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Ecological stoichiometry: An elementary approach using basic principles

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Abstract

Based on the observation that organism-specific elemental content creates ecologically relevant mismatches such as between plant and animal tissue, it was postulated—and experimentally verified—that this would profoundly affect trophic efficiency and nutrient fluxes in ecosystems. From its beginnings as a *Daphnia*-centered perspective, the field of ecological stoichiometry (ES) has widened to include many organism groups, and ecosystem types, and the questions it addresses have broadened. We address some of the development of ES in aquatic sciences especially over the past 10 yr, focusing on homeostasis and mass balance in the consumer, and its effect on trophic efficiency and nutrient recycling in aquatic communities. We also discuss how ES has provided novel insights into genomic, proteomic, and cellular responses at one end of the biological scale as well as into large-scale effects related to biogeochemical couplings at the ecosystem level. The coupling of global C, N, and P cycles via their biotic interactions and their responses to climate change accentuate ES as an important toolkit for ecosystem analysis. We also point to some of the major topics and principles where ES has provided new insights. For each of these topics we also point to some novel directions where the ES concepts likely will be useful in understanding and predicting biological responses.

Ecological stoichiometry (ES)—theory and development

Concepts and history—Nothing takes its beginning from itself, and so it also is for ecological stoichiometry (ES), which rests foremost upon the Law of the Minimum principle originally formulated by the botanist Carl Sprengel and later popularized by Justus von Liebig (1840). In one of its forms, this law states that in a finite universe organism growth will become limited by the one element in lowest environmental supply relative to organismal demands. Today's research on elemental limitation and optimal elemental ratios thus represents a long tradition in plant or agricultural sciences. Simultaneously, a parallel idea of animals (or heterotrophs in general) being generally limited by energy prevailed among the majority of ecologists. This focus on energy gained strength from Lindeman's seminal ideas on trophic levels (Lindeman 1942), where energy was the key currency transferred between food-web compartments, and where trophic efficiency equaled energy use efficiency. If all organisms had identical requirements for energy and all possible materials, any of these parameters would be mathematically redundant to the others, and a single dimension would suffice for representing biological dynamics. ES, however, is based on departures from this assumption (Sterner and Elser 2002). In this review we will focus on recent developments related to the question of homeostatic control, which is central to mass balance estimates as well as trophic efficiency analysis, nutrient recycling, and cell biology or systems biology. The contrast in potential range of variability of element ratios in autotrophs vs. herbivores is a critical contrast, affecting community composition, population dynamics, and stability (Urabe et al. 2002; Andersen et al. 2004). Since the scope of ES extends from cellular responses in terms of P and N regulation of growth rate and protein synthesis, metabolism, and genomic responses to biogeochemical couplings at the ecosystem and global levels, we will also touch upon these issues, but less comprehensively. For each major topic we also point to some novel directions where the ES concepts likely will be useful in understanding and predicting biological responses. Our goal here is not for complete coverage, which would require much more space, but to provide a selective update to some topics compared with where the field was approximately 10 yr ago when the field was comprehensively described (Sterner and Elser 2002).

Historically, most of this line of thinking was foreshadowed by the work of Redfield et al. (1963) and by studies on copepod elemental mass balance (Corner et al. 1976). This line of study did not flourish at this time, perhaps because the variability in marine C : N : P ratios is less than what is observed in freshwater. A finding important to the development of ES was that pelagic consumer-driven recycling was a strong trophic link and was affected by both algal and zooplankton characteristics (Lehman and Naumoski 1985; Olsen et al. 1986). Subsequently, recycling rates and ratios were explicitly linked to a stoichiometric and predictive mass balance approach in context of consumer–food interactions that came to be known as *ecological stoichiometry* (Sterner 1990; Elser and Hassett 1994).

Over the past three decades ES has expanded greatly. The number of ISI Web of Science citations including to the word "stoichiometry" in this journal has increased from 0 in 1990 to almost 400 by 2010, and the annual citations

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for the phrase "ecological stoichiometry" in ecological journals has gone from 0 to 2000 over the same period. The building blocks summarized above provided the foundational material for an integrated synthesis published just over 10 yr ago (Sterner and Elser 2002), and ES has continued to expand. New habitats, new organisms, and new approaches are being added to the field. Both in sheer volume and diversity of themes ES has grown well beyond a point where a complete coverage of the literature can be provided in one review. A pertinent question in fact is what should most usefully be considered as part of ES, because the term is sometimes now used to indicate any consideration of nutrient elements. We encourage the use of the definition of ES given by Sterner and Elser (2002): "The balance of multiple chemical substances in ecological interactions and processes, or the study of this balance. Also sometimes refers to the balance of energy and materials." This definition is true to the spirit of the use of the term in balancing chemical reactions in chemistry.

Stoichiometry in producer–herbivore interactions—To what extent are energy flux in ecosystems and the shape of trophic pyramids governed by quantity or quality of primary producers? Is herbivore production limited by food quantity or quality? And how, eventually, do food quality constraints propagate up the food chain? Answers to these questions have broadened from focus solely on food quantity to an increased emphasis on multiple dimensions of food quality. ES has offered a complementary perspective (Reiners 1986) to one based purely on energy.

Living matter consists of more than 20 mostly unsubstitutable elements, and whichever of these is in shortest supply relative to demands may in principle be rate limiting to growth—not just to plants, but to all organisms. The biomass of all living species shares a common core recipe, but diversity in terms of biological function also means chemical elements are not found in precisely the same proportions in all organisms. That metazoans and other heterotrophs often seem to face an unbalanced diet relative to their demands for growth made the insights of Liebig relevant also for consumers. Since plants consist of relatively more C-rich structural or storage matter than animals, herbivores must typically ingest a diet deficient in nutrients such as nitrogen (N) and phosphorus (P) relative to their own somatic demands, which may lead to surplus C even when respiration is accounted for. This stoichiometric mismatch between plants and herbivores is more pronounced in terrestrial than in aquatic systems, meaning that trophic efficiency or carbon use efficiency is higher in aquatic relative to terrestrial systems, with forests and pelagic ecosystems representing the extremes of a continuum (Cebrian 1999). The higher efficiency in pelagic systems partly reflects less need for C-rich supportive tissue such as lignin and cellulose, but also the minute size of the phytoplankton as well as a more favorable stoichiometry from a consumers' point of view. Hence while herbivores certainly are worse off in terrestrial systems, they also frequently suffer stoichiometric imbalance in aquatic habitats. As shown in Fig. 1, there are major disparities

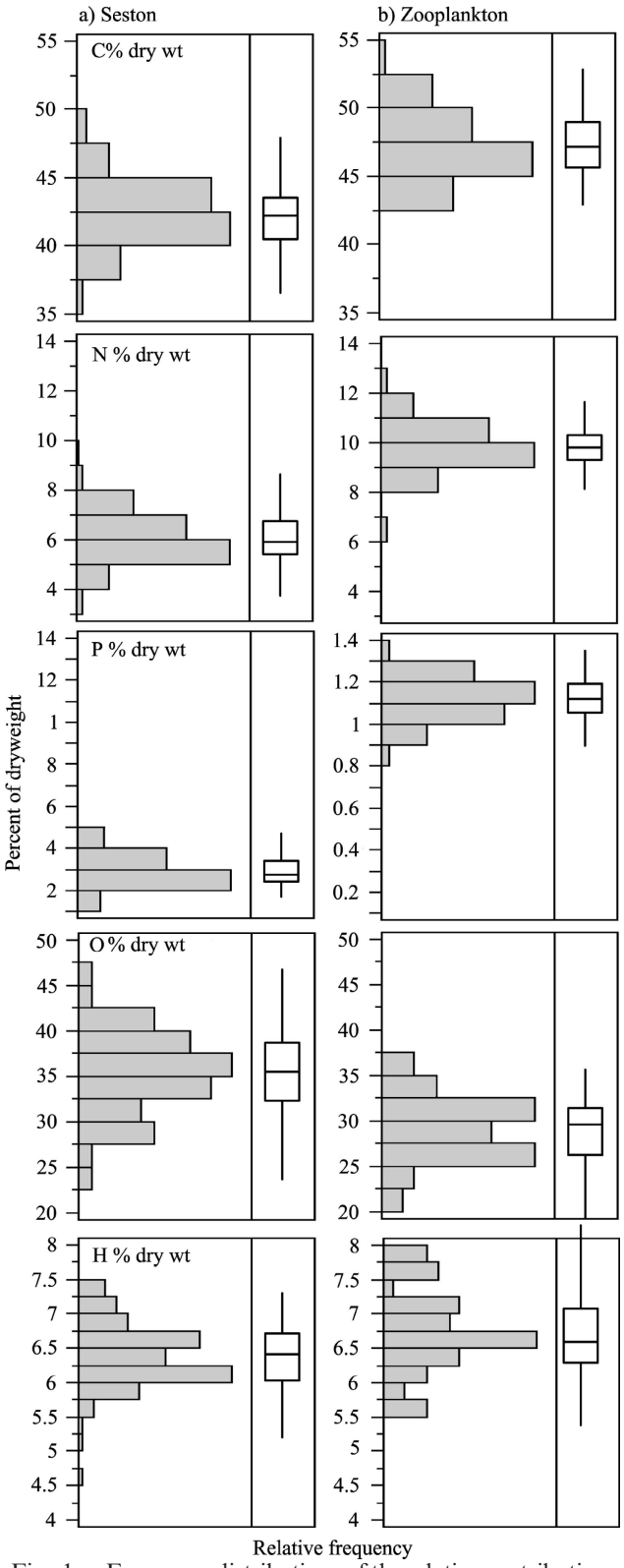

Fig. 1. Frequency distributions of the relative contributions of carbon, nitrogen, phosphorus, oxygen, and hydrogen to the mass of bulk lake (a) seston and (b) zooplankton, and the disparities in their elemental content based on monthly samples over 3 yr from Lake Vuntus, the Netherlands. Box plots represent median (horizontal line), 25% confidence interval (box), and 95% confidence interval (vertical lines; from Hessen 2008).

in the major elements between zooplankton and their food (seston), with the strongest deviation for P, followed by N, which also has been suggested to be a key limiting element for consumers (White 1993, but *see* Cease et al. 2012). Stoichiometric work on N has continued this line of thinking, but the main focus in ES, however, has been on P. Reasons for this are (1) that the C : P ratio is often more variable in biomass than C : N; (2) that P can be explicitly linked to growth via the biosynthetic machinery; and (3) that P limitation of grazer growth gained support both from theoretical and empirical studies.

A first strong indication of P limitation in aquatic animals came from studies of *Daphnia* P release along a gradient of P : C in their algal food (Olsen et al. 1986). Grazer P-release rate was negatively correlated with algal P content, and tellingly approached zero release at low, but still ecologically realistic, P : C ratio. This more efficient P housekeeping under stronger P deficiency suggested they could suffer from direct P limitation. At about the same time, a dissimilarity in elemental composition in stoichiometric ratios between lake seston and zooplankton in natural systems was observed, and studies on C : N : P ratios in a range of zooplankton species suggested a more stable elemental ratio *within* species (indicative of a homeostatic regulation) than *between* species (Andersen and Hessen 1991). Cladocerans, especially daphnids, had higher P and lower N contents (as a percentage of dry mass) compared with copepods. These lines of reasoning were brought together (Sterner et al. 1992) to explain how food-web changes affect grazer P limitation (Hessen 1992; Sterner and Hessen 1994) and altered nutrient use and recycling of N and P previously observed by Elser et al. (1988).

An early critique of ES was that metazoans, unlike osmotrophs, ingest packages of food containing macromolecules of mixed origin and thus would be unable to discriminate between specific elements and accordingly adjust the uptake of specific elements over the gut wall (Brett 1993). Moreover, several studies indicated that growth rates and fitness of aquatic grazers responded strongly to specific macromolecules such as certain polyunsaturated fatty acids (Müller-Navarra 1995), essential amino acids (White 1993; Anderson et al. 2004), or sterols (von Elert et al. 2003), rather than single elements or bulk carbohydrates, proteins, or lipids.

Since the biochemical makeup in autotrophs often depends on their degree of nutrient limitation and thus may be confounding factors, establishing causality from these correlations has been problematic. Some insights into the molecule vs. element conundrum has come from studies of digestive enzymes. Up-regulation of specific digestive enzymes such as alkaline phosphatases in response to dietary P restriction (McCarthy et al. 2010; Wojewodzic et al. 2011) strongly suggest that uptake of P over the gut wall is adjusted to meet stoichiometric demands. However, the point is that, even if a major share of elements is assimilated along with other elements in molecular packages, they can be redirected to different purposes within the body; e.g., P will be directed to maintenance and construction of nucleic acids, phospholipids, or energy carriers such as adenosine triphosphate (ATP; Ventura 2006). For metazoans, P is also imperative for exoskeletons or bones, while N will be directed to proteins, while C is most prevalent in carbohydrates or lipids (for a more comprehensive overview, *see* Sterner and Elser 2002). Neither biochemical nor elemental limitations necessarily subsume the other, and they may well operate at different places and times (Sterner and Schulz 1998). There are no irreconcilable inconsistencies between a molecular and an elemental approach, but the latter has the distinct advantage that it can take advantage of mass balance.

Confirmation of direct, P-based stoichiometric constraints on consumer growth has accumulated in recent years, mostly from laboratory experiments. Early evidence came from the experiments of Urabe et al. (1997), in which P-limited *Daphnia* gained increased growth when "soaked" in high concentrations of inorganic P, clearly demonstrating the direct potential of P alone to boost growth rate. These inferences were further supported by the elegant "spiking" experiments in which P-deficient algae were supplied with inorganic P immediately before being fed to *Daphnia* (providing elevated cellular P without changes at the macromolecular level), stimulating animal growth and demonstrating clearly that low P alone is sufficient to limit animal growth (Rothhaupt 1995). These, and a suite of follow-up experiments in lab and field settings (Elser et al. 2001), have demonstrated convincingly that P deficiency can constrain the growth rate of consumers. This is not to say, however, that other nutritional deficiencies cannot coincide, and at times override, such P-based constraints. Multiple algal characteristics—cell size, biochemical make-up, and cell wall thickness—enter into the transfer of energy and materials from plant to herbivore. Studies have shown how P-deficient algae can be resistant to digestion so that grazers may be limited by energy even when consuming high C : P food (DeMott and Tessier 2002). Such results point to complications in interpreting field stoichiometric data, but they do not reject a stoichiometric perspective per se. If digestion resistance affects the relative uptake of C vs. P, it has stoichiometric implications for the potential shifts between energy and mineral nutrient limitation. We will address this situation further in the section dealing with threshold elemental ratios.

Recent evidence from marine systems has demonstrated that not only primary but also secondary and tertiary consumers in pelagic food webs can be potentially constrained by insufficient nutrient content in their prey (Malzahn et al. 2010). This means that a bottom-up effect of poor stoichiometric quality may propagate up the food chain, not only because of poor production of the herbivores, but also because of direct, stoichiometric constraints. By and large, however, carnivores should be less likely than herbivores or detritivores to face N or P deficiency (Gaedke et al. 2002).

Also consumers from within, i.e., endoparasites, are also potentially affected by the stoichiometry of their host. Hosts and pathogens potentially compete for many resources (Smith and Holt 1996). Under nonstrict homeostasis, reduction in element content within a host may affect the competition between hosts and their parasites. Experiments

testing the effect of low resource quality on the growth and reproduction of bacterial (Frost et al. 2008) and fungal (Hall et al. 2009) parasites of *Daphnia* suggest that parasites indeed also suffer from host nutrient limitation. Thus, reduction in parasite virulence may partially offset food quality penalties on consumers or, alternatively, the drain of limiting elements to parasites might further amplify the effects of nutrient limitation on hosts (Andersen et al. 2004). Understanding the interactions between parasites and food quality may be essential in predicting the outcome of infectious disease in aquatic ecosystems in the face of changing nutrient loading and represents a promising frontier for future work.

Homeostasis, mass balance, and trophic transfer efficiency

Homeostasis, a general view—Homeostasis is central to life itself—for example it has been said that a living cell must be capable of three basic things: metabolic homeostasis, cellular reproduction, and Darwinian evolution (Shuler et al. 2012). To achieve positive fitness, organisms must obtain a complement of $\sim$ 20 elements from their surroundings. Because there is a limited range of recipes where all those element building blocks are found in their necessary and available forms for life, the elemental content of living organisms is a highly ordered state compared with the surroundings. The concept of "stoichiometric homeostasis" refers to the regulatory processes that allow such an ordered state of elements to exist; it is a form of negative feedback applied to elemental composition.

In ES, stoichiometric homeostasis is a quantifiable parameter. It was defined (*see* Sterner and Elser 2002) as

$$H = (dx/x)(dy/y) \tag{1}$$

where H (eta) = is a homeostatic regulation coefficient, x = a stoichiometric property of an organism's resources (e.g., %P, P : C, etc.), and y = that same stoichiometric property within the organism itself.

As written here, one can see that stoichiometric homeostasis is the proportional change in the chemical makeup of an organism's resources relative to the proportional change in that same chemical property of the organism itself. Note that if the variation associated with the organism (dy/y) is much less than that associated with the resources (dx/x), $H \gg 1$. The integral form of Eq. 1 is

$$y = cx^{(1/H)} \tag{2}$$

H is expected to range between 1 (no regulation) and ∞ (no change in y relative to x, or "strict homeostasis"). When originally presented, this model was simply an algebraic description of the "flattening" of a slope of y vs. x relative to the null expectation of a slope = y/x (Sterner and Elser 2002), and it seemed to fit the available data. Having no "real" physiological foundation beyond that, there were no underlying biological or physiological reasons to expect this model to fit. Even today, we are unaware of any work that attempts to put this model onto more mechanistic footing; this seems to offer a potentially new research area in organism-level ES.

Several additional convincing cases of where the model fits empirical data have since appeared (Hood and Sterner 2010; Yu et al. 2010). On the other hand, questions have arisen about whether this model (Eq. 1) is likely to apply to the full range of resource stoichiometry (Persson et al. 2010), and it does seem reasonable to expect breakdown of homeostatic regulation in consumers under extreme conditions, such as the many experiments with monoculture food with atomic C : P > 1000. Clear lack of fit has recently been observed, with the identity of the limiting substance important in controlling the degree of homeostasis as measured by H (Scott et al. 2012). The limit of infinity (strict homeostasis) can be inconvenient during statistical analysis, so transforms such as 1/H (Hood and Sterner 2010; Persson et al. 2010) may sometimes be preferable. Another potentially important sense of homeostasis, not explicitly described by this model, is the range of resource stoichiometry under which an organism can regulate its own stoichiometry. The basic model (Eqs. 1 and 2) was not intended to capture all possible senses of homeostatic regulation but to provide a single, simple description appropriate for thinking about stoichiometric homeostasis quantitatively over intermediate, ecologically meaningful ranges of the data. It provided an approach to make "apples to apples" comparisons of the homeostatic regulation of elements in different organisms.

Since Eq. 1 was originally suggested, it has been applied to new cases, and its use seems to be growing. However it was true in 2002 and remains true today that there are major gaps in our knowledge of elemental homeostatic regulation, while some organisms (*Daphnia*, particularly) are much, much better studied from a stoichiometric standpoint than are other organisms. Indeed, many major groups have still not been studied at all. Perhaps the longest standing hypothesized contrast in stoichiometric homeostasis is that autotrophs are generally less homeostatic than heterotrophs. An almost archetypal contrast between C : N : P ratios of chlorophytes, which seem to exhibit a total lack of homeostasis (Fig. 2), and strictly homeostatic animals has often been referred to in the literature. However, these two conditions actually represent endpoints and not necessarily the most typical situations.

This archetypal contrast has since been examined quite extensively, and accumulating evidence indicates that heterotrophs are indeed generally more homeostatic than autotrophs, but many intermediate cases also now are known. Hall et al. (2005) called the generality of the high degree of flexibility associated with *Scenedesmus* into question by looking at patterns of field seston C : N : P as a function of nutrient loading. Klausmeier et al. (2008) also considered this question and suggested that there was an important growth rate dependence of phytoplankton homeostasis—as populations approach their physiological maximum they demonstrate less and less stoichiometric variability and cell composition approaches the "optimal nutrient ratio" (defined there as the ratio of minimal cell quotas). The degree of autotroph homeostasis for any given situation seems to depend on the species composition as well as on conditions. A lack of regulation of stoichiometric homeostasis in plants is probably not generally an accurate assessment.

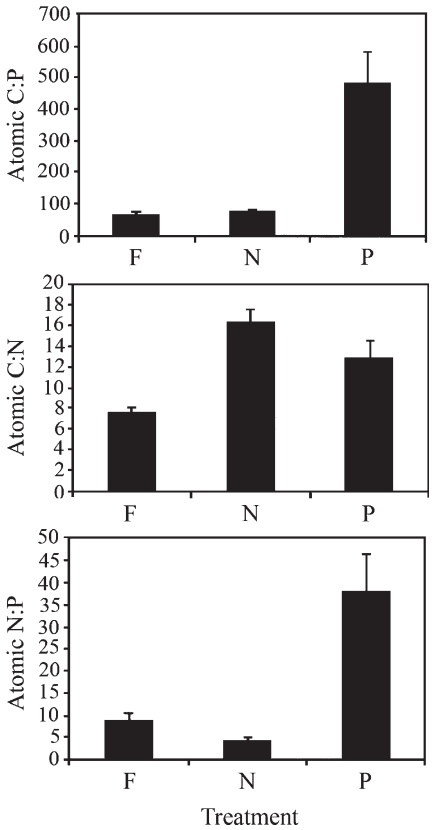

Fig. 2. Stoichiometric flexibility in the chlorophyte *Selenastrum capricornutum* grown under either full medium (F, no limitation), N limitation (N), or P limitation (P). Note the 10-fold variation in C : P in contrast to only twofold variation in C : N, and that P limitation also affects C : N ratio (middle panel). (For details on experimental setup, *see* Hessen et al. 2002).

It is also important to note that the category of "heterotrophs" includes a diverse range of organisms. Is it appropriate to paint all these with a single brush? Several studies have provided new information on the homeostatic regulation within prokaryotic heterotrophs ("bacteria"). Makino et al. (2003) suggested that bacteria were strongly homeostatic but also that they nevertheless exhibited considerable variability associated with strain identity. In this way, they considered bacteria to be, indeed, more like animals than plants. Cotner et al. (2010) reported that field-collected, bacterial-sized lake particles exhibited stoichiometric flexibility, but this could in fact represent species or strain replacements. Scott et al. (2012) showed that homeostasis of C : P was high under P deficiency but weak under P sufficiency (carbon limitation). Other, recent studies on bacterial stoichiometry are adopting new techniques to measure elemental and macromolecular composition of individual cells (Hall et al. 2011*a*,*b*). Overall these microbial studies suggest that some degree of homeostasis is indeed closely associated with a heterotrophic mode of nutrition, even in unicellular osmotrophs.

In this early state of data accumulation it may be premature to characterize typical homeostatic regulation for many groups, but there are more data available now than in 2002. Persson et al. (2010) recently performed a literature review followed by a meta-analysis on the degree of stoichiometric regulation of N and P content for a diverse set of organisms. They identified 132 datasets from 57 studies where the degree of stoichiometric homeostasis could be estimated. Phytoplankton, zooplankton, and fish were all relatively well represented in the studies; although, owing to absence of C data for fish, not all element ratios were able to be examined in that group. In addition, bacteria, fungi, and freshwater as well as terrestrial invertebrates were represented with smaller numbers of cases. The meta-analysis did provide robust support for the generalization that heterotrophs are more stoichiometrically homeostatic than autotrophs, but there was some overlap between the groups. Strict homeostasis was well represented in the data (for N : P, 6 of 20 algal datasets, 2 of 4 bacterial datasets, and 6 of 7 aquatic macroinvertebrates).

Homeostatic relaxation and models—The strict homeostasis condition has often been adopted in mathematical modeling (Sterner 1990; Loladze et al. 2000; Andersen et al. 2004) primarily to improve analytical tractability. Marine food-web models also typically incorporate fixed elemental proportions both in food and consumers, which clearly is an oversimplification, and predictions on productivity, nutrients cycling, and sequestration of transport of C in three-dimensional models are highly sensitive to the degree of homeostasis or flexibility in food and grazer stoichiometry (Anderson et al. 2013). Numerous studies now show nonstrict homeostasis in diverse heterotrophic consumers (DeMott et al. 1998; Chrzanowski et al. 2010; Small and Pringle 2010). Although the most pronounced departures from homeostasis are obtained under a truly extreme range of C : P in the diet (< 80 to > 1000), it must be asked whether previous theoretical results are fragile to relaxation of the strict homeostasis assumption. One study (Wang et al. 2012) concluded that dynamic properties of models were retained even after some relaxation of the strict homeostasis assumption, with the sensitivity of the model to altered homeostasis depending on parameter choice such as herbivore turnover rate, but more model analyses are needed where the effects of modulating the degree of homeostasis are studied.

Mechanisms of elemental adjustments—There are multiple potential points of homeostatic control in organisms (Frost et al. 2006; Wagner et al. 2013). Indeed, genome-enabled approaches have now documented the incredibly large set of biochemical adjustments that are made by organisms under different limitation regimes (Carlson and Taffs 2010; Jeyasingh et al. 2011). With so many adjustments happening at once, it may be quite difficult to characterize in simple terms how organisms maintain stoichiometric homeostasis. Homeostasis can in principle be maintained via food selection (Simpson and Raubenheimer 1995; Raubenheimer and Jones 2006); via elemental intake adjustments with energetic expenditures by feeding behavior (Plath and Boersma 2001); or via regulating assimilation across the cell membrane or gut wall, postassimilative metabolism, or any combination of the above (Anderson et al. 2005; He and Wang 2008; Suzuki-Ohno et al. 2012). For the presumably

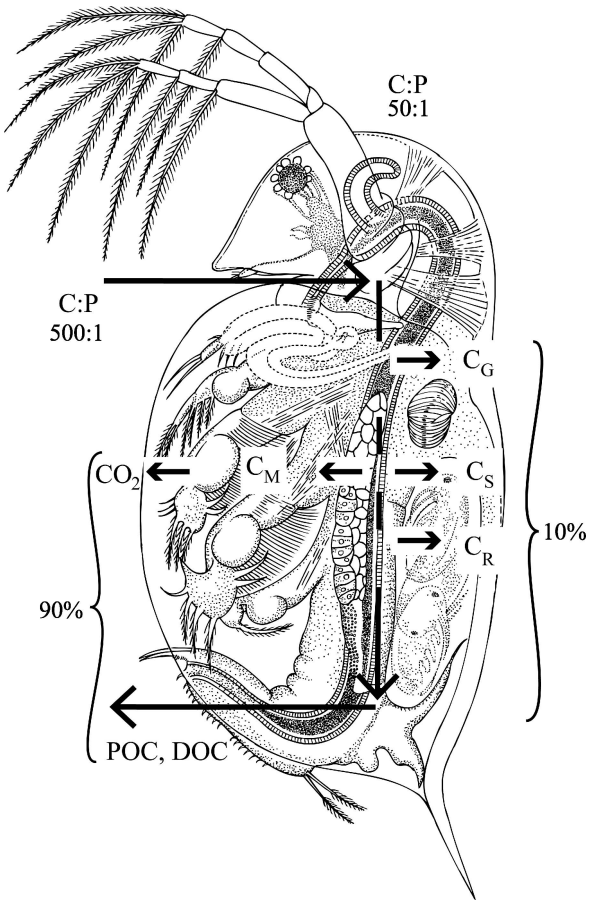

Fig. 3. Intake and potential fates of C in a consumer related to the C : P ratios in consumer (50) and food (500). Ingested C may be used for growth (C_G), storage (C_S), or reproduction (C_R), or disposed of as respiratory metabolic costs (C_M) or as undigested particulate or dissolved organic C. In this tentative example, growth efficiency will be 10%.

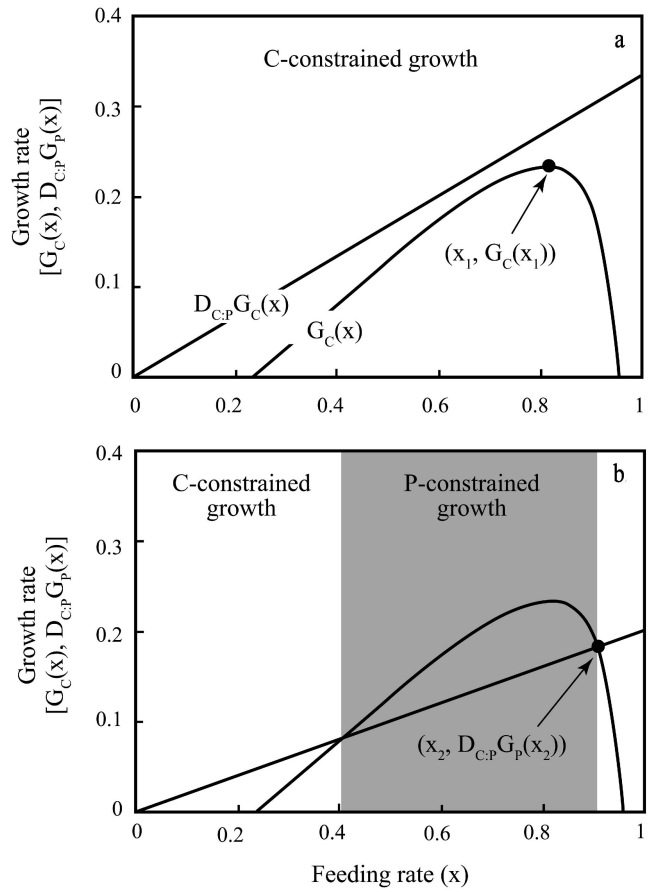

Fig. 4. An optimal feeding model illustrating the relationship between growth rate and feeding rate (x) of consumers at (a) P-rich food conditions and (b) P-poor food conditions. The growth rate in terms of C ($G_C(x)$) increases with feeding rate to a level where energetic cost exceeds C acquired by ingestion and assimilation. On the other hand, there is no P cost accompanying feeding effort. Accordingly, growth rate estimated from acquired P and rescaled by the C : P ratio of consumer ($D_{C:P}G_P(x)$) increase linearly with feeding rate. The slope of this line is determined by the relative P content and assimilability of the food. Depending on shape of the convex curve ($G_C(x)$) and slope of the linear line ($D_{C:P}G_P(x)$), the growth rate at a feeding rate is constrained either by C ($G_C(x) \leq D_{C:P}G_P(x)$) or P ($G_C(x) \leq D_{C:P}G_P(x)$). After Suzuki-Ohno et al. (2012).

common case of "excess C" (Hessen and Anderson 2008), there are various routes for disposal (Fig. 3). In addition to increased respiration, consumers may temporarily store C in energy and C-rich storage compound (lipids), dispose of excess C as dissolved organic forms as shown in recent studies (Darchambeau et al. 2003; He and Wang 2008), or deposit excess C as nonviable eggs that are rich in C relative to nutrient elements (Urabe and Sterner 2001).

How organisms adjust feeding rate in the context of element excess was recently examined by combining ES with functional response and optimal feeding theory (Suzuki-Ohno et al. 2012). From the standpoint of C or energy alone, at any given food level there is one feeding rate that maximizes net intake because the increased feeding rate involves disproportionately increased energetic costs and thus eventually net loss of C (x_1 in Fig. 4). However, this optimal feeding rate will not necessarily also optimize acquiring other elements because loss of these other elements is not necessarily proportional to metabolic energy losses. Hence, an optimal feeding rate on P-deficient food should be higher compared with P-sufficient food because it should be the rate maximizing net growth rate by satisfying P demand relative to C demand (x_2 in Fig. 4). Such a feeding response implies that consumers compensate for deficient elements by increasing feeding rate (compensatory feeding). However, this response is not necessarily observed at high food abundance because there is an upper limit on feeding rate by a finite handling time (Suzuki-Ohno et al. 2012).

The precise route that excess elements take during homeostatic regulation has a strong bearing on their effects "downstream" through nutrient cycling (Hessen and Anderson 2008; Sistla and Schimel 2012). At a broader level still, the effect of resource stoichiometry on growth rate and thus size structure of consumer populations, as well as on the success or failure of individual genotypes or species within a guild, all can potentially operate simultaneously in natural

populations (Sistla and Schimel 2012). For these reasons, it is not often advisable to use field-collected data on consumer and resource stoichiometry as a good test for homeostatic regulation. Observing high P consumers in locations with high P resources may reflect local adaptation or even shifts in cryptic species, not an inability for a given organism to regulate in the presence of variation. Applying stoichiometric models to the landscape scale must incorporate such sorting processes as well as physiological adjustments, but the resulting biogeographic patterns may arise from many more sources of stoichiometric flexibility.

Thermodynamics tells us that the maintenance of order can occur only through expenditure of energy. Thus, homeostasis must generate costs that should result in some loss of fitness. Only when energy is in excess or is truly free can we completely overlook this cost of maintaining stoichiometric homeostasis. Benefits of plasticity in stoichiometric composition seem clear—they reduce a fundamental thermodynamic penalty. Some consumers exposed to stoichiometrically varying resources do have greater long-term growth rates when stoichiometric flexibility allows them to integrate resources across those episodes (Hood and Sterner 2010). Given homeostasis is widespread and has an easily identified cost, there must be offsetting benefits of maintaining homeostasis. Perhaps there is some generalist–specialist dichotomy that can help explain the evolution of a presumably expensive trait. This topic is an area in need of further study.

It is important to note that any quantification of H strictly applies only to that life stage of that species (or even genotype) and that resource and under those conditions. Though broad generalizations such as "animals are more homeostatic than plants" seem to apply widely, any given measurement of H has uncertain and quite probably limited applicability. During ontogeny changed macromolecular makeup is inevitable even at the species level, and the different stoichiometry of these macromolecules will necessarily be reflected in onotogenetic variation in element ratios (Laspoumaderes et al. 2010). For example, juveniles typically have higher specific growth rates than adults, clearly reflected in higher P content and lower N : P (Carrillo et al. 2001; Villar-Argaiz et al. 2002). Temporal adjustments are also expected in that animals may store lipids either as a result of surplus food or as a result of stoichiometric imbalance with excess C in the diet (Hessen and Anderson 2008). This will cause elevated C : P or C : N. Consequently, under low food conditions, animals consume previously stored carbon, resulting in a decrease of C : P or C : N (Matthews and Mazumder 2005; Shimizu and Urabe 2008). For species with pronounced, seasonal lipid-storage like high-latitude marine copepods, optimum food stoichiometry may differ substantially between periods of active growth in juvenile stages to periods (late summer and fall) when growth has ceased and the copepods build up extensive stores of C-rich storage lipids (Aubert et al. 2013). This implies that stoichiometric optima may change with stage and season, especially for species with prolonged and rather complex life cycles.

Also, different tissues and body compartments will differ in their elemental ratios (Færøvig and Hessen 2003), e.g., storage tissues like liver in vertebrates or hepatopancreas in crustaceans will be more C rich than muscle tissue, while gonads and bony structures may be more P rich. Thus, further studies into these kinds of ontogenetic bottlenecks (Nakazawa 2011) or tissue-specific allocations will refine our understanding of stoichiometric strategies in organisms relative to the more simplified studies based integrated or pooled analysis of ground-up whole organisms.

Threshold elemental ratio—A conceptual cornerstone in ES is the threshold elemental ratio (TER), which is the elemental ratio where grazers enter from a primarily C or energy limitation into elemental limitation. TER itself is intimately linked to the degree of homeostasis. In its simplest form, the TER considers the difference in elemental ratios of food relative to consumer, corrected for the (assumed) utilization efficiency for the elements involved, including the contribution of respiration in the case of C. Generally, the TER for C vs. P is set as

$$\mathrm{TER}_{\mathrm{C:P}} = \{A_P/[(I_C A_C - R_C)/I_C]\} \times Q_C/Q_P \qquad (3)$$

where A_P and A_C are maximum net incorporation efficiencies for P and C, respectively; I_C is the mass-specific ingestion rate of C; R_C is the mass-specific respiration loss; and Q_C/Q_P is the consumer's C : P ratio (i.e., the mass-specific content of C over P). Typically, the assimilation efficiency and excretion rate for the scarcest element is set to 100% and 0%, respectively, for simplicity, while elements in excess will be assimilated with lower efficiency and also potentially disposed of after assimilation.

Estimates of TER are available for a limited number of organisms, however. For *Daphnia* a TER of 200–300 C : P by atoms (Olsen et al. 1986; Urabe and Watanabe 1992; Sterner and Hessen 1994) has been proposed, while other organisms present a much wider range of TERs, reflecting their feeding mode, growth rate, and body stoichiometry (Frost et al. 2006; Doi et al. 2010). In fact, for aquatic invertebrates, a strong, negative correlation has been found between $\mathrm{TER}_{\mathrm{C:P}}$ and maximum specific growth rate (Frost et al. 2006), which likely reflects the increased demands for ribonucleic acids (RNA), and thus P at high growth rate (*see* discussion of growth rate and stoichiometry below), and identifies sensitivity to food quality as a key trade-off on rapid growth. Evidence suggests that organism TER is more related to body stoichiometry than metabolic characteristics (Doi et al. 2010).

Using the TER we can see how ES theory incorporates both food abundance and quality. At very low C concentration, a major fraction of ingested C is allocated to maintenance costs (Sterner and Robinson 1994); hence, the relative fraction of ingested C that can be used for growth (e.g., $[(I_C A_C\text{-}R_C)/I_C]$) will decrease, and consequently the threshold C : P ratio will increase. However, the threshold C : P ratio is not likely to rise to infinity even under very low food quantities if excess C is used as energy to increase feeding activities (Boersma and Kreutzer 2002; Suzuki-Ohno et al. 2012; *see* below) or if P is lost as maintenance cost even under zero-growth conditions (Anderson and Hessen 2005). Thus, the energetic and

elemental expenditures due to feeding and metabolism have come to be seen as key in shaping growth response of consumers to changes in food quantity under different food quality conditions (Iwabuchi and Urabe 2012*a*), and the conceptually most important key linking P to growth is the Growth Rate Hypothesis (*see* below).

Transfer efficiency and digestion resistance—In its original and simplest form, the TER is based on the assumption that the element in least supply is assimilated with maximum efficiency. In the case of P, this is often set to 1 (100%), while for C it may be set to 0.6, linearly declining toward zero with increasing C : P. These values may be representative for highly edible food, but other factors determine assimilation efficiency and therefore can modulate TER from these simple assumptions. Clearly, assimilation efficiency for P (AE_P) can never be exactly 1, and notably AE for C (AE_C) may be affected by induced plant defenses, including digestion resistance, as well as gut passage time (DeMott et al. 2010). In the case of complete digestion resistance (where algae survive gut passage), AE will clearly be zero for all elements for that specific algae.

The experiments of DeMott and van Donk (2013) clearly demonstrated how strong P limitation of algal growth enhanced digestion resistance, promoting energy limitation in consumers feeding on P-deficient algae. Thus, TER may be affected both by food quantity as well as by community composition and digestion defenses.

While digestion defenses clearly add complexity to ready application of ES in the field, in some ways digestion defense theory can be considered complementary to ES and can be analyzed within a stoichiometric context. For example, reduced assimilation efficiency of C (AE_C) in response to P deficiency should make P limitation less likely if AE_P remains unaffected. However, reduced digestibility may also affect the consumer's ability to access P as well as C in some cases. If it affects AE_P relative to AE_C proportionately, it would not affect TER.

In a mixed phytoplankton community, the trade-off between grazing resistance and high growth will depend on whether or not the grazers are selective or not. In the case on nonselective filter feeders like *Daphnia*, density-dependent effects may also occur via facilitation by grazing and release of nutrients; thus shifting between high biomass of P-limited autotrophs to low biomass of P-sufficient autotrophs (Sommer 1992), which subsequently could switch between element and energy limitation of the consumers. This kind of feedback at the population level is a fertile ground for ES-explicit population modeling (*see* below).

The issue of AE and digestion defenses may also have bearings on TER in subtler ways, if grazer defenses among plants make digestion more energy demanding (e.g., by demanding a higher energy use to access the energy). Thus, while the issue of energy transfer efficiency is a core aspect of ES, the complex world of real food highlights potential pitfalls and unresolved issues. More importantly, however, it clearly points to fertile field of future research in food-web analysis.

Too much of a good thing?—ES theory, as described by Sterner and Elser (2002), considered the disposal of elements in excess but did not take into account any costs associated with that disposal. Without such costs, growth rate should simply level off when C : P is below the TER. However, observations of maximal growth rate at intermediate food P content in diverse organisms (Boersma and Elser 2006; Elser et al. 2012; Morehouse et al. 2012) suggest that, in one range of the data (high C : P), consumer P limitation occurs, but in another range (low C : P), there are significant negative effects of excess P (Fig. 5). Such a growth curve has been referred to as a "stoichiometric knife-edge." These negative effects of P-rich food may be behavioral (lowered feeding), metabolic (costs associated with excretion), or even overt toxicity. For example, it has been observed that *Daphnia* feeding on high P food reduced their feeding rate for unknown reasons and thus received insufficient C or energy (Plath and Boersma 2001), again stressing that energy limitation and stoichiometric quality limitation are not always easily disentangled.

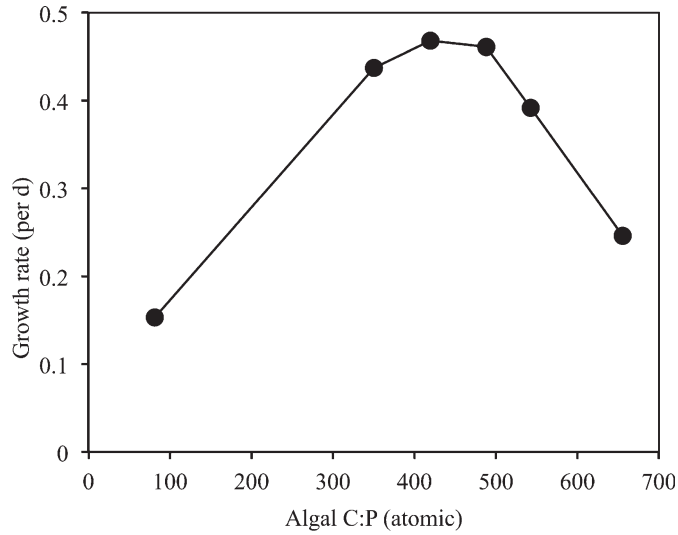

Fig. 5. Effects of high and low food C : P ratio on somatic growth of *Daphnia magna* (Plath and Boersma 2001). Decreased growth at high C : P ratio reflects the well-established effects of low food P content on *Daphnia* growth, while the reduced growth at low C : P was interpreted as a result of low animal feeding rates leading to C limitation. Figure redrawn from Plath and Boersma (2001).

These knife-edge studies indicate that P acts not just as an essential resource but also functions ecologically as if it were a kind of toxin at high dietary concentration. The stoichiometric knife-edge has been observed in diverse situations, but we still lack a good sense of its general applicability. We need more studies to document the ranges of resource stoichiometry where growth and performance decline in diverse organisms, and not the least under field conditions. Still the knife-edge concept, as well as stoichiometric responses in general, is heavily biased toward experimental studies. We also need to better identify the mechanistic basis associated with impaired performance under excess P, and we need to assess its importance under field conditions. Effects of excess dietary P may be an underappreciated factor contributing to reduced performance of food webs and loss of consumer

Table 1. Summary of literature survey of laboratory and field studies examining relationships relevant to the Growth Rate Hypothesis in aquatic heterotrophs (invertebrates and bacteria). Two studies of algae are included that measured RNA in conjunction with P. *See* Web Appendix (www.aslo.org/lo/toc/vol_58/issue_6/2219a.html), for full summary of the individual experiments and studies.

Relationship	%P vs. μ	%RNA vs. μ	%RNA vs. %P
Positive	27	12	15
Nonsignificant	12	6	6
Negative	1	0	0
Total	40	18	21
% agreement	68	67	71

diversity under eutrophication. We also need to determine if knife-edge dynamics hold for other elements than P. Recent documentation of effects of high plant N on a terrestrial herbivore (locusts; Cease et al. 2012) suggests the knife-edge is not just about P. Insights and tools from nutritional geometry may be especially useful in integrating these multiple dietary constituents into a single framework connecting behavioral and physiological mechanisms to ecological outcomes (Simpson et al. 2010).

The growth rate hypothesis

Elements, growth rate, and protein synthesis—Early observations of variation in consumer C : N : P ratios due to variation in biomass P contents (Hessen 1992; Andersen and Hessen 1991) naturally raised the question of the underlying biochemical basis of this variation, leading to the development of the Growth Rate Hypothesis (GRH; Elser et al. 1996). The GRH proposes a three-part causal linkage (with corresponding correlations) among growth rate, P content, and RNA content. As we will see below, the GRH is most applicable to small (< 1 g dry mass) heterotrophs under P-determined growth at a given temperature. It was also later proposed that maintaining high rates of ribosome synthesis would entail necessary changes in the genes that code for ribosomal RNA (rRNA; Elser et al. 2000*b*). Specifically, the length of intergenic spacers (the regulatory region where RNA polymerase binds to synthesize rRNA) and the number of copies of the rRNA genes contained in the genome were hypothesized to be positively associated with high rates of growth and high RNA and P contents.

Evidence for GRH? A meta-analysis—Studies over the past decade have provided broad, but not unanimous, support for these proposed linkages among ribosomes, P content, and growth rate. Table 1 provides an overview of much of this work of closest relevance to aquatic biota, emphasizing small heterotrophs (bacteria, crustacean zooplankton, and other invertebrates). Considerably more studies have evaluated growth (μ) and P content (or P : C) relationships than have examined RNA vs. μ or RNA vs. P. Nevertheless, more than two-thirds of these published studies reported the expected positive relationships among the triumvirate of parameters. Furthermore, based on the results of studies that measured both RNA and P on paired samples, RNA can indeed contribute significant quantities of P to overall biomass P content (25–90%) in this group of biogeochemically significant organisms (Elser et al. 2003). Closer examination of the approximately one-third of studies that did not support the GRH provides insight into the conditions under which the GRH is likely to hold.

(1) Some of these cases involve relatively large-bodied animals. Specific growth rate, P content, and RNA content all scale negatively with body size, so that the contribution of RNA to overall biomass P content also declines, as other, non–growth-related, pools of P (e.g., deoxyribonucleic acid (DNA), phospholipids, and eventually, bone) become dominant in larger organisms (Gillooly et al. 2005). RNA contributes ~ 25% of body P for 1 mg dry mass organisms but 6% or less for organisms 1 g or larger. In fish, almost all the variation in body P content is associated with bone (Hendrixson et al. 2007).

(2) Other cases where predictions of the GRH were not observed involve organisms grown under limitation by nutrients other than P, such as N (protein)-deficient food, food lacking in essential sterols, or simply low food abundance. In this sense these heterotrophs conform to the same stoichiometry–growth rules that autotrophs do: their growth rates depend on the cell quotas, which depend on the growth-limiting constituent and not the nonlimiting one.

(3) Some cases included in Table 1 involve interspecific comparisons of various species isolated from different habitats and compared for field conditions or for controlled laboratory conditions. In either case such assessments of the GRH are problematic because growth response to a food C : P ratio may differ among species due to species-specific differences in assimilation and metabolic activities. Moreover, when interspecific comparisons were made, one should consider the appropriate phylogenetic corrections that would be needed to isolate growth rate differences per se from differences in growth, RNA, or C : N : P that reflect phylogenetic history.

(4) In some cases the GRH has been evaluated for cases in which growth rate differs due to differences in temperature. This is problematic because the GRH was formulated for consideration of differences in growth rate for biota at a *given* temperature (Elser et al. 2000*b*). Indeed, various studies have shown that low-temperate acclimation leads to *increased* RNA and P contents. It has been hypothesized that organisms increase production of biosynthetic machinery in order to compensate for reduced rates of reaction at low temperature (Woods et al. 2003).

Along with homeostatic regulation and threshold elemental ratio, the GRH constitutes a general concept of

ES that should be broadly applicable to many organisms. While clearly valid for bacteria, heterotroph protists, and invertebrates, its applicability to autotrophs is less obvious (Matzek and Vitousek 2009; Flynn et al. 2010) and demands further studies. One important aspect of the GRH, and the broader, "biological stoichiometry" that it encompasses, is that it has made it easier to integrate biogeochemical studies with evolutionary studies, a step that may be especially useful given recent realizations that "ecological time" and "evolutionary time" are not as disjunctive as had previously been assumed (Schoener 2011).

The genomic basis of the GRH—Similar to how ribosome content can limit and affect protein synthesis, the genomic elements responsible for rRNA (the "rDNA" in eukaryotes or "rrn" genes in prokaryotes) can control the production of ribosomes. Fast-growth, RNA- and P-rich lifestyles should be associated with particular variations in rDNA, namely, long intergenic spacers (IGS) in the rDNA operons (reflecting increased numbers of promoter or enhancers to increase binding of RNA polymerase) or increased copy numbers of the operon (to increase overall translational capacity for RNA production; Elser et al. 2000*b*).

There have been few tests of the genomic basis to the GRH to date, and these offer some mixed experimental support. In one study, fast-growing juvenile *Daphnia* (produced as a pleiotropic effect of selection on adult fecundity) had high RNA contents and higher P contents and carried a single, long version of the IGS. In contrast, populations of slower growing animals were dominated by genotypes with a short-spacer IGS variant (Gorokhova et al. 2002). In another study, a long-spacer (presumably P-demanding) clone of *Daphnia pulex* was eliminated in competition with a short-spacer clone when algal food had a high C : P ratio; however, the long-spacer clone won when food was P rich (Weider et al. 2005). However, another study found that, although the RNA : DNA ratio and growth rate were correlated positively with IGS length within three *Daphnia* species, no associations with C : P or N : P ratios were observed (Weider et al. 2004). DeMott and Pape (2005) tested for the food quality trade-off of high growth rate, assessing the GRH in a set of field-collected *Daphnia* species raised on foods of contrasting C : P ratio. These authors did not find the expected positive correlation between growth on P-sufficient food and body P : C ratio, nor did they find evidence for a strong trade-off between high growth rate and sensitivity in high C : P food. On the other hand, a more recent study by Seidendorf et al. (2010) took closer account of phylogenetic relationships among the study species and showed that, across 12 species of *Daphnia*, those with high maximum somatic growth rates showed larger declines in performance on P-deficient diets, most likely linked with their high demands for RNA. Nevertheless, it is clear that more studies are needed to test these ideas more comprehensively.

Although DNA does not make up as large a cellular pool as does RNA in most situations, under P scarcity there should be selective pressure to minimize any waste of P. When comparing the relatively slow-growing copepods with fast-growing cladocera, not only was the smaller genome size per se observed, but also a higher RNA : DNA ratio was seen in cladocerans (Hessen et al. 2008; Fig. 6). The commonly observed small genome size in fast-growing organisms could reflect an evolutionary reallocation of P from noncoding DNA to RNA (Hessen et al. 2008). The implication is that there may be causal links between growth rate, cell-specific (or biomass-specific) RNA content, and genome size. Hence, streamlining the genome may be a consequence of selection against noncoding DNA (transposons, pseudogenes, repetitive elements), since these elements may hold a significant fraction of cellular P. Interestingly, swapping resources (P) from DNA to RNA not only maximizes growth via protein synthesis but also reduces cell volume and increases cell division rate because genome size generally scales positively with cell volume and negatively with growth rate (Gregory 2005). Hence, in a growth rate context, it would be a doubly favorable strategy.

Similarly, chronic N deficiency may apply evolutionary pressure to reduce the N costs of making proteins. Since the elemental composition of the amino acids themselves displays pronounced differences (C : N ratios range from 1.5 in arginine to 9 in tyrosine and phenylalanine), it would make sense for organisms suffering chronic N deficiency to substitute wherever possible toward low-N amino acids—a response that indeed seems to occur (Baudouin-Cornu et al. 2001; Elser et al. 2011). However, these patterns have not yet been widely examined for aquatic biota, largely due to a paucity of whole-genome information.

Feedbacks, nutrient regeneration, and multiple elements

Nutrient regeneration—Mass balance principles dictate that release or regeneration of nutrient elements like N or P necessarily reflect the balance between net intake and somatic and reproductive demands. For a homeostatic consumer, this can be calculated with high accuracy. With the proviso that consumers generally do not obey *strict* homeostasis (*see* previous discussions), some qualitative and quantitative predictions can be made for various resource-consumer scenarios. An early, striking example of this was the trophic cascade–induced shift from large cladocerans (low N : P) to copepods (high N : P) that caused a shift from P to N limitation for autotrophs (Sterner et al. 1992), formally linking the classical, trophic cascade to stoichiometric-driven effects in food webs.

A comprehensive and valuable review of various stoichiometrically explicit feedbacks has recently been provided by Hall (2009); thus we need not reiterate all those issues here. However, in brief, while classical Lotka-Volterra type predictions are based on quantity-related null-clines for both food (prey) and consumer, ES gives different predictions for several reasons (Andersen et al. 2004; Elser et al. 2012). First of all, grazing and nutrient regeneration combined will yield highly flexible autotroph stoichiometry with typically low biomass but high quality (i.e., low C : P) under periods of high consumer biomass and high grazing pressure. Under this scenario, grazers face

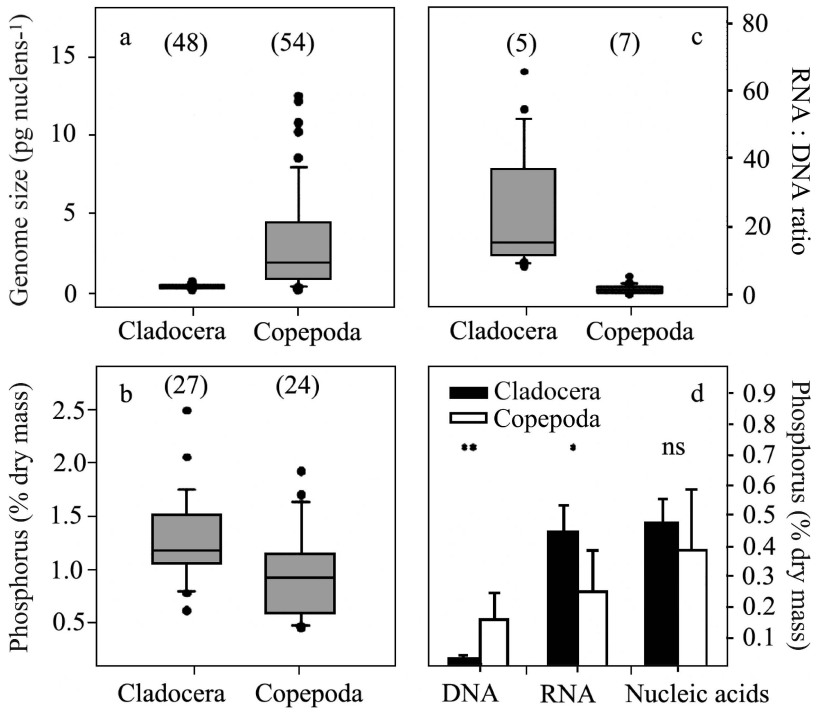

Fig. 6. Allocation of P to nucleic acids in cladocerans and copepods. (a) Diploid genome size, (b) Phosphorus as % of dry weight, (c) RNA : DNA ratio, and (d) the fraction of total P allocated to either DNA or RNA. Boxes represent medians, and 75% percentiles, vertical lines the 95% percentiles. The number of species for each group given in parentheses above (from Hessen et al. 2008).

population decline due to quantity starvation, similar to classic density dependence. However, high biomass of autotrophs and low grazing (and recycling) give high C : P ratios and quality starvation of consumers (Sommer 1992), which contrasts with classical density-dependent relations for consumer populations. In such scenarios, nutrient return from consumers may change the strength and outcome of competition, and in some cases nutrient return makes sustained coexistence of consumer species possible (Loladze et al. 2004; Hall 2009), a prediction supported by experimental evidence in which improved stoichiometric food quality via nutrient recycling by consumers subsequently promoted coexistence of two consumer species rather than competitive exclusion of inferior species (Urabe et al. 2002).

According to the resource ratio theory of competition, the outcomes of exploitative competition among algal species under limited supplies of two nutrients can be predicted by zero-net growth isoclines (ZNGIs). For consumer animals, the threshold food level (TFL) at which the individual growth rate is zero can be viewed as a minimum food level determining ZNGI. TFL in terms of C (TF_C) has often been measured for zooplankton, and a simplistic model of competitive dominance between copepods and cladocera as a function of their TFL and TER values was proposed by Sterner and Hessen (1994). Recently, Iwabuchi and Urabe (2012*a*) measured TFL in terms of P (TF_P) for several daphnid species and graphically analyzed ZNGIs in the plane of food P and C concentrations (Fig. 7). In this plane, a diagonal line passing the origin is a consumption vector and thus represents the P : C ratio of food. The analysis predicts the outcome of competitive interactions among these daphnid species will change depending on elemental ratios of the food. Indeed, this prediction was experimentally substantiated (Iwabuchi and Urabe 2012*b*), indicating that differential outcomes of exploitative competition between the same two consumers due to differences in food quality can be understood by this extension of resource ratio theory to consumer species.

Elements beyond C, N, and P—While, for reasons outlined above, the focus in ES has been on P and N, there is in principle a range of elements that could limit growth or performance in organisms; it is simply a matter of the balance between demand and availability. For example, Fe deficiency causing high C : Fe in phytoplankton could induce Fe limitation in consumers, as supported by recent experiments with the marine copepod *Acartia tonsa*, which suffered increased naupliar mortality and decreased egg production when feeding on low-Fe diatoms (Chen et al. 2011).

Thus again in principle a suite of elements that serve as obligate constituents of macromolecules could fit within the concept of ES. Indeed, it is likely that there are specific environments and specific organisms for which other elements than N or P (or Fe) may represent the stoichiometric bottleneck. While the main focus of ES likely will remain the key elements C, N, and P, and thus the balance between energy demands and biosynthesis, there is a largely unexplored niche for stoichiometry

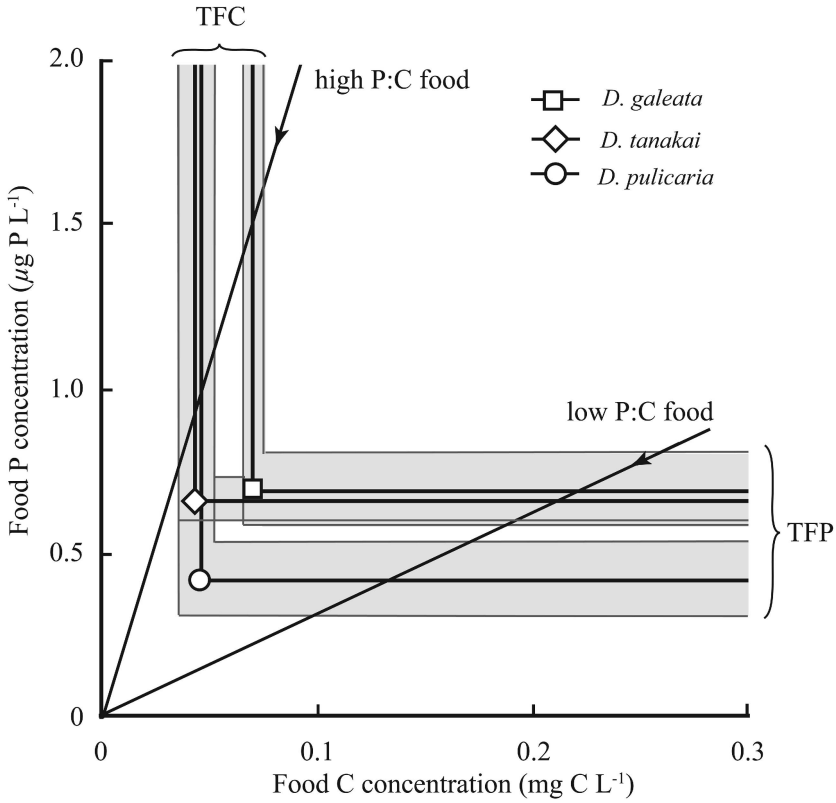

Fig. 7. Graph of competitive interactions between three *Daphnia* species when fed high and low P : C algae. The horizontal and vertical lines represent ZNGI for C and P, respectively. The gray areas represent 1 standard deviation. The two diagonal lines with arrows represent high and low P : C food lines, respectively. An x-value on those lines represents a food quantity, and their slope represents the P : C ratio of algal food. Arrows represent consumption vectors, which move toward the origin along the food lines as grazers consume the food. (From Iwabuchi and Urabe 2012*a*.)

beyond C, N, and P. Since most elements form part of particular organic or inorganic macromolecules with an explicit, stoichiometric build-up, this is one of the trajectories for further development of ES. Indeed, the novel work of Karimi et al. (2010) and Bradshaw et al. (2012) already points in this direction.

Large-scale effects, biogeochemistry, and global change

Stoichiometry of pelagic systems—In systems dealing with interactions between grazers and higher plants, the stoichiometric mismatch can be estimated with good accuracy. The same holds for experimental setups where a well-defined, single-species phytoplankton diet is offered. However, for pelagic systems under natural conditions there are some specific challenges. Here, the food consists of a heterogeneous mix of dead and live particles collectively labeled "seston." We now know that seston differs strongly in its elemental ratios both within and between localities, and particularly the C : P ratio shows pronounced variability (Hecky et al. 1993; Elser et al. 2000*a*). Since seston quality is imperative for pelagic grazers, their nutrient cycling, community composition, and transfer efficiency to higher trophic levels, understanding the causes for this strong variability is of considerable interest. Various biotic and abiotic causes have been advocated to account for the more than 10-fold variation in seston C : P observed across pelagic ecosystems.

For most lakes and marine systems there are seasonal fluctuations in phytoplankton stoichiometry reflecting light, nutrient, and community dynamics. High autotroph biomass with low turnover rates will typically have high C : element ratios, while increased zooplankton grazing and faster turnover of N and P would correspondingly be expected to reduce the sestonic C : element ratios. In addition, the seston composition per se will influence its stoichiometry in various ways. Live autotrophs often contribute a relatively modest fraction of bulk seston both in freshwater (Hessen et al. 2003) and marine (Frigstad et al. 2011) ecosystems, where detritus or heterotrophs of mixed origin may dominate at least temporarily and thus strongly affect seston stoichiometry. Furthermore, different algal taxa have different C : N : P ratios; notably, Chlorophyceae seem to be evolutionary geared toward higher C : P compared with other groups (Quigg et al. 2003). These causes of variable seston quality and stoichiometry should be kept in mind when assessing drivers and consequences of autotroph stoichiometry and are also relevant for the

understanding of the maintenance of Redfield ratio (*see* below).

A recent meta-analysis (Sterner et al. 2008) included data on elemental stoichiometry for seston in both freshwater and marine systems and is the most comprehensive view to date. They found variable C : N : P stoichiometry when analyzing the data on smaller spatial scales (e.g., between ocean basins) and proposed a revised global ratio of C : N : P = 166 : 20 : 1 (atomic). This elevation of C : nutrient ratios above the canonical values may simply reflect the larger and more diverse dataset but could also be caused by recent environmental trends such as increased CO_2 as well as warming, increased thermal stability, and more shallower mixing depths (implying more severe nutrient scarcity in photic layers).

These "large-scale" stoichiometry issues related to the pelagic system highlight the need for models capable of coupling biogeochemical cycles in a stoichiometric-explicit way to understand and predict ecosystem effects such as productivity, trophic transfer, C sequestration and thus the "biological pump." The need for such approaches may be especially acute in marine biogeochemistry, which has long been dominated by the hegemony of the Redfield ratio (Redfield et al. 1963), as most studies have assumed a fixed Redfield ratio for simplicity in modeling of C sequestration and food-web dynamics. Various studies (Klausmeier et al. 2004; Anderson et al. 2013), as well as work already reviewed in this paper, reveal that such an approach is a considerable oversimplification.

While an in-depth evaluation of various aspects of the Redfield ratio is beyond the scope of this review, we note that there are clear unresolved issues dealing with the stoichiometric coupling between elemental ratios in dissolved and particulate fractions, as well as stoichiometric control of C sequestration (Anderson et al. 2013). Future modeling and empirical studies are needed to establish how our emerging understanding of variation in seston C : N : P stoichiometry impinges on large-scale C sequestration in a changing biosphere.

Light and CO_2 as stoichiometric drivers—One of the most intriguing and counterintuitive interpretations of ES that links energy flux, food quantity, and food quality of pelagic food webs is that the light : nutrient ratio serves as a main determinant of trophic efficiency (Sterner et al. 1997). The crux is that shifts in the intensity of photosynthetic active radiation (PAR), especially at low concentrations of P, elevate cellular C : P ratios in autotrophs due to a disproportionately high uptake of C relative to P at high light (Urabe and Sterner 1996; Sterner et al. 1997; Hessen et al. 2002). This could be a common scenario in many oligotrophic lakes, in particular during summer, when water transparency is high and there is a general shortage of inorganic, bioavailable P. Sterner et al. (1997) used field data to test these effects, calculating a ratio of light : P for a number of lakes by putting mixed-layer mean light in the numerator and total P concentration in the denominator and demonstrating that the light : P ratio was strongly correlated with the C : P ratio of seston.

Thus, by modulating epilimnetic light intensity, mixing depth will strongly affect both biomass and the elemental ratio of autotrophs, since there will be less light available in lakes with deep mixing layers (Huismann et al. 1999; Diehl 2007). Based on these associations, it was proposed (Sterner et al. 1997; Diehl 2007) that increased solar energy input could paradoxically negatively affect grazers due to reduced stoichiometric quality despite increased autotroph biomass (later termed the "paradox of energy enrichment," Loladze et al. 2000).

While increased PAR promotes C fixation, there is growing evidence not only for reduced photosynthesis under ultraviolet (UV) exposure but also for enhanced uptake of P (Xenopoulos et al. 2002). This means that UV works in the opposite direction of PAR by causing not only decreased biomass but also decreased C : P ratios (Hessen et al. 2010). Since the intensities of PAR and UV are in general positively correlated, high levels of both PAR and UV could conceivably cancel out strong C : P responses in the autotrophs, although changes in the UV : PAR ratio could shift autotroph C : P in either direction. The next step in this context would be focused studies of the mechanistic coupling between light intensities, spectral properties, inorganic C, and nutrients, building on what is known about nutrient requirements for the photosynthetic machinery, growth, and storage. Also needed are more studies that examine how stoichiometric food quality modulates consumer response to direct UV exposure (Souza et al. 2010). This again could then feed into models predicting large-scale effects of ecosystem changes in CO_2, light intensity and quality, and nutrients, all of which are changing simultaneously but in different proportions.

In a similar fashion as with high levels of PAR, elevated CO_2 may yield increased autotroph biomass and C : nutrient ratios due to a skewed accumulation of C relative to P (or N), leading to reduced consumer productivity due to quality starvation (Urabe et al. 2003). While the huge variability in $[CO_2]$ or the partial pressure of CO_2 (P_{CO_2}) in lakes is driven more by in-lake processing of terrestrially derived dissolved organic carbon (DOC), rising atmospheric CO_2 could affect algal stoichiometry in oligotrophic lakes as well as in offshore, marine systems with minor effects of allochthonous DOC.

Biogeochemical cycles at the catchment scale—The stoichiometry of recipient waters bears the fingerprints of the catchment. Large-scale monitoring has shown that C : N : P : Si ratios in pristine, boreal lakes can be largely explained by N deposition, terrestrial vegetation as inferred from Normalized Differential Vegetation Index, temperature, runoff, and the fraction of bogs in the catchment (Fig. 8; Hessen et al. 2009). Not only elevated N deposition but also anthropogenic changes in various biogeochemical cycles have become a major concern both on the local and global scale. The global biochemical cycles interact in various ways; thus, a stoichiometric approach is valuable in assessing possible effects. For example, in a global change context, increased deposition of N might affect CO_2 sequestration and vice versa (Schindler and Bayley 1993). Also, other major elements for biological productivity, notably P and Si, could interact with the C and N cycles in various ways, and thus the fluxes of these four elements

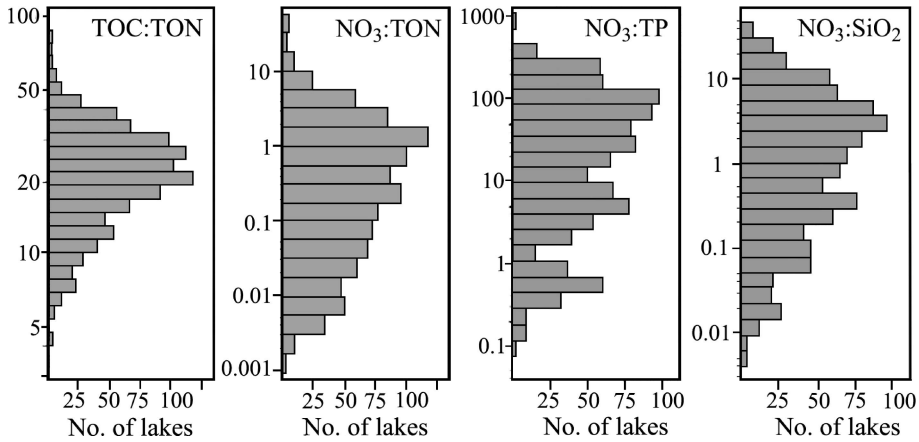

Fig. 8. Distribution plots for elemental ratios (mol : mol) for (a) total organic carbon : total organic nitrogen (TOC : TON), (b) NO_3-N : TON, (c) NO_3-N : total phosphorus (TP), and (d) NO_3-N : SiO_2 in nearly 1000 pristine Norwegian lakes. Note the modest span in TOC : TON, and the extremely wide span in NO_3 to TON, TP, and SiO_2, all mostly driven by the atmospheric N deposition, hydrology, and vegetation density of the catchments (from Hessen et al. 2009).

from watersheds both in absolute and relative terms will be affected by both temperature and precipitation.

Stoichiometric coupling in biomass implies that alterations in the N and P cycles interact closely with each other and, in turn, these can alter C sequestration in both terrestrial and aquatic ecosystems to produce large-scale effects on biogeochemical cycles. For example, since P does not have a gaseous phase and thus is distributed mostly by wind-eroded particles, the average global N : P deposition ratio is > 20 times higher than the Redfield ratio (Sardans et al. 2012). While the deposition ratio will vary depending on proximity to sources of airborne N and P, there is little doubt that we should see a shift toward less stringent N limitation or more intense P limitation in systems receiving high N deposition (Elser et al. 2009). While increased N deposition appears to promote drawdown of C via the biological pump in coastal marine systems (Kim et al. 2011), nutrient limitation of production in lakes over wide regions of elevated N deposition may have shifted from N limitation or N and P colimitation toward a more intense P limitation (Elser et al. 2009), with subsequent effects on stoichiometric food quality (Elser et al. 2010). This latter effect was inferred from elevated levels of alkaline phosphatase in *Holopedium* and *Daphnia*; however, the role of this enzyme as diagnostic marker of specific P deficiency is not finally settled (Wagner and Frost 2012).

Insights from ecological stoichiometry may be critical in building next-generation global biogeochemical models that take into account the complex interactions and that can forecast the effects of biogeochemical disruptions on ecosystems.

Synthesis and prospectus

In this review and synthesis, we have seen that a cornerstone in ES, the fact that all organisms are made of the same "stuff," provides a mechanistic link from atoms to molecules, organisms, and processes that is applicable at scales from the cell to the biosphere. In some ways it can be seen as complementary to other "laws" and first principles in biology. ES has passed its stage of infancy and embraced new topics, disciplines, and ecosystems since its departure from what was basically a *Daphnia*–chlorophyte interaction 25 years ago and, while a full coverage of the topic has been beyond the reach of this review, we have tried to address its current status and development for some major concepts and processes at various scales, focusing on aquatic ecosystems. We have pointed to further directions where ES could and should develop to broaden its general validity. One important realization is that the cycling of life's essential elements is, to a large extent, the outcome of processes and feedbacks that originate in individual cells and organisms and whose ecological and evolutionary implications come into closer focus through the lens of stoichiometric theory. We currently experience major changes in the cycling and C, N, and P (plus multiple other elements) on Earth. We see diminishing resources of P, which at the same time still cause major eutrophication problems, a global fertilization experiment due to N_2 converted to bioavailable N, and levels of atmospheric CO_2 now exceeding 0.04%. Realizing the intimate connection between these elements, we see literally fertile grounds for ES in the years to come.

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