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LETTER

Early Cambrian food webs on a trophic knife-edge? A hypothesis and preliminary data from a modern stromatolite-based ecosystem

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Abstract

Here we use the theory of ecological stoichiometry to propose and provide a preliminary test of a novel hypothesis that the Cambrian 'explosion' may have been triggered by changes in circulating P availability in the biosphere. We exposed living stromatolites from a spring-fed stream in Mexico to a gradient of P enrichment to examine subsequent effects on stromatolite C : P ratio and on the primary grazer, an endemic snail. Consistent with a previously hypothesized stoichiometric 'knife-edge', snail performance was maximal at intermediate P-enrichment, indicating *in situ* stoichiometric constraints because of high stromatolite C : P ratio along with high sensitivity to excessive P intake. These results are consistent with the idea that stoichiometric constraints may have delayed the evolutionary proliferation of animals in ancient stromatolite-dominated ecosystems and also suggest that high food P content can significantly impair consumers. We propose that ecosystem P availability may have impacted both the expansion and decline of animal taxa in the history of life.

Keywords

Cambrian explosion, ecological stoichiometry, phosphorus, snails, stromatolites.

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INTRODUCTION

One of the most dramatic events in the history of life on Earth was the sudden expansion and diversification of metazoan animals *c.* 520 million years ago (Ma), the so-called 'Cambrian explosion'. These events have long-fascinated scientists, leading to the production of a variety of hypotheses regarding possible intrinsic and extrinsic (environmental) triggers responsible for this rapid diversification. These include causes involving increasing atmospheric oxygen (Towe 1970; Valentine 1973; Runnegar 1982; Thomas 1997), the appearance of key genomic innovations (Erwin 1993; Raff *et al.* 1994; Davidson *et al.* 1995), and increases in total ecosystem productivity (Cook & Shergold 1984; McMenamin 1988; Brasier 1990).

With respect to the latter, considerations of the role of nutrients in potentially constraining the development of ancient food webs have focused entirely on overall rates of total productivity. Low productivity has been proposed to somehow limit the success of large-bodied animals likely to occupy trophic levels high in food webs (Cook & Shergold 1984; McMenamin 1988; Brasier 1990; but see Butterfield 1997). However, various findings emerging from studies of ecological stoichiometry (the study of the balance of energy and multiple chemical elements in ecological interactions, Sterner & Elser 2002) highlight the importance of mismatches in the elemental composition of food items (i.e. biomass of autotrophic algae or higher plants) and consumers (i.e. herbivores) as major factors affecting consumer growth and production (Sterner & Elser 2002). In general, autotrophic biomass has variable but generally high C : nutrient ratio, while animals have low C : nutrient ratios in their bodies that they homeostatically regulate around taxon- or stage-specific values (Sterner & Elser 2002). These physiological contrasts lead to frequent situations in which herbivores face strong stoichiometric constraints on growth and reproduction (Elser *et al.* 2000). In fact, stoichiometric models of autotroph–consumer interactions predict the existence of stable equilibria dominated by autotroph biomass into which consumers cannot invade ('deterministic extinction' of the consumer; Andersen *et al.* 2004).

The preceding discussion suggests a hypothetical scenario (Elser 2003) for the Precambrian Eon, when, for billions of years, Earth's ecosystems were dominated by microbial biomass (heterotrophic Bacteria and Archaea, autotrophic Cyanobacteria and, eventually, eukaryotic algae), in the absence of large metazoan herbivores such as those that play a key role in modern trophic transfer. Perhaps microbial mats during the Precambrian, such as those generating widespread stromatolites (bio-laminated sediments; see Walter 1994), suffered severe nutrient (PO_4) limitation, resulting in extremely high biomass C : P ratio that excluded large metazoan herbivores because of stoichiometric constraints. According to this hypothesis, this state was maintained until environmental changes altered microbial nutritional status (lowered microbial C : P ratio) in such a way as to improve its ability to support large, fast-growing, metazoans (Elser 2003). Paleoenvironmental evidence suggesting the feasibility of such a scenario exists and will be discussed below.

Thus, application of stoichiometric theory might help reduce existing uncertainty (Martin 2003) surrounding the ecological events that may have triggered the Cambrian 'explosion'. Testing causal hypotheses about events of the distant past is, of course, very difficult. Generally, there are two ways to proceed: one can make predictions about things in the fossil or geological record that would be observed if the hypothesis were correct or make predictions about the outcomes of experiments that can be conducted with modern systems similar to those of the past (i.e. 'analogues'). In this paper, we take both of these strategies. First, the stoichiometric constraint hypothesis predicts that the geological record should show major changes in the global P cycle at the time of the Cambrian explosion. As discussed below, this is, in fact, what is reported by Cook & Shergold (1984). Second, the stoichiometric constraint hypothesis predicts certain outcomes in studies performed in modern ecosystems analogous to the ancient situation: laminating microbial mats (living stromatolites) should have high biomass C : P ratios and P addition should stimulate animal growth and performance, at least in some part of the range.

A preliminary evaluation of these ideas comes from our studies in the Chihuahuan desert near Cuatro Ciénegas, Coahuila, Mexico (see Methods for detailed site description). Here, a diverse array of active stromatolite-generating microbial communities ('stromatolites', hereafter) occupy desert springs discharging warm and highly mineralized waters rich in Ca, Mg, CO_3, SO_4 and NO_3 but relatively low in PO_4 (Elser *et al.* 2005a). Furthermore, these stromatolites often co-occur with several metazoan consumers, including an endemic hydrobiid snail *Mexithauma quadripaludium*, and thus offer a unique opportunity to examine the interactions between actively growing stromatolites and metazoan herbivores. Previous work (Elser *et al.* 2005a) indicates that, compared with values exhibited by aquatic and terrestrial autotrophs in other ecosystems (Elser *et al.* 2000), the P-content of microbial biomass comprising the surficial layers (< 1 mm) of these stromatolites is extremely low (high C : P ratios ranging from *c.* 500 to 5000; N : P ratios are also correspondingly high). Since animal biomass is generally much more P-rich (Elser *et al.* 2000), P-limitation of growth for herbivores consuming this material seems likely. During a preliminary 21-d experiment performed in 2001, enrichment of PO_4 to stromatolites dramatically lowered its C : P ratio from *c.* 3000 to *c.* 700. Consistent with P limitation of snail growth (Elser *et al.* 2003), the P- and RNA-contents of *M. quadripaludium* were significantly higher in the + P treatment (Elser *et al.* 2005a,b), consistent with higher growth rate in response to the P enrichment. However, in 2002 a similar but longer-term experiment yielded conflicting results: P-enrichment lowered stromatolite C : P ratio from *c.* 800 to < 100 but snails in the + P treatment exhibited significantly lower RNA content, growth rate, and survivorship than in unenriched controls. These conflicting results, coupled with similar laboratory observations recently reported for the crustacean *Daphnia* (Plath & Boersma 2001), led to the hypothesis (Elser *et al.* 2005b) that snails at Cuatro Ciénegas live on a kind of 'stoichiometric knife edge', in which, in nature, their growth is limited by the high C : P ratio of stromatolite biomass but their performance can be impaired by unnaturally P-rich (low C : P) food.

Thus, we propose that new developments in ecological stoichiometry, both the existence of stoichiometric constraints because of low food nutrient content as well as negative effects of high food nutrient content (Boersma & Elser in press), may help in understanding important events in the history of life. To bolster this suggestion we present the results of a field experiment in which stromatolites were exposed to a range of experimental P enrichment from 0 to 10 μM P, after which various aspects of snail performance were assayed. Given the 'knife edge hypothesis', we expected P fertilization to lower stromatolite C : P ratio and, in turn, to stimulate snail growth, survivorship, and production but only over a limited range of P enrichment. At higher levels of P enrichment, the hypothesis predicts decreased snail performance. Our data are consistent with these predictions and lead us to suggest that consideration of stoichiometric food quality may improve our understanding of the evolution of early food webs.

METHODS

Study system and organisms

Our experiment involved studies in the Rio Mesquites (Fig. 1a), a stream fed by warm springs in the Cuatro Ciénegas basin (CCB hereafter), Coahuila, Mexico. The

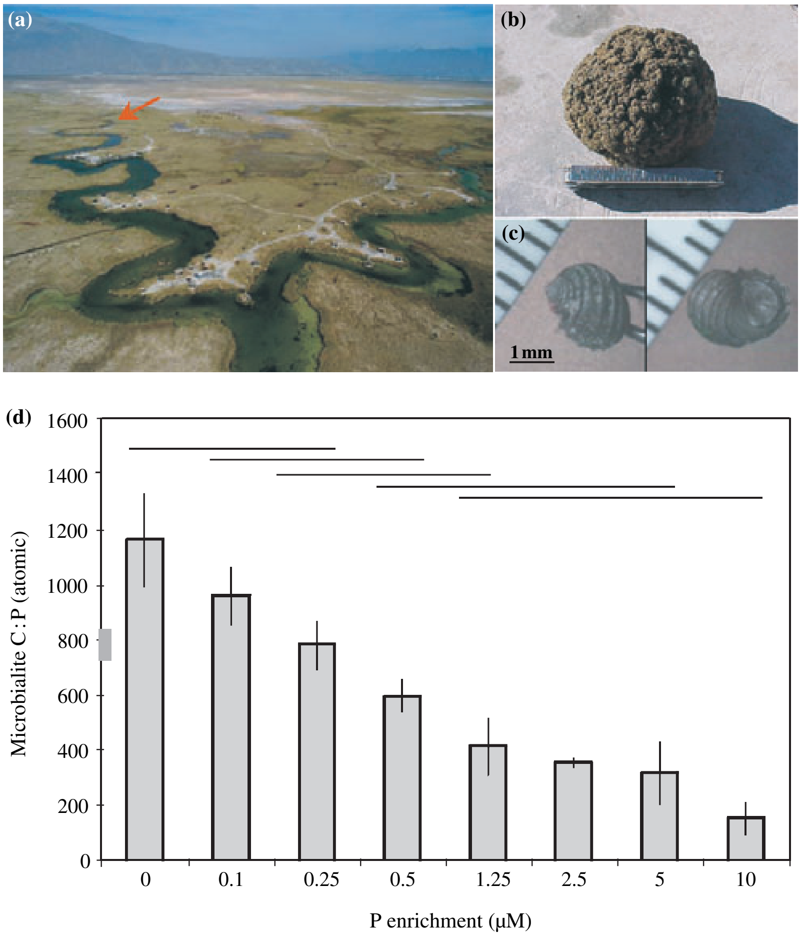

Figure 1 (a) Aerial photograph showing our study site (red arrow) in the Rio Mesquites, Cuatro Ciénegas, Coahuila, México. For scale, the width of the stream in the foreground is *c.* 5 m. (b) Typical oncoid stromatolite used in the experiment. For scale, the pocket knife is about 10 cm in length. (c) Dorsal and ventral views of the hydrobiid snail *Mexithauma quadripaludium*, the dominant metazoan grazer on hard benthic surfaces in Cuatro Ciénegas. This genus is endemic to Cuatro Ciénegas. The horizontal bar in the left-hand image indicates a 1-mm scale. (d) C : P ratios of surficial organic biomass of oncoid stromatolites after 39 d across a range of PO_4 enrichment from 0 to + 10 μM. Averages for three replicate stromatolites for each treatment are shown. Error bars on the columns reflect ± 1 SE and horizontal bars join treatment means that are not significantly different from each other according to Tukey's HSD multiple comparison test (overall ANOVA: $F_{7,16} = 18.57$, $P < 0.0001$). The grey bar on the *y*-axis indicates the mean and ± 1 SE for determinations made on three stromatolites freshly collected from the Rio Mesquites at the start of the experiment. Photographic credits: (a) La Venta Exploration Team (http://www.laventa.it); (b) J.J. Elser, and (c) J.H. Schampel.

CCB is an area administered by the Mexican Instituto Nacional de Ecologia as an 'Area de Protección de Flora y Fauna'. At CCB, mildly thermal springheads are small to large (< 1.0 m^2 surface area to > 1.0 ha), with surface outflows ranging from seepage to *c.* 1.0 m^3 s^{-1}, temperatures from 24 to *c.* 40 °C, total dissolved solids (TDS) from 0.3 to 2.0 mg L^{-1} and chemistry varying from $CaSO_4$ to $Ca(HCO_3)_2$-dominated. Of particular interest for our studies is the presence in the CCB of more than a dozen hydrobiid snail species (Hershler 1985). Among these, the endemic species *Mexithauma quadripaludium* (Fig. 1c) was the focus of our experiments, as it grazes primarily on hard surfaces such as oncoid stromatolites (Fig. 1b).

In general (Elser *et al.* 2005a), waters of Rio Mesquites are warm (average temperature 27 °C), neutral to alkaline (average pH *c.* 7.6), well-buffered (average alkalinity *c.* 4 meq L^{-1}), and high in conductivity (average *c.* 2900 μS). Dissolved inorganic nitrogen (DIN) concentrations are relatively high, with an average NO_3 concentration of *c.* 20 μmol L^{-1} (always > 6 μmol L^{-1}). However, P concentrations are low, with total P (TP) *c.* 0.6 μmol L^{-1}. Reflecting high inorganic N and low P concentrations, DIN (NO_3 + NH_4): soluble reactive P (SRP) and TN : TP ratios are high (> 35 and > 100, respectively, on average), indicating likely P-limitation of microbial growth. Calcium and sulphate levels are also high (average *c.* 309 and

1200 mg L^{-1}, respectively). The high alkalinity and Ca^{2+} concentrations promote efficient and rapid photosynthesis-driven calcification in the stromatolites, with areal rates similar to those found in corals (Garcia-Pichel *et al.* 2004).

The oncoid stromatolites we studied (Fig. 1b) are formed by rolling transport along the bottom of the Mesquites River. At a finer scale, the stromatolites exhibit finely branching columnar growth, with documented accretion rates of *c.* 1 mm yr^{-1} (Garcia-Pichel *et al.* 2004). Accretion is mediated by calcifying cyanobacterial – diatom biofilms that produce mostly clotted (thrombolitic) internal fabrics, with occasional laminated (stromatolitic) domains. When these oncoids reach a diameter > 35 mm, they become stabilized and grow into rounded columnar forms that may eventually merge to form flat-topped bioherms. The thrombolitic microfabrics seen in the Rio Mesquites oncoids, columnar forms, and bioherms become common in the fossil record during the early Cambrian (Grotzinger & Knoll 1999), suggesting that the Cuatro Ciénegas systems provide useful analogues for ecological conditions present at the time of metazoan diversification. Indeed, a previous study (Garcia-Pichel *et al.* 2004) has considered relative rates of calcification and metazoan bioerosion in these systems and interpreted these in terms of their implications for the role of grazers in initiating stromatolite decline during the early Cambrian.

Experimental conditions

Oncoid stromatolites of *c.* 10-cm diameter were collected from the Rio Mesquites and were randomly assigned to eight treatments representing levels of P enrichment (0, 0.1, 0.25, 0.5, 1.25, 2.5, 5 and 10 μM). Two stromatolites were suspended in each 20-L translucent container (four replicate containers per treatment) holding *c.* 15 L of stream water that was exchanged and freshly enriched twice daily. Water in the containers was circulated intermittently to reduce the formation of boundary layers. All snails were removed from stromatolites at the beginning of the experiment. Individual *M. quadripaludium* were collected from these stromatolites, and from others from the stream, given individual paint marks, weighed (wet weight including their shell), and then randomly returned to graze for the duration of the experimental period (39 days during July to August 2003). Eight snails were placed on each stromatolite, approximately the same as ambient densities (Elser *et al.* 2005b). Suspending the stromatolites in the experimental containers (rather than, for example, merely placing them on the bottom) prevented the snails from leaving the stromatolite and thus not experiencing the stromatolite food environment. At the end of the experiment, surficial biomass of each stromatolite was sampled for determination of organic matter content and elemental composition (see below) and individual snails were removed, recorded, and re-weighed.

Stromatolite elemental composition

Following previous approaches (Elser *et al.* 2005a), biomass elemental composition (C and P) was estimated by scraping three replicates of *c.* 100 mg of material from the surface of the stromatolite and combining the surficial material for later analysis. The organic matter content of the samples was estimated based on an established method (Hedges & Stern 1984): the sample was dried for 12 h at 60 °C, weighed, and then combusted for 2 h at 525 °C using a muffle furnace, re-equilibrated to room temperature for 30 min to account for loss of hydration by $CaCO_3$, and re-weighed. The loss of mass upon combustion was converted to organic C by assuming that organic matter was 42% C by dry weight (Elser *et al.* 2000). P was estimated by dissolving the post-combusted sample in 5 N H_2SO_4 for 30 min, bringing the sample to a known volume with deionized water, and then analysing the solution for PO_4 using colorimetric analysis (APHA 1992). Biomass C : P was estimated by dividing the %C by %P values for each sample and then converting to an atomic ratio by multiplying by 2.54.

RESULTS AND DISCUSSION

Before describing our data in detail and considering their potential implications, we note here that these particular findings pertain only to the modern stromatolite-based ecosystems at our Cuatro Ciénegas study site. Their relevance to other such systems and to the early food webs of the Cambrian remains to be determined. We also note that, while the microscopic morphometry of these oncoids resembles various microstructures recorded in the fossil record (see Methods) and suggests that our study system is a reasonable analogue for at least some ancient environments, extrapolation from modern ecological experiments to ancient contexts should be done with caution. Thus, we present these data only as an assessment of the feasibility of the hypothesized stoichiometric mechanisms affecting past evolutionary events. This, in turn, may stimulate further research evaluating these mechanisms in other stromatolite-based food webs on the modern Earth and, importantly, in the paleontological and paleoenvironmental record.

The organic matter content of the surficial stromatolite materials was unaffected by P-enrichment ($P > 0.50$ in ANOVA), indicating that the absolute quantity of food available to the snails was uniform across P-enrichment treatments. PO_4 enrichment significantly decreased stromatolite C : P ratio from *c.* 800 for freshly collected stromatolites and *c.* 1100 for those in the unenriched treatment to *c.* 150 in the + 10 μM treatment (Fig. 1d;

$P < 0.0001$ in ANOVA). Note that C : P values in unenriched and low-enrichment (< 1.25 μM) treatments are high, considerably exceeding, for example, the C : P ratio (*c.* 300) that induces P-limitation in crustacean herbivores (Sterner & Elser 2002). Effects of P-enrichment were strongly transmitted to the herbivore trophic level, both under conditions of low and high stromatolite C : P (Fig. 2). To evaluate these effects, it was necessary to account for the inherent size dependence of growth rate by first regressing growth rate against log-transformed body mass, yielding an estimate of the slope of the body size relation. Treatments with animals performing robustly at small sizes would exhibit steep negative slopes, while those in which animals were doing poorly would produce shallow slopes. Body–size relationships were generally very strong within each P-enrichment treatment (Fig. 2a,b; $P < 0.001$; $R^2 > 0.90$). A distinct unimodal relationship between the slope of the body size function and stromatolite C : P ratio in the eight treatments was observed (Fig. 2b). Increased growth was observed when small amounts of P were added (0 vs. 0.1–0.5 μM P; stromatolite C : P > 400) but substantial declines were observed when P-enrichment exceeded 1.25–2.5 μM (C : P < 400). Snail survivorship also responded to P-enrichment (Fig. 2c): survivorship increased moderately as P was added up to 1.25 μM (C : P *c.* 400), after which survivorship decreased strongly. Combined effects on

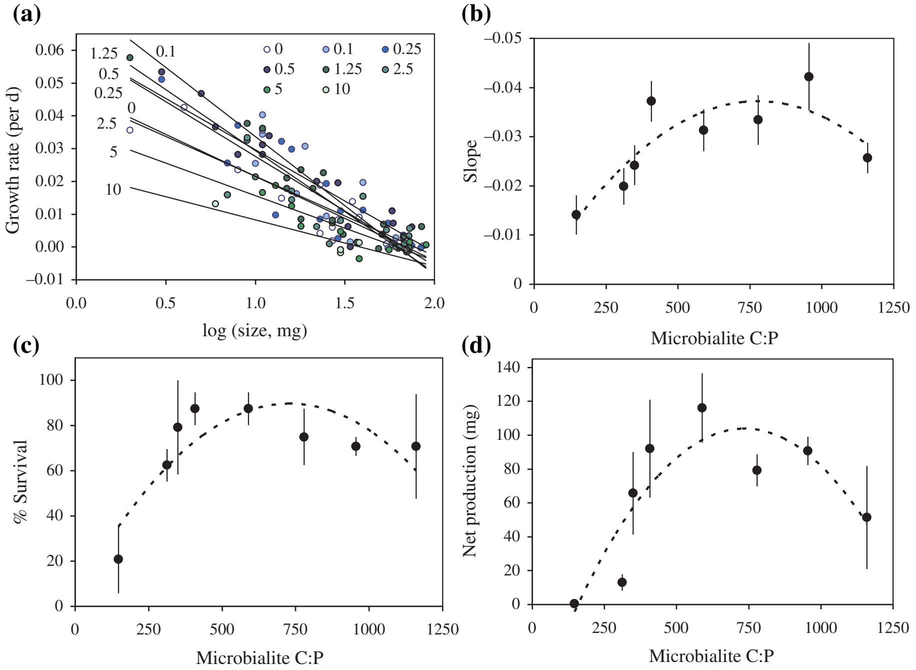

Figure 2 Response of the primary stromatolite grazer, the hydrobiid snail *Mexithauma quadripaludium*, to experimental P-enrichment at Rio Mesquites, Cuatro Ciénegas. (a) Size-dependence of individual growth rate in the eight experimental treatments. All snails obtained from replicate stromatolites in a given treatment were combined in a single regression analysis for that treatment. All regression relationships were highly significant ($P < 0.001$, $R^2 > 0.90$). (b) Unimodal response of size-corrected growth rate to stromatolite C : P ratio. In this figure, the slope of the body size relationship of growth (a) for snails in each P-enrichment treatment is plotted against that treatment's corresponding stromatolite C : P ratio. A quadratic function fit to the data was statistically significant ($P < 0.05$, $r^2 = 0.71$) and yielded the following equation: $Y = -5 \times 10^{-4} - 9.4 \times 10^{-5}X + 6.1 \times 10^{-8}X^2$. Error bars indicate ± 1 SE for the regression slopes from (a). (c) Unimodal response of snail survivorship to stromatolite C : P ratio generated by the different P-enrichment treatments. Survivorship was determined by recovery of individually marked snails at the end of the 39-day experimental period. A quadratic function fit to the data was marginally significant ($P < 0.06$, $r^2 = 0.68$) and yielded the following equation: $Y = 4.32 + 0.23X - 1.6 \times 10^{-3}X^2$; both the linear and quadratic terms were statistically significant ($P < 0.05$). The overall effect of P-enrichment treatment on snail survivorship (square-root transformed) was statistically significant (ANOVA, $F_{7,16} = 2.99$, $P < 0.04$). Error bars indicate ± 1 SE for percentage survivorship in each treatment. (d) Unimodal response of average 'net' snail production (the sum of mass increments for all marked snails still present at the end of the experiment, averaged over all replicates for each treatment), a metric that integrates both growth of individual snails (b) and survivorship (c). A quadratic function fit to the data was statistically significant ($P < 0.03$, $r^2 = 0.76$) and yielded the following equation: $Y = -64.8 + 0.46X - 3 \times 10^{-3}X^2$; both the linear and quadratic terms were statistically significant ($P < 0.05$). Error bars indicate ± 1 SE for mean net snail production in each treatment. The overall effect of P-enrichment treatment on net snail production was highly significant (ANOVA, $F_{7,16} = 4.33$, $P < 0.008$). In all cases shown in (b)–(d), linear fits to the data were non-significant and quadratic regressions were significantly better than linear functions according to a likelihood ratio test ($0.04 < P < 0.09$).

growth and survivorship were strongly apparent in the 'net' production of snail biomass during the experimental interval (Fig. 2d; estimated by summing, in each replicate, the mass changes in all marked snails surviving until the end of the experiment): production increased for small amounts of PO_4 enrichment but declined precipitously when enrichment exceeded 1.25 μM and stromatolite C : P ratio was *c.* <400.

Thus, our data suggest that snails at Cuatro Ciénegas live on a 'stoichiometric knife edge', with a significant constraint because of the low P content of stromatolites under natural conditions but with high sensitivity to negative effects of high P supply. At high stromatolite C : P, the increase in snail performance as stromatolite C : P was lowered by P enrichment is easily interpreted as a relaxation of stoichiometric constraints imposed by the low P content of the food base, as in a variety of studies published in recent years (Stelzer & Lamberti 2002; Sterner & Elser 2002, and references therein). However, the decline in snail performance with further lowering of C : P is a more novel result requiring explanation. Before discussing this, we note that similar unimodal growth responses to dietary P content have been reported previously including studies with abalone (Tan *et al.* 2001), shrimp (Dy Penaflorida 1999), the waterflea *Daphnia* (Plath & Boersma 2001), and mayflies (Frost & Elser 2002), although the significance of such responses in natural settings has not yet been widely considered by physiological ecologists (Boersma & Elser in press).

A variety of possibilities for the decline of snail growth and survivorship at low stromatolite C : P exist. In a laboratory study of the crustacean *Daphnia*, decreased growth rate at very low food C : P was shown to be the result of decreased animal feeding rate, as animals apparently fed to meet their physiological P demand and, in doing so when C : P was unnaturally low, failed to meet their energy requirements. Another possibility is that the species composition or biochemical composition of the microbial community in the stromatolite shifted such that toxic effects because of various defensive molecules were imposed. However, our previous work (Elser *et al.* 2005a) has shown that P addition in these systems shifts the microbial community from dominance by cyanobacteria, normally viewed as unnutritious and potentially toxic, to dominance by diatoms, normally considered a higher quality food (Brett & Muller-Navarra 1997). However, it is conceivable that behavioural responses such as selective feeding by the snails contributed at least partially to the observed responses if, for example, preferred food taxa in the microbial community had their highest abundance or production under conditions of intermediate P supply. We have no data to resolve this possibility.

Another alternative for the decline of snail performance in high P enrichment treatments is that high P intake itself was toxic to the snails. It is well known that cytoplasmic PO_4 levels are tightly regulated (Dzeja & Terzic 2003), as these are metabolically coupled to a variety of key biochemical transformations (such as bioenergetic synthesis of ATP). It may be the case that, for a taxon whose normal environment presents food with very low P content, highly efficient P assimilation and retention mechanisms have evolved but that there has been little selective pressure on homeostatic mechanisms for releasing excess P. Thus, exposure to foods with high P content may present a metabolic overburden to the consumer such that growth and survivorship are significantly impaired. Considerably more research will be required to evaluate these alternatives.

To the extent that responses under conditions of low P supply are representative of ancient conditions, our data have various implications for a possible role of stoichiometric constraints in evolutionary events surrounding the Cambrian radiation. Here we present a hypothetical sequence of events that we hope can stimulate explicit tests (Fig. 3). At least some paleoenvironmental data suggest that stromatolitic microbes in the Precambrian grew under conditions that would generate severe P-limitation and high biomass C : P ratio. First, the oceans of the early Earth do appear to have had extremely low levels of circulating P, suggesting wide-spread P-limitation (Cook & Shergold 1984; Brasier & Lindsay 1998; Bjerrum & Canfield 2002) that would favour production of biomass with high C : nutrient ratio in autotrophs (Sterner & Elser 2002). Second, processes of calcification associated with stromatolite morphogenesis (Garcia-Pichel *et al.* 2004) may also have further lowered PO_4 availability to stromatogenic microbes because of sorption of PO_4 by carbonates (Hinedi *et al.* 1992) or trapping of organically bound P in rapidly accruing carbonates, as in modern calcareous environments (Fourqurean *et al.* 1992; McGlathery *et al.* 1994; Koch *et al.* 2001). Finally, the global P cycle showed significant changes during the Precambrian–Cambrian transition, as a geochemical signal of the Cambrian transition in the sedimentary record is the appearance of massive phosphorite deposits (Cook & Shergold 1984; Cook 1992; Lambert *et al.* 1992; Shen *et al.* 2000). Although the precise timing of phosphorous increases during the late Proterozoic is not well constrained, it seems clear that, as with the modern Earth, increases in circulating P could have been promoted locally by wind-driven upwelling systems that would have developed at subtropical latitudes along the western margins of the late Proterozoic supercontinent, Rodinia (Cook 1992; Donnadieu *et al.* 2004). In addition, the appearance of fungal and algal life in on-land ecosystems (McMenamin & McMenamin 1994), sometime between *c.* 700 and 600 Ma based on molecular clock estimates (Hedges *et al.* 2004), is likely to have accelerated biogenic weathering of continental rocks and soils. While this would have been balanced against

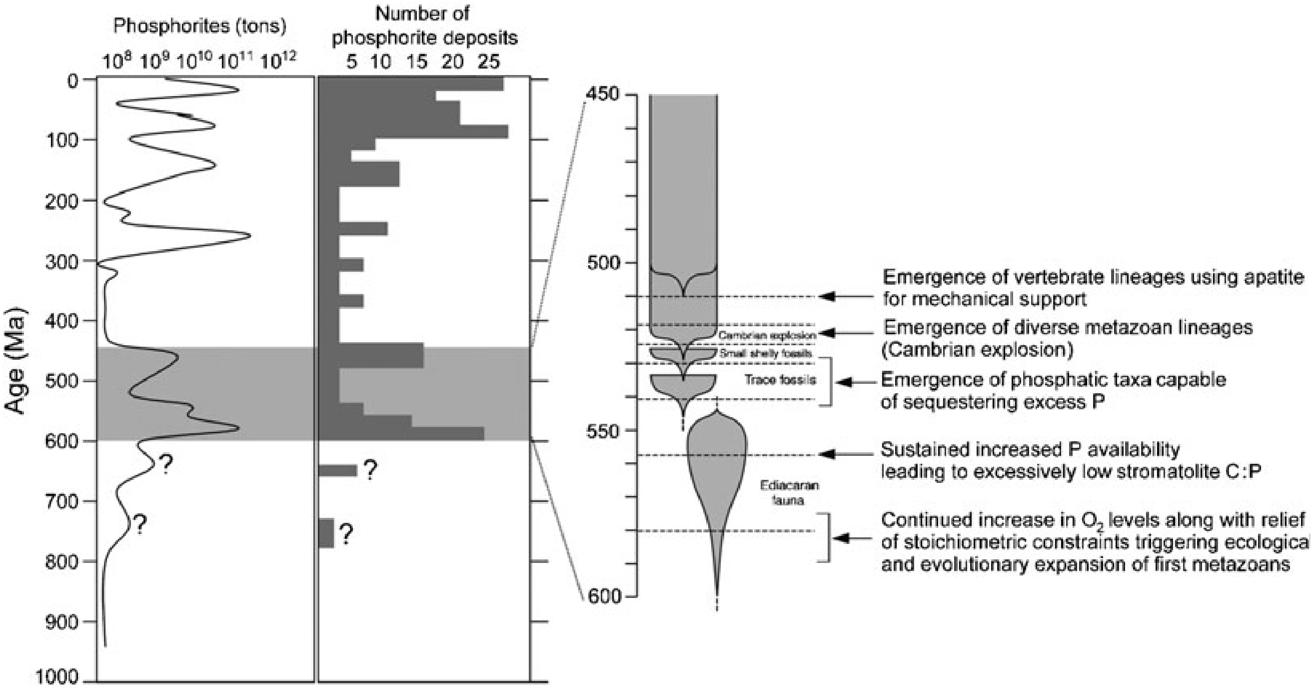

Figure 3 Hypothesized sequence of events surrounding the Precambrian–Cambrian transition via changes in circulating P availability (Cook & Shergold 1984; Brasier 1990) and subsequent impacts on food quality according to the 'stoichiometric knife edge' demonstrated in Fig. 2. The geologic record of phosphorite deposits (left) shows massive increases in the biosphere *c.* 600 Ma at the time of the diversification of the Ediacaran fauna. Note the logarithmic scale for phosphorite tonnage. Question marks indicate uncertainty about the validity of reported phosphorite deposits during the late Precambrian (Cook & Shergold 1984). In the proposed scenario, the initial increase in circulating P at the end of the Proterozoic relieved stoichiometric constraints and contributed to the evolutionary expansion of primary consumers, including the expansion of the first Metazoa. With sustained increases in circulating P, stromatolite C : P ratios would continue to decline, eventually exceeding the threshold for negative effects such as those shown for snails at Cuatro Ciénegas in the low C : P regions of Fig. 2b–d. According to our tentative proposal, this may have contributed to the mass extinction of the Ediacaran fauna and favoured the eventual emergence of metazoan taxa capable of sequestering excess P in mineral form internally or externally. Subsequently, this ability to form mineral apatite was used by early vertebrates in the evolution of bone needed for mechanical stiffening as they evolved large body size. Figure adapted from Cook & Shergold (1984).

the tendency of these same ecosystems to tie up P and other nutrients on land (see McMenamin & McMenamin 1994), a net increase in the delivery of P to the oceans appears plausible and is supported by the accumulation of massive marine phosphorite deposits at this time. The break-up of Rhodinia at the end of the Proterozoic would have also enhanced nutrient fluxes to the oceans because of elevated runoff and delivery of soluble weathering products to an increasing number of coastal margins.

Thus, as with Cuatro Ciénegas snails under high stromatolite C : P ratio, we suggest a scenario in which the period preceding the Precambrian–Cambrian boundary (> 600 Ma, Fig. 3) was a time of severe stoichiometric constraints on the ecological and evolutionary expansion of primary consumers, in which the evolution of the metazoan trophic strategy was limited not by an insufficient total quantity of ecosystem productivity but instead by its poor quality (high C : P). These constraints were relieved by the first substantial increases in circulating P *c.* 600 Ma (Cook & Shergold 1984; Cook 1992; Baturin 2003) that acted in concert with higher oxygen levels to stimulate the radiation of the first metazoans (the Ediacaran fauna at *c.* 580 Ma, Fig. 3) by permitting higher metabolic rates and enhanced production of P-rich ribosomal RNA needed for sustained high growth rates (Elser *et al.* 2003). This expansion of grazing animals during the lower Cambrian (530 Ma) is correlated in time with the major late-Proterozoic decline in stromatolite diversity and abundance (Awramik 1971; Walter & Heys 1985; Grotzinger & Knoll 1999). Conversely, our data showing negative effects of low food C : P ratio on snail growth and survivorship suggests a novel mechanism for how major increases in nutrient availability might precipitate mass extinction, as occurred with Ediacaran fauna at the end of the Vendian (Martin 1999). While it is unclear whether the rapid decline of the Ediacaran fauna beginning *c.* 550 Ma (Weiguo 1994, Fig. 3) was because of widespread extinction (Narbonne 2005) or reflects taphonomic bias, our data suggest that sustained high levels of P supply could also have contributed to the observed decline. More specifically, it is possible that these taxa, having diversified from lineages with a history of strongly P-limited food conditions, were highly susceptible to negative effects of low C : P ratio in their food base during the end Proterozoic increase in available P.

These mechanisms may also shed light on subsequent events. For example, following the demise of most of the Ediacaran taxa, at least some of the first metazoans to proliferate at the base of the Cambrian (Fig. 3) were small-bodied taxa having phosphatized exoskeletons (Bengtson *et al.* 1992; Kirschvink & Hagadorn 2000). We tentatively propose that these some of these forms were favoured, and may have given rise to extant vertebrate lineages, because they could sequester P in mineral form and thus avoid the negative effects of excess dietary P intake. In our suggestion, this ability to direct the deposition of mineral apatite may then have laid the groundwork for the appearance of ancestral vertebrates from among these early forms (*c.* 525 Ma, Fig. 3). In such a scenario, bone, now necessary for the mechanical support of large body plans, can be seen as an exaptation (*sensu* Gould & Vrba 1982), from a previous function of PO_4 detoxification, consistent with previous discussions of the role of exaptation in biomineralization (Kirschvink & Hagadorn 2000).

While tentative, our proposal provides a complementary perspective in relation to prevailing explanations for the Cambrian explosion (i.e. increased oxygen levels, Runnegar 1982; Thomas 1997), in the sense that it suggests that higher oxygen levels were necessary, but perhaps not sufficient, for the evolution of the diversity of metazoans that now dominate the scene. While our evidence indicating a role for the key nutrient P in metazoan evolution is preliminary and necessarily indirect, ecological stoichiometry does add a new mechanistic dimension to the impacts of eutrophication in the history of life. These hypotheses require further testing, both in the geological record via paleoindicators of biomass stoichiometry (Watanabe *et al.* 2000) or biotic P processing (Blake *et al.* 2001) and in longer-term field experiments in other modern stromatolite-based ecosystems. However, by integrating ecosystem ecology with nutritional physiology and evolutionary biology, analyses based on ecological stoichiometry may help illuminate the multivariate mechanisms that underpinned one of life's most important episodes.

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