetic group was not significant ($F_{2,11} = 0.001$, $P = 0.990$) and was removed from the model. However, P content did differ with phylogenetic grouping ($F_{2,13} = 13.10$, $P < 0.001$, Fig. 3a) and body size (log transformed; $F_{1,13} = 21.42$, $P < 0.001$). All phylogenetic groups differed significantly from each other (arachnids vs diplopods: $F_{1,6} = 16.10$, $P = 0.007$; arachnids vs hexapods: $F_{1,11} = 4.95$, $P = 0.048$; diplopods vs hexapods: $F_{1,8} = 96.17$, $P < 0.001$). Interestingly, the slope of the allometric relationship between log body %P and log body size was nearly identical for all three groupings (slope estimates: arachnids = -0.151, diplopods = -0.145, hexapods = -0.156, Fig. 3b).

Calculating the mismatch between cave resources and cave species

The C:P mismatch between cave detritus and troglobiotic millipedes was nearly 50–75% more than the mismatch between that of stream detritus and shredders or between terrestrial plants and herbivores reported elsewhere
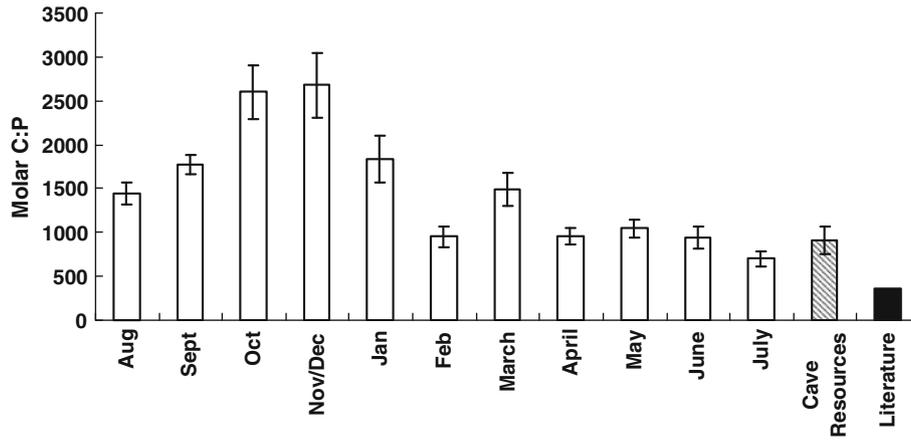
Fig. 1 The quality [average molar carbon:phosphorus (C:P) ratio] of organic material removed from 12 caves (*hatched bar*) compared to the quality of the litter collected monthly on the surface. Also included for comparison is previously published data (*black bar*) for the quality of the fermentation layer of the soil (see text)
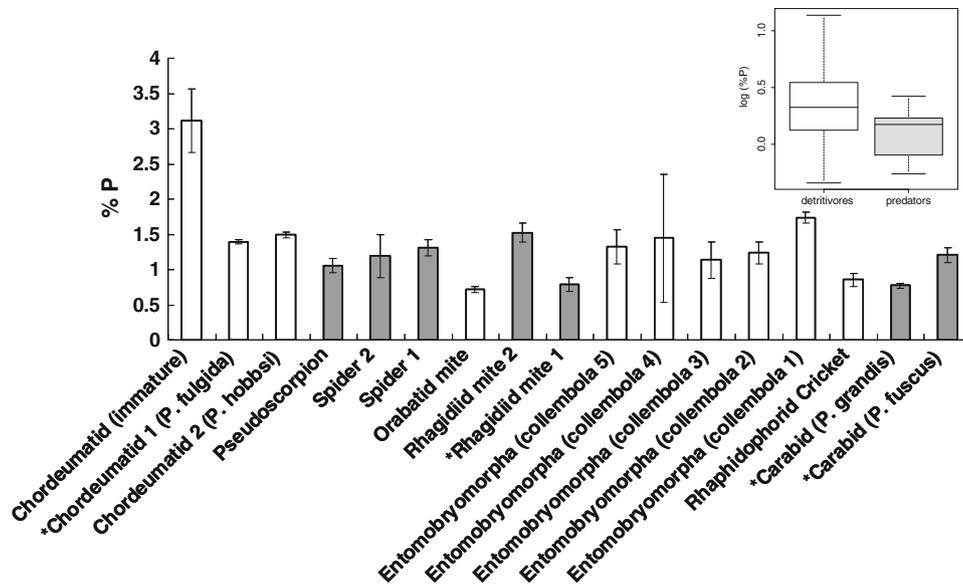

Fig. 2 A community-wide comparison of the P content of 17 arthropods collected from Buckeye Creek Cave. Predatory species are shaded in *gray*. *Asterisks* denote species that are restricted to caves (troglobionts). *Inset* Average %P for detritivores compared to predators

(Table 1). In fact, the imbalance between cave detritus and the detritivorous millipede, which is driven primarily by the very low C:P of the millipede, was higher than any other stream resource/consumer or the terrestrial herbivore/vegetation comparison reported in Cross et al. (2003). Cave predators, on the other hand, match closely with their food source, considering either millipedes or collembola as potential prey items. This mismatch between beetles and detritivores is negligible in comparison to the mismatch between the detritivores and detritus (Table 1).

Phosphorus content of cave carabids and millipedes

The average P content of the surface carabids compiled from the literature was 0.617 ± 0.07% ($n = 5$ species),

which was lower than that of the troglobiotic carabids (0.815 ± 0.04 %P; $n = 18$ individuals). This difference in %P between habitats was significant, with a significant interaction between species and size ($F_{1,19} = 16.35$, $P < 0.001$). There was a marginal negative allometry of body %P for the large troglobiotic beetle species ($F_{1,15} = 4.104$, $r^2 = 0.2148$, $P = 0.061$), but no relationship could be observed for the smaller troglobiotic beetle species (Fig. 4a). An allometric relationship with %P was observed for surface carabids taken from Woods et al. (2004) when one outlier was removed ($F_{1,2} = 27.10$, $P = 0.035$).

The average P content for the troglophilic millipede was 1.50 ± 0.04% ($n = 19$ individuals), which was slightly higher than that of the troglobiotic millipede (1.39 ± 0.03 %P; $n = 27$). There was no effect of size
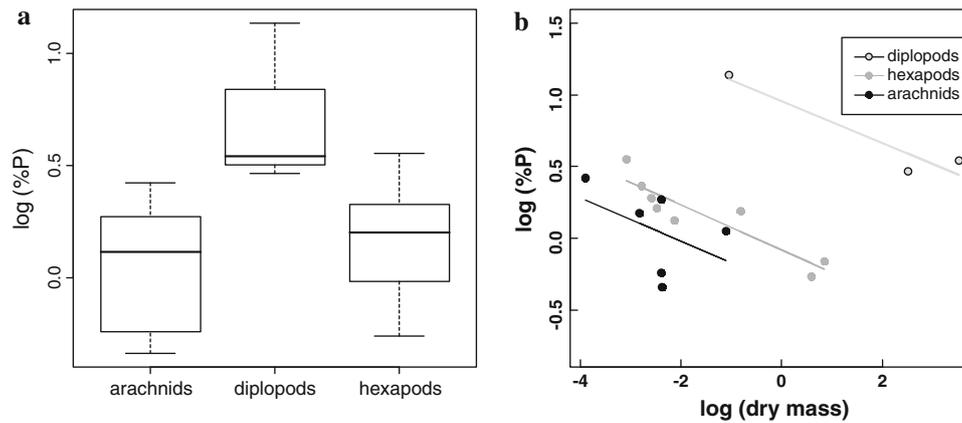
Fig. 3 The P content (log transformed) of **a** three major phyloge-netic groups (arachnids, diplopods, hexapods), all of which are significantly different from each other, and **b** these three major groups plotted against average log (dry mass). Each *point* represents a species in that grouping, and the *lines* represent the regression of %P on body size for that group

Table 1 Comparisons of percentage carbon (%C), percentage phosphorus (%P) and molar C:P among detritus removed from caves and obligate cave invertebrates. Numbers in parentheses are sample sizes for invertebrate analyses. The troglophilic millipede, *Pseudotremia hobbsi*, is included for comparison. Also included are previously published values of stream and terrestrial resource-consumer pairs (data from Cross et al. 2003)

	%C	%P	C:P	C:P mismatch[a]
Detritus				
Resources removed from caves[b]	34.77	0.22	913.11	
Detritivores				Detritus/detritivore
Pseudotremia fulgida	28.84 (7)	1.39 (27)	60.51	15.09
Pseudotremia hobbsi	32.31 (5)	1.50 (19)	62.82	14.53
Collembola[c]	47.52 (6)	1.36 (18)	101.91	8.96
Predators				Detritivore/predator
Pseudanophthalmus fuscus	44.54 (3)	1.21 (2)	107.28	0.59 (Millipede)
				0.94 (Collembola)
Pseudanophthalmus grandis	50.83 (13)	0.77 (17)	192.51	0.33 (Millipede)
				0.53 (Collembola)
C:P mismatches in other systems[d]				
Leaf detritus/stream shredders				9.76
Stream epithilion/stream scrapers				4.72
Stream prey/stream predators				1.45
Terrestrial plants/terrestrial herbivores				8.34

[a]C:P mismatch calculated as the ratio of C:P (resource)/C:P (consumer)
[b]Average molar C:P of cave resources is the grand average across the average C:P for each of the 12 caves
[c]The average C concentration for collembola is taken from data from Teuben and Verhoef (1992)
[d]Data for other systems taken from Table 1 in Cross et al. 2003. We used their raw numbers of C:P to calculate mismatch values (whereas they used arithmetic differences)

or the size × species interaction when comparing adults of the two species. With size removed from the model, the species were marginally different in %P ($F_{1,43} = 3.59$, $P = 0.065$) when one influential outlier was excluded [without the removal of the outlier, the troglophile had significantly higher %P ($F_{1,44} = 5.21$, $P = 0.028$)]. The subadult millipedes, which could not be assigned to species, had exceptionally high P content, with an average of 3.11% (Fig. 2). Across all millipedes sampled, we found a negative allometric relationship ($F_{5,46} = 84.82$, $P \leq 0.001$, Fig. 4b), but there was a significant size × species interaction ($F_{2,46} = 61.87$, $P < 0.001$). When we examined each species alone, we found negative allometry in the subadults ($F_{1,4} = 30.85$, $P = 0.005$), but not among adults of either species (*P. hobbsi* $F_{1,17} = 0.220$, $P = 0.645$; *P. fulgida* $F_{1,25} = 0.088$, $P = 0.770$).

Biochemical content of cave millipedes

Without accounting for species' size, the only biochemical component that differed between troglobiotic and troglophilic millipedes was the RNA/DNA ratio
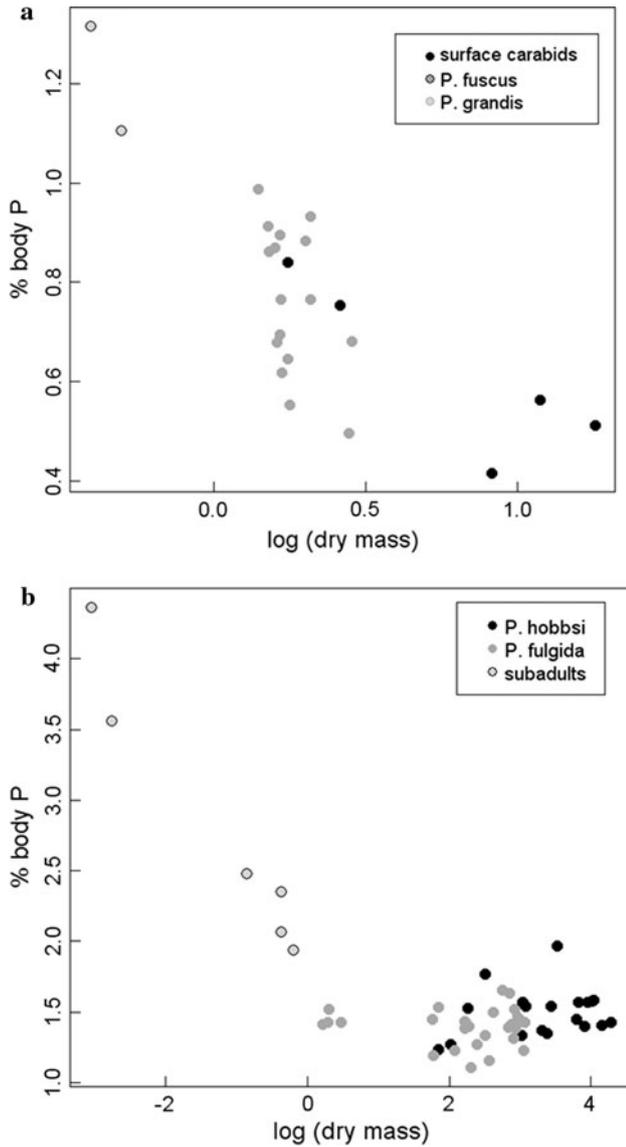
Fig. 4 Percent P allometry of **a** carabid beetles, including surface carabids (compiled from the literature) and two congeneric troglobiotic beetles (*Pseudanopthalmus fuscus* and *P. grandis*), and **b** chordeumatid millipedes, including two congeneric millipedes (troglobiont = *Pseudotremia fulgida*, troglophile = *P. hobbsi*) and subadult stages of either *P. hobbsi* or *P. fulgida*

Fig. 5 Concentrations of DNA and RNA (as percent dry mass), and the RNA/DNA ratio of the troglobiotic and troglophilic millipedes

(Fig. 5). To analyze the biochemical content of the millipedes statistically, we fitted three separate linear models of biochemical content (log transformed %DNA, %RNA or RNA/DNA ratio) with the predictor variables of species, size (log transformed), and their interaction. DNA content (as a percentage of dry mass) differed between millipede species, with a significant interaction between species and size ($F_{1,12} = 6.49$, $P = 0.026$). In contrast, RNA content (% dry mass) did not differ between species ($F_{1,13} = 1.67$, $P = 0.218$), though there was a significant size effect ($F_{1,13} = 9.69$, $P = 0.008$). For the RNA/DNA ratio, there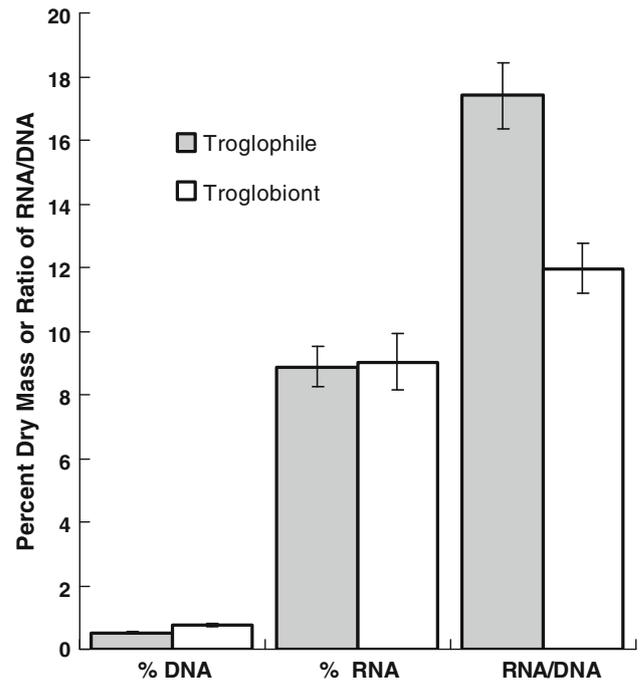 was no effect of size and the RNA/DNA ratio of troglophilic millipedes was 45% higher than that of the troglobiotic millipedes ($F_{1,13} = 21.60$, $P < 0.001$).

Discussion

Our goal was to examine the potential for resource quality to constrain the biochemistry of cave arthropod consumers. Although the C:P of cave resources was higher than the surface resources for at least a portion of the year, the overall C:P content of cave resources was not significantly lower than that of above-ground detritus as we predicted. Seasonal variation in the quality of allochthonous resources entering caves and the potential for bacterial enrichment of detrital resources in caves may both contribute to the overall lack of difference between surface and in-cave resources. Although our findings about the relative nutrient content of basal resources are equivocal, we did observe that obligate cave animals have less body %P than closely related surface-dwelling relatives (as predicted from known cave adapted life-history traits). Overall, our results suggest that although the surface and cave resources did not differ using our methods, the great nutritional mismatch between resources and cave-obligate consumers may contribute to a mechanistic explanation for the differences in %P. However, at this time we cannot rule out the hypothesis that the relatively low P content in cave arthropods may result from selection for low-P phenotypes (e.g., low growth rate) rather than from constraints due to P scarcity.

Characterizing the elemental stoichiometry of cave resources

Although, on average, cave detrital material was nutrient poor (0.22 %P), some of the caves had rather P-rich detrital resources (e.g., 0.95 %P). This material was likely nutrient-rich fecal material, fungus, or bacterial films on these decomposing resources (Maraun and Scheu 1996; Cross et al. 2003). Despite the variation between caves in detrital %P, the average C:P ratio of cave resources (913.1) was within the range of the C:P ratio of surface litter (July 698.7; November/December 2,679.6). This result suggests that P limitation may not be particularly important for consumers in this cave ecosystem; however, conclusions from our results should be drawn cautiously for several reasons. First, our results contrast with comparisons from other systems. For example, the bulk litter on the forest floor of a geographically and ecologically similar mature oak forest in New Jersey was much richer than our findings for the surface, with a C:P ratio of 360 (Lang and Forman 1978; Fig. 1). In addition, our surface and cave samples were collected at different decomposition stages, and further, the cave resources we collected may have contained more organic-rich material such as feces or bacteria. Future comparisons of surface versus cave resources should include the litter layer of the surface soil to afford a more complete comparison. For example, examining different forest ecosystems in Greece, Kavvadias et al. (2001) collected all litter on the forest floor at the three horizons of the humus profile [litter (L), fermentation (F), and humus (H)], and found that the litter layer was lower quality than the fermentation and humus layers (average C:P 658 L compared to average C:P 367 F, H). Such a sampling strategy, if applied to the surface above the caves, is likely to yield higher quality, decomposing resources, than the freshly fallen litter that we used for this comparison.

Cave resources were of lower quality than the surface resources collected at the same time of year as the initial cave resource removal (July). Though not significant, the cave resource C:P was 31% higher than the surface litter collected at the same time (Fig. 1). Because cave resources are of poor quality even during the summer, we suspect that cave resource quality would only degenerate during the fall and winter months, when surface resources are of poor quality and there is the greatest potential for input of these nutrient-poor leaves into caves. In addition, it is important to note that the removed resources were collected at the bottom of the vertical pits; a cave can extend much farther than the resources are often carried, and cave animals far within a cave may only encounter "islands" of these resources (Gibert and Deharveng 2002).

Differences between cave-dwelling and surface species

In two cases where recognizable pairs of troglobiotic and troglophilic species existed, (chordeumatid millipedes and rhagidiid mites), the troglobionts were both lower in %P than their troglophilic counterparts. Given the typically slower growth rates in obligate cave-dwelling species, these results provide preliminary support for the growth rate hypothesis (Sterner and Elser 2002) in this system. When troglobiotic carabid beetles were compared to literature data for surface carabid beetles, we found a significant interaction between species and size. However, because the literature data included many different carabid species, phylogenetic and environmental variation are likely to influence this result.

The connection between P content and growth rate in millipedes requires further investigation. Obligate cave millipedes did have lower %P and RNA/DNA ratios than troglophilic millipedes, consistent with the growth rate hypothesis. However, we found no difference in RNA content between millipede species; instead, the between-species difference in RNA/DNA ratio was due to the lower DNA content in the troglophilic species. Although RNA/DNA ratio has been shown to be positively associated with actual growth rates in comparisons among conspecifics (Vrede et al. 2002), it is unclear whether a similar pattern exists in interspecific comparisons. Instead, differences in RNA/DNA ratio among species may often be associated with differences in DNA content (e.g., Hessen et al. 2007) rather than in investment in rRNA for increasing protein synthesis rates. The difference in DNA content between species in our study is small (0.24% dry mass) and accounts for only 1.78% of the difference in their P content, suggesting differences in allocation to other traits influences P levels. One such trait may be the cuticle, which is thinner in troglobiotic millipedes—a pattern widely considered to reflect relaxed selection for water retention in humid cave environments (Culver 1982). Our results suggest that a broad-scale comparison is needed before RNA/DNA ratio can be used as a surrogate for synthetic capacity in terrestrial arthropods.

Because of their overall high body %P (1.39%) compared to other arthropods, troglobiotic millipedes are greatly out of stoichiometric balance with their food resource. The great disparity between C:P of cave detritus and C:P of cave millipedes (resource:consumer ration of ~15 for both the troglophilic and troglobiotic millipedes) is larger than any previously reported stoichiometric mismatch (Table 1). This strong mismatch suggests that cave millipedes may be faced with extreme nutrient constraints. Some of this mismatch may be offset by millipedes selectively feeding on particular nutrient-rich components of the detrital resource base. For example, although millipedes have been reported to feed directly on dead wood (which is extremely nutrient-poor, Kerkhoff et al. 2006), they also may feed on comparatively nutrient-rich fecal material (Shear 1969; Kaneko 1999) and have been associated with animal remains (Tibbett and Carter 2008). Nevertheless, to offset the unusually large dietary stoichiometric mismatch reported here, such selectivity in millipede feeding would have to be quite extensive. To the degree that the

stoichiometry of the millipedes' realized diets approximates the stoichiometry of within-cave resources, the observed mismatch between detritus quality and millipedes' needs would certainly provide a reasonable explanation for the slow growth rates (Cross et al. 2003) and reduced protein synthetic capacities (RNA/DNA; Fig. 5) of troglobiotic millipedes.

It is also possible that other nutrients, aside from P, may be limiting in this system. Limitation of C or N has been shown to decouple the relationship between RNA and P (Elser et al. 2006). Nitrogen limitation was not investigated here, but Studier (1996) found that cave crickets do indeed have less N than surface crickets, which may be due to the thin exoskeleton of cave species. The possibility of N-limitation in cave species and its biochemical ramifications are currently under investigation. The possibility of C-limitation due to the overall lack of resources in caves is also an interesting area of research (see Simon et al. 2007).

Phosphorus content of terrestrial cave invertebrates

Consistent with previous studies of terrestrial arthropods (Woods et al. 2004; Martinson et al. 2008; Hambäck et al. 2009), we did not find a significant difference in body %P between detritivorous (millipedes, collembola and oribatid mites) and predaceous (spiders, carabid beetles, rhagidiid mites and pseudoscorpions) species. The lack of a difference between trophic groups may be due in part to the large variation in %P content of detritivores, as some species contained very high levels of P (millipedes) while others did not (oribatid mites). Subadult millipedes, which as outliers were excluded from our analysis, were extremely rich in P. Higher P levels in juvenile individuals have also been seen in *Daphnia* and *Drosophila*, in which juvenile stages have higher growth rate and P requirements than adults (Boersma and Kreutzer 2002; Vrede et al. 2002; Cross et al. 2003; Elser et al. 2006). The P content of the adult cave millipedes was nearly twice as high as the reported average for other arthropods (Woods et al. 2004; Martinson et al. 2008), but was within the range reported for decaying millipede carcasses on the surface (ranging from 1.07 %P at death to 1.59 %P during the first month of decomposition, Seastedt and Tate 1981) and was similar to mealworms and waxworms (Barker et al. 1998). The relatively high body content of P in millipedes may result from the maintenance costs of their rigid, generally heavily calcified cuticle (Cloudsley-Thompson 1950), which may influence their specific P content (Shimizu and Urabe 2008). As in vertebrate bones, calcium (Ca) and P appear to co-occur in arthropod cuticles, where they may operate jointly to increase cuticular strength and durability. For example, analysis using electron microprobes has found Ca and P embedded in the cuticle of a ground-dwelling fly larva (Cribb et al. 2005), a burrowing species for which a strong cuticle would be especially important. Cuticular P content may also explain the high %P in terrestrial isopods (Tibbets and Molles 2005) and stream crustaceans (Evans-White et al. 2005). Such a situation would also explain the high P content we have observed for aquatic obligate cave isopods (1.88 ± 0.16%, *n* = 9, unpublished data). In addition, the low C content of the millipedes (28–32%), which is much lower than most reported terrestrial arthropods (typically 45–50%), is consistent with terrestrial detritivorous isopods reported in Tibbets and Molles (2005). This result also suggests that the cuticular structure of these animals largely determines their atypical stoichiometric signature.

We found no allometric pattern of body %P and dry body mass in the adult millipedes. Such a lack of P allometry in detritivores has recently been reported in Martinson et al. (2008), where the authors compared the P content of species (not individuals, as we examined here). Allometric scaling with P has been suggested because of the relationship between body size and growth rate, as well as the theory that arthropod exoskeletons should scale with body mass (Woods et al. 2004). The lack of P allometry in adult millipedes may be because P is predominantly important in the immature millipedes, which above and beyond their cuticular needs also require P for rapid growth and do not yet have the body composition of an adult. Once adulthood is reached, there may be a threshold amount of P needed for maintaining body composition (namely cuticular structure), and less required for everyday maintenance (as the adults do not grow). Similar ontogenetic transitions in body composition have been found in *Drosophila* (Watts et al. 2006), *Daphnia* (DeMott 2003), and the copepod *Mixodiaptomus* (Carillo et al. 2001). Between the two adult millipedes, however, the lack of an allometric relationship may be attributed to the low phosphorus content of the troglobiotic species. This species, which is smaller than the troglophilic species, would be expected to have a higher phosphorus content if the pattern of allometry held.

We did, however, see interesting allometric patterns across large phylogenetic groupings, finding that across broad groups (arachnids, diplopods, and hexapods) there exist nearly identical allometric slopes between log size and log %P (Fig. 3b). We also discovered an interesting allometric pattern with the troglobiotic beetles. As other studies have found for predators (Woods et al. 2004), the smaller species (*P. fuscus*) had higher %P than the larger species (*P. grandis*). *Pseudophthalmus grandis*, the larger species, also has a very large geographic range compared to the smaller species, a phenomenon also reported for other groups of troglobiotic beetles (Barr 1967). It is possible that the less nutrient-demanding, larger species is able to survive in a greater range of locations. Further examination of the P content of other *Pseudophthalmus* species may yield insights into cave biogeography.

Conclusions and future directions

The nutrient-limited environment of caves is an ideal system in which to investigate questions focused on the interplay between resource quality and generalized adaptations to cave life. These adaptations include morphological changes such as a lack of pigment and thin cuticles, as well as physiological characteristics, such as slow reproductive and developmental rates. Though we have focused here on the terrestrial cave environment, recent syntheses concerning aquatic cave organisms and their habitats suggest some potential routes for future research, especially as the differences between growth and metabolic rates of subterranean and surface animals are more pronounced in the aquatic habitat (Hüppop 1985). For example, subterranean aquatic habitats are monopolized by amphipods, isopods, and copepods, yet aquatic insects (which dominate many surface habitats) are scarcely represented in caves (Gibert and Deharveng 2002). Perhaps stoichiometric theory could potentially explain what makes for a good cave colonizer and allow us to investigate which species "succeed" in different environments (Michaels 2003), such as the nutrient-limited cave habitat. Perhaps the nutritional constraints imposed by cave environments are sufficiently strong as to exclude certain types of consumers, such as those that cannot efficiently store or assimilate limiting nutrients, although more research is needed to substantiate this possibility.

References

APHA (1992) Standard methods for the examination of water and wastewater, 18th edn. American Public Health Association, Washington, DC

Barker D, Fitzpatrick MP, Dierenfeld ES (1998) Nutrient composition of selected whole invertebrates. Zool Biol 17:123–134

Barr TC (1967) Observations on the ecology of caves. Am Nat 191:475–491

Barr TC (1968) Cave ecology and the evolution of troglophiles. In: Dobzhansky T, Hecht MK, Steere WC (eds) Evolutionary biology. Appleton-Century-Crofts, New York, pp 35–102

Boersma M, Kreutzer C (2002) Life at the edge: is food quality really of minor importance at low quantities? Ecology 83:2552–2561

Buckley BA (1984) RNA–DNA ratio: an index of larval fish growth in the sea. Mar Biol 80:291–298

Buckley BA, Szmant AM (2004) RNA/DNA ratios as indicators of metabolic activity in four species of Caribbean reef-building corals. Mar Ecol Prog Ser 282:143–149

Carillo P, Villar-Argaiz M, Mendina-Sanchez JM (2001) Relationship between N:P ratio and growth rate during the life cycle of Calanoid copepods: an in situ measurement. J Plankton Res 23:537–547

Christman MC, Culver DC (2001) The relationship between cave biodiversity and available habitat. J Biogeogr 28:367–380

Cloudsley-Thompson JL (1950) The water relations and cuticle of *Paradesmus gracilis* (Diplopoda, Strongylosomidae). Q J Microsc Sci 91:453–464

Cook SC, Davidson DW (2006) Nutritional and functional biology of exudate-feeding ants. Entomol Exp Appl 118:1–10

Cribb BW, Rasch R, Barry J, Palmer CM (2005) Distribution of calcium phosphate in the exoskeleton of larval *Exeretonevra angustifrons* Hardy (Diptera: Xylophagidae). Arthropod Struct Dev 34:41–48

Cross WF, Benstead JP, Rosemond AD, Wallace JB (2003) Consumer-resource stoichiometry in detritus based streams. Ecol Lett 6:721–732

Culver DC (1982) Cave life: evolution and ecology. Harvard University Press, Cambridge

DeMott W (2003) Implications of element deficiencies for zooplankton growth. Hydrobiologia 491:177–184

Denno RF, Fagan WF (2003) Might nitrogen limitation promote omnivory among carnivorous arthropods? Ecology 84:2522–2531

Denno RF, Raupp MJ, Tallamy DW, Reichelderfer CF (1980) Migration in heterogeneous environments: differences in habitat selection between the wing forms of the dimorphic planthopper, *Prokelisia marginata* (Homoptera: Delphacidae). Ecology 61:859–867

Denno RF, Gratton C, Peterson MA, Langellotto GA, Finke DL, Huberty AF (2002) Bottom-up forces mediate natural-enemy impact in a phytophagous insect community. Ecology 83:1443–1458

Elser JJ (2006) Biological stoichiometry: a chemical bridge between ecosystem ecology and evolutionary biology. Am Nat 168:S25–S35

Elser JJ, Urabe J (1999) The stoichiometry of consumer-driven nutrient recycling: theory, observations, and consequences. Ecology 80:735–751

Elser JJ, Dobberfuhl D, Mackay NA, Schampel JH (1996) Organism size, life history, and N:P stoichiometry: towards a unified view of cellular and ecosystem processes. Bioscience 46:674–685

Elser JJ, Fagan WF, Denno RF, Dobberfuhl DR, Folarin A, Huberty A, Interlandi S, Kilham SS, McCauley E, Schulz KL, Siemann EH, Sterner RW (2000a) Nutritional constraints in terrestrial and freshwater food webs. Nature 408:578–580

Elser JJ, Sterner RW, Gorokhova E, Fagan WF, Markow TA, Cotner JB, Harrison JF, Hobbie SE, Odell GM, Weider LJ (2000b) Biological stoichiometry from genes to ecosystems. Ecol Lett 3:540–550

Elser JJ, Watts T, Bitler B, Markow TA (2006) Ontogenetic coupling of growth rate with RNA and P contents in five species of *Drosophila*. Funct Ecol 20:846–856

Evans-White MA, Stelzer RS, Lamberti GA (2005) Taxonomic and regional patterns in benthic macroinvertebrate elemental composition in streams. Freshw Biol 50:1786–1799

Faerovig PL, Hessen DO (2003) Allocation strategies in crustacean stoichiometry: the potential role for phosphorus in the limitation of reproduction. Freshw Biol 48:1782–1792

Fagan WF, Denno RF (2004) Stoichiometry of actual vs potential predator–prey interactions: insights into nitrogen limitation for arthropod predators. Ecol Lett 7:876–883

Fagan WF, Lutscher F, Schneider K (2007) Population and community consequences of spatial subsidies derived from central-place foraging. Am Nat 170:902–915

Fink P, Von Elert E (1996) Physiological responses to stoichiometric constraints: nutrient limitation and compensatory feeding in a freshwater snail. Oikos 115:484–494

Frost PC, Evans-White MA, Finkel ZV, Jensen TC, Matzek V (2005) Are you what you eat? Physiological constraints on organismal stoichiometry in an elementally imbalanced world. Oikos 109:18–28

Gibert J, Deharveng L (2002) Subterranean ecosystems: a truncated functional biodiversity. Bioscience 52:473–481

Hambäck PA, Gilbert J, Schneider K, Martinson HM, Kolb G, Fagan WF (2009) Effects of body size, trophic mode and larval habitat on dipteran stoichiometry: a regional comparison. Oikos 118:615–623

Hessen DO, Jensen TC, Kyle M, Elser JJ (2007) RNA responses to N- and P-limitation; reciprocal regulation of stoichiometry and growth rate in *Brachionus*. Funct Ecol 21:956–962

Hoffman R (1950) A preliminary list of the cleidogonid millipedes, with descriptions of a new genus from Guatemala and a new species from Virginia. J Wash Acad Sci 40:87–92

Huberty AF, Denno RF (2006) Consequences of nitrogen and phosphorus limitation for the performance of two planthoppers with divergent life-history strategies. Oecologia 149:444–455

Hüppop K (1985) The role of metabolism in the evolution of cave animals. Natl Speleol Soc Bull 47:136–146

Hüppop K (2005) Adaptation to low food. In: Culver DC, White WB (eds) Encyclopedia of caves. Elsevier, Burlington, pp 4–9

Kaneko N (1999) Effect of millipede *Parafontaria tonominea* Attems (Diplopoda Xystodesmidae) adults on soil biological activities: a microcosm experiment. Ecol Res 14:271–279

Kavvadias VA, Alifragis D, Tsiontsis A, Brofas G, Stamatelos G (2001) Litterfall, litter accumulation and litter decomposition rates in four forest ecosystems in northern Greece. For Ecol Manag 144:113–127

Kay AD, Ashton IW, Gorokhova E, Kerkhoff AJ, Liess A, Litchman E (2005) Toward a stoichiometric framework for evolutionary biology. Oikos 109:6–17

Kerkhoff AJ, Fagan WF, Elser JJ, Enquist BJ (2006) Phylogenetic and growth form variation in the scaling of nitrogen and phosphorus in the seed plants. Am Nat 168:E103–E122

Kunter M, Sket B, Blejec A (1999) A comparison of the respiratory systems in some cave and surface species of spiders (Araneae, Dysderidae). J Arachnol 27:142–148

Kyle M, Watts T, Schade J, Elser JJ (2003) A microfluorometric method for quantifying RNA and DNA in terrestrial insects. J Insect Sci 3:1. Available online: http://www.insectscience. org/3.1

Lang GE, Forman RTT (1978) Detrital dynamics in a mature oak forest: Hutcheson Memorial Forest, New Jersey. Ecology 59:580–595

Lee KP, Simpson SJ, Raubenheimer D (2004) A comparison of nutrient regulation between solitarious and gregarious phases of the specialist caterpillar, *Spodoptera exempta* (Walker). J Insect Physiol 50:1174–1180

Loomis HF (1943) New cave and epigean millipeds of the United States, with notes on established species. Bull Mus Comp Zool 92:373–410

Makino W, Cotner JB, Sterner RW, Elser JJ (2003) Are bacteria more like plants or animals? Growth rate and resource dependence of bacterial C:N:P stoichiometry. Funct Ecol 17:121–130

Maraun M, Scheu S (1996) Changes in microbial biomass, respiration and nutrient status of beech (*Fagus sylvatica*) leaf litter processed by millipedes (*Glomeris marginata*). Oecologia 107:131–140

Martinson HM, Schneider K, Gilbert J, Hines JE, Hambäck PA, Fagan WF (2008) Detritivory: stoichiometry of a neglected trophic level. Ecol Res 23:487–491

McGlynn TP, Salinas DJ, Dunn RR, Wood TE, Lawrence D, Clark DA (2007) Phosphorus limits tropical rain forest litter fauna. Biotropica 39:50–53

Michaels AF (2003) The ratios of life. Science 300:906–907

Mira A (2000) Exuviae eating: a nitrogen meal? J Insect Physiol 46:605–610

Mitchell RW (1969) A comparison of temperate and tropical cave communities. Southwest Nat 14:73–88

Moe SJ, Stelzer RS, Forman MR, Harpole WS, Daufresne T, Yoshida T (2005) Recent advances in ecological stoichiometry: insights for population and community ecology. Oikos 109:29–39

Poulson TL (1963) Cave adaptation in amblyopsid fishes. Am Nat 70:257–290

Poulson TL, Lavoie KH (2000) The trophic basis of subterranean ecosystems. In: Wilkens H, Culver DC, Humphries WF (eds) Ecosystems of the world 30: subterranean ecosystems. Elsevier, Amsterdam, pp 231–249

Poulson TL, White WB (1969) The cave environment. Science 165:971–981

Schade JD, Espeleta JF, Klausmeier CA, McGroddy ME, Thomas SA, Zhang L (2005) A conceptual framework for ecosystem stoichiometry: balancing resource supply and demand. Oikos 109:40–51

Schneider K, Culver DC (2004) Estimating subterranean species richness using intensive sampling and rarefaction curves in a high cave density region in West Virginia. J Cave Karst Stud 66:39–45

Schulz KL, Sterner RW (1999) Phytoplankton phosphorus limitation and food quality for *Bosmina*. Limnol Oceanogr 44:1549–1556

Seastedt TR, Tate CM (1981) Decomposition rates and nutrient contents of arthropod remains in forest litter. Ecology 62:13–19

Shear WA (1969) A synopsis of the cave millipeds of the United States, with an illustrated key to genera. Psyche 76:126–143

Shimizu Y, Urabe J (2008) Regulation of phosphorus stoichiometry and growth rate of consumers: theoretical and experimental analyses with *Daphnia*. Oecologia 155:21–31

Simon KS, Pipan T, Culver DC (2007) A conceptual model off the flow and distribution of organic carbon in caves. J Cave Karst Stud 69:279–284

Simpson SJ, Simpson CL (1990) The mechanisms of nutritional compensation by phytophagous insects. In: Bernays EA (ed) Insect–plant interactions, vol 2. CRC, Boca Raton, pp 111–160

Slansky F (1993) Nutritional ecology: the fundamental quest for nutrients. In: Stamp NE, Casey TM (eds) Caterpillars: ecological and evolutionary constraints on foraging. Chapman & Hall, New York, pp 29–91

Sterner RW, Elser JJ (2002) Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, Princeton

Studier EH (1996) Composition of bodies of cave crickets (*Hadenoecus subterraneus*), their eggs, and their egg predator, *Neaphaenops tellkampfi*. Am Midl Nat 136:101–109

R Development Core Team (2008) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. http://www. R-project.org

Tibbets TM, Molles MC Jr (2005) C:N:P stoichiometry of dominant riparian trees and arthropods along the Middle Rio Grande. Freshw Biol 50:1882–1894

Tibbett M, Carter D (2008) Soil analysis in forensic taphonomy. CRC, Boca Raton

Teuben A, Verhoef HJ (1992) Direct contribution by soil arthropods to nutrient availability through body and faecal nutrient content. Biol Fertil Soils 14:71–75

Urabe J, Watanabe Y (1992) Possibility of N or P limitation for planktonic cladocerans: an experimental test. Limnol Oceanogr 37:244–251

Urabe J, Clasen J, Sterner RW (1997) Phosphorus limitation of *Daphnia* growth: is it real? Limnol Oceanogr 42:1436–1443

Valentine JM (1931) New cavernicole Carabidae of the subfamily Trechinae Jeannel. J Elisha Mitchell Sci Soc 46:247–258

Valentine JM (1932) A classification of the genus *Pseudophthalmus* Jeannel (fam. Carabidae), with descriptions of new species and notes on distribution. J Elisha Mitchell Sci Soc 48:261–280

Villar-Argaiz M, Sterner RW (2002) Life history bottlenecks in *Diaptomus clavipes* induced by phosphorus-limited algae. Limnol Oceanogr 47:1229–1233

Vrede T, Persson J, Aronsen G (2002) The influence of food quality (P:C ratio) on RNA:DNA ratio and somatic growth rate of *Daphnia*. Limnol Oceanogr 47:487–494

Watts T, Woods HA, Hargand S, Elser JJ, Markow TA (2006) Biological stoichiometry of growth in *Drosophila melanogaster*. J Insect Physiol 52:187–193

Weider LJ, Elser JJ, Crease TJ, Mateos M, Cotner JB, Markow TA (2005) The functional significance of ribosomal (r)DNA variation: impacts on the evolutionary ecology of organisms. Annu Rev Ecol Evol Syst 36:219–242

Woods HA, Fagan WF, Elser JJ, Harrison JF (2004) Allometric and phylogenetic variation in insect phosphorus content. Funct Ecol 18:103–109