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To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs

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Homeostasis of element composition is one of the central concepts of ecological stoichiometry. In this context, homeostasis is the resistance to change of consumer body composition in response to the chemical composition of consumer's food. To simplify theoretical analysis, it has generally been assumed that autotrophs exhibit flexibility in their composition, while heterotrophs are confined to a constant (strictly homeostatic) body composition. Yet, recent studies suggest that heterotrophs are not universally strictly homeostatic. We examined the degree to which autotrophs and heterotrophs regulate stoichiometric homeostasis (P:C, N:C, N:P, or %P and %N). We conducted a quantitative review and meta-analysis using 132 datasets extracted from 57 literature sources which examined the dependence of organismal stoichiometry on resource stoichiometry. Among individual datasets, there was a wide range of responses from strictly homeostatic to non-homeostatic. Even within heterotrophic organisms, varying levels of homeostasis were observed. Comparing the degree of homeostasis between organisms based on large-scale habitat types using meta-analysis indicated some significant differences between groups. For example, aquatic macroinvertebrates were significantly more homeostatic in terms of P:C than terrestrial invertebrates. Our meta-analysis also confirmed that, with regard to N:P, heterotrophs are significantly more homeostatic than autotrophs. Furthermore, our analysis indicated that the homeostasis parameter 1/H, despite being a potentially useful predictive metric, has to be utilized with caution since it oversimplifies some important aspects of the responses of organisms to elemental imbalances. This critical evaluation of stoichiometric homeostasis contributes to a better understanding of many food-web interactions, which are commonly driven by elemental imbalances between consumers and their resources.

Ecological stoichiometry is an approach where elements are used as a common currency to examine the scaling of trophic dynamics across all scales of organization within a system (Sterner and Elser 2002). It concerns potential imbalances in the relative supply of elements between organisms and their resources. The primary focus lies on nitrogen (N) and phosphorus (P) as essential nutrients, often being related to carbon (C) as an energy source. By supplementing energy as the central unit of measure with the ratios of C:N, C:P or N:P, our understanding of processes at a variety of scales, ranging from macromolecular content to ecosystem processes has greatly increased (Elser et al. 2000b, Sterner and Elser 2002, Hessen and Anderson 2008). For example, accumulating empirical and theoretical studies have shown that imbalances among C:N, C:P or N:P between consumers and their resources can strongly constrain consumer growth and reproduction (Anderson et al. 2004, Frost et al. 2005).

The concept of stoichiometric homeostasis, that organisms regulate their elemental composition, was central to the development of the ecological stoichiometry framework (Koojiman 1995, Elser and Urabe 1999, Loladze et al. 2000, Sterner and Elser 2002). Stoichiometric homeostasis may be regulated through a variety of pre-ingestive (e.g. food selection) or post-ingestive (e.g. differential assimilation or excretion) mechanisms (reviewed by Anderson et al. 2005, Frost et al. 2005). Organisms that maintain constant stoichiometry regardless of fluctuations in resource stoichiometry are considered strictly homeostatic, while those whose composition varies in direct proportion to changes in the composition of their resources are non-homeostatic (Sterner and Elser 2002). Elemental homeostasis refers only to the variability in consumer nutrient content that is driven by variation in resource nutrient content. Not all variation in elemental content, however, is related to stoichiometric homeostasis. For example, when resource supply ratios were held constant, growth rate and temperature both shaped the C:N:P content of bacteria (Chrzanowski and Grover 2008).

There is a dichotomy between autotrophic and heterotrophic organisms with regard to the strength of homeostatic regulation. Autotrophic organisms are normally considered to be plastic because their stoichiometric composition can vary widely with fluctuations in nutrient supply and light (Sterner et al. 1998). In contrast, heterotrophs are generally thought to be strictly homeostatic. Some studies found that variability in resource stoichiometry had little to no effect on heterotroph body elemental composition (Andersen and Hessen 1991, Fagan et al. 2002), but others call the generality of this pattern into question (DeMott 2003, Fink and Von Elert 2006, Mulder and Bowden 2007, Chrzanowski and Grover, 2008). DeMott and Pape (2005), for example, assessed P homeostasis for eleven *Daphnia* species and hybrids and found that these species varied from weakly homeostatic (*D. magna*) to strongly homeostatic (*D. dentifera*).

Where consumers fall on the continuum from non-homeostatic to strictly homeostatic has important consequences for consumer–resource interactions, the supply of C and nutrients to higher trophic levels, and nutrient recycling (Elser and Urabe 1999, Grover 2003, Malzahn et al. 2007, Mulder 2007, Mulder and Bowden 2007). We analyzed datasets from published studies reporting information about the nutrient content of an organism in relation to the nutrient content of its resources and performed meta-analyses to address the following questions: 1) Are autotrophs less homeostatic than heterotrophs? 2) Are the majority of heterotrophic organisms strictly homeostatic? 3) Does the degree of homeostasis differ among organisms based on broad taxonomic groupings? There is a large body of research on the stoichiometric properties of zooplankton, but our objective was to assess the generality of patterns in homeostasis as broadly as possible by searching for data from major taxonomic groups, including autotrophs, microorganisms, invertebrates and vertebrates. This analysis aimed to increase the understanding of food-web interactions in a stoichiometric context, as imbalances in the elemental composition between consumers and their resources can underlie many trophic interactions (Sterner and Hessen 1994, Grover 2003, Malzahn et al. 2007).

Methods

Data extraction

We collected data from scientific articles published before July 2009. Beforehand the authors already knew of many relevant studies, especially involving aquatic organisms or terrestrial insects. We searched for additional articles using internet literature databases (ISI Web of Knowledge, Wiley InterScience, SCIRUS, HighWire and Google Scholar). Once an article providing appropriate information was found, we also searched the articles that where cited by, or cited this article.

To be included in our analyses, articles had to report the dependence of organism stoichiometry on resource stoichiometry (primarily for P:C, N:C, N:P, but also for %N, or %P), or provide data from which this relationship could be calculated. When possible we used mean C content to convert %N or %P data into N:C or P:C ratios; however, resource nutrient to carbon ratios could not be calculated for the fish studies identified, therefore we only examined percent N and P homeostasis for this group. In addition, datasets had to meet the following criteria: 1) only one species or strain per dataset (no community data), 2) consumer elemental content had to be given for the whole organism, 3) the study had to include at least three levels of resource quality (in order to allow the calculation of consumer vs resource regressions on more than two points, as the raw data were generally not available), 4) the study had to examine the influence of resource stoichiometry on consumer stoichiometry, while controlling for, to a reasonable degree, changes in food identity or other aspects of diet quality, 5) data had to come from studies where life stage, temperature, and other environmental factors were controlled. Many studies failed to meet these criteria. For example, we were not able to find any datasets on mammals, birds, reptiles or terrestrial autotrophs (plants) that satisfied criterion two. On a few carefully considered occasions we relaxed the fifth criterion in order to include underrepresented taxa; most commonly, for field experiments with terrestrial invertebrates which could not carefully control environmental variables (marked in Supplementary material Appendix 1 Table A1).

In several cases we assumed consumer %C of dry weight in order to calculate the ratios with N or P. *Daphnia* %C was assumed to be 45% (according to Andersen and Hessen 1991). Gastropod data from Elser et al. (2006) was converted to P:C using 37% C as suggested by the authors, for other gastropods 42% C was assumed (Liess and Hillebrand 2006). When insect %C was not directly reported, we used the average %C values reported for the appropriate order by Elser et al. (2000a). Since resource %C is variable, studies that did not report resource ratios were excluded, except for the fish studies where we examined %N and %P homeostasis. When only percent protein was reported (primarily insect and fish studies), percent N was calculated by dividing percent protein by 6.25 (Block and Bolling 1946, Deutsch and Seabra 1955). In the majority of the fish studies, percent protein itself had been estimated from %N using this same conversion factor. In some instances when an article did not provide enough data, the authors were contacted and graciously provided additional data.

Data analysis

There were 132 datasets from 57 studies that met our inclusion criteria (Supplementary material Appendix 1 Table A1). We augmented these with unpublished data for 19 additional datasets (Supplementary material Appendix 1 Table A1). Taxa were divided into broad taxanomic groups: bacteria, phytoplankton, fungi, invertebrates, and fish. The invertebrates were further sub-divided based on habitat use into zooplankton (dominated by *Daphnia*), aquatic macroinvertebrates, and terrestrial invertebrates. The aquatic macroinvertebrate group included studies conducted on the aquatic larval stage of species that are terrestrial as adults. The terrestrial insect group included adults of species that are terrestrial throughout their life cycle.

An organism's degree of stoichiometric homeostasis was characterized by the homeostasis coefficient H (eta):

$$H = \frac{\log_{10}(x)}{\log_{10}(y) - \log_{10}(c)}$$

where x is the resource nutrient stoichiometry (e.g. P:C or % P), y is the organism's nutrient stoichiometry (same units as resource) and c is a constant (Sterner and Elser 2002). Therefore, 1/H is the slope of the regression between log(x) and log(y) and should take values between zero and one. Strictly homeostatic organisms have an H of infinity, which presents a number of analytical problems; therefore, we used the regression slope, 1/H, in all analyses. Regression analyses were conducted for P:C ($1/H_{P:C}$), N:C ($1/H_{N:C}$), N:P ($1/H_{N:P}$), %N ($1/H_N$), or %P ($1/H_P$) for each dataset as appropriate for the data provided in the articles or otherwise provided by the article's authors. Since the slope was expected to be greater than or equal to 0, one-tailed tests with $\alpha=0.1$ were used. If the regression relationship was non-significant ($p>0.1$), 1/H was set to zero (as in Makino et al. 2003) and the organism considered 'strictly homeostatic'. Species with $1/H=1$ were not homeostatic. All datasets with significant regressions and $0<1/H<1$were arbitrarily classified as: $0< 1/H <0.25$ 'homeostatic', $0.25< 1/H <0.5$ 'weakly homeostatic', $0.5< 1/H <0.75$ 'weakly plastic', $1/H >0.75$ 'plastic'.

In the context of this study, the stoichiometry of a strictly homeostatic species is by definition tightly constrained across wide variation in resource stoichiometry (Sterner and Elser 2002); for these species, analytical error would, ideally, be the only source of variation in X:C. Operationally, we classified cases as strictly homeostatic ($1/H = 0$) when the least squared regression slope was insignificant ($p>0.1$). Unfortunately, this criterion does not distinguish cases of strict homeostasis from those where consumer X:C is highly variable, a result of especially noisy data or biological independence of consumer and resource stoichiometries. There were no clear expectations of how much variability to expect in strictly homeostatic cases. Here, we used the residual error in the plastic cases (root mean squared error, RMS, Zar 1998) to estimate the expected degree of background variation (RMS ± 95% CI). We compared the mean RMS for each nutrient ratio to the variation in each strictly homeostatic case as described by its standard deviation. Mathematically, these metrics are similar (Zar 1998), although calculation of the degrees of freedom differs slightly between equations (SD: DF = n–1; RMS: DF = n–2). When these two metrics differed, we rejected the null hypothesis that all strictly homeostatic species were correctly classified. This hypothesis was tested for each nutrient ratio separately using a t-test.

To statistically test the degree of homeostasis between taxanomic groups, we conducted a mixed-model meta-analysis per Gurevitch and Hedges (2001) with modifications for the use of regression slopes (Supplementary material Appendix 1 Table A1) following Becker and Wu (2007) and Gurevitch and Hedges (1999). First, we calculated weighted mean slopes in the fixed-effects model by weighting each slope by the reciprocal of its sampling-error variance for each study. Then we employed the mixed-models approach to attribute within study variation to fixed effects and between-study variation to random effects. Mixed-model weighted mean slopes and 95% confidence intervals were similarly calculated as in the fixed effect analysis, but each slope was weighted by the reciprocal of its unconditional variance for each study (Gurevitch and Hedges 2001). Non-overlapping 95% confidence intervals for the weighted mean slopes were interpreted as a significant difference. In addition to conducting a meta-analysis of each taxanomic group, we also calculated an overall mean weighted slope for all heterotrophic organisms excluding fish. Because the only group of autotrophs in our analysis was algae, we were able to compare the weighted mean slopes of algae to heterotrophs to assess the patterns of homeostasis associated with organisms at the autotroph versus heterotroph level of classification.

Regression analyses were conducted using SAS ver. 9.1 (SAS Inst.); the meta analyses were done in R (R Development Core Team); Statistica (StatSoft) was used for all other statistical analyses.

Results

P:C homeostasis

Datasets of $1/H_{P:C}$ were available for most groups except for algae, fungi and fish (Fig. 1A, 2). The distribution across groups, however, was not even; nearly half of these observations focused on the genus *Daphnia*. In general, zooplankton and terrestrial insects consumed diets lower in P content than themselves; that is, regression lines for these species fell above the one to one line (Fig. 2). Bacteria were grown in media ranging from low to high P, relative to tissue chemistry. Aquatic macroinvertebrates consumed either a P rich or P poor diet, relative to their tissue chemistry; regression lines in this group rarely crossed the one to one line (Fig. 2). In general, the heterotrophs tightly regulated P:C homeostasis. For the zooplankton, 1/H varied from 0 to 0.2 (Fig. 1A), and strict homeostasis was observed in 19 of 34 zooplankton datasets (Fig. 1A, Supplementary material Appendix 1 Table A1). Two of the marine zooplankton exhibited a significant negative relationship between body P:C and resource P:C (Supplementary material Appendix 1 Table A1), however, the range of resource P:C in these datasets were narrow. Seven of the eight aquatic invertebrate datasets were classified as strictly homeostatic. Two of the four terrestrial invertebrates were classified as strictly homeostatic, and the two others homeostatic or weakly homeostatic. Two of the four bacterial datasets were strictly homeostatic, while the two other strains were homeostatic ($1/H_{P:C}$: 0.16–0.19).

Terrestrial invertebrates and zooplankton had weighted mean $1/H_{P:C}$ significantly higher than zero (Fig. 3). In contrast, aquatic macroinvertebrates and bacteria had $1/H_{P:C}$ that included zero within the 95% confidence intervals. Heterotrophs combined also had a mean $1/H_{P:C}$ higher than zero. Terrestrial invertebrates had a relatively high mean $1/H_{P:C}$ (0.26), significantly higher than aquatic macroinvertebrates (0.04) and zooplankton (0.08). There were no other significant differences among groups.

N:C homeostasis

We calculated $1/H_{N:C}$ for all broad groups except algae and fish (Fig. 1B, 4). However, the low number of datasets limited across group comparisons. Bacterial, fungi, aquatic

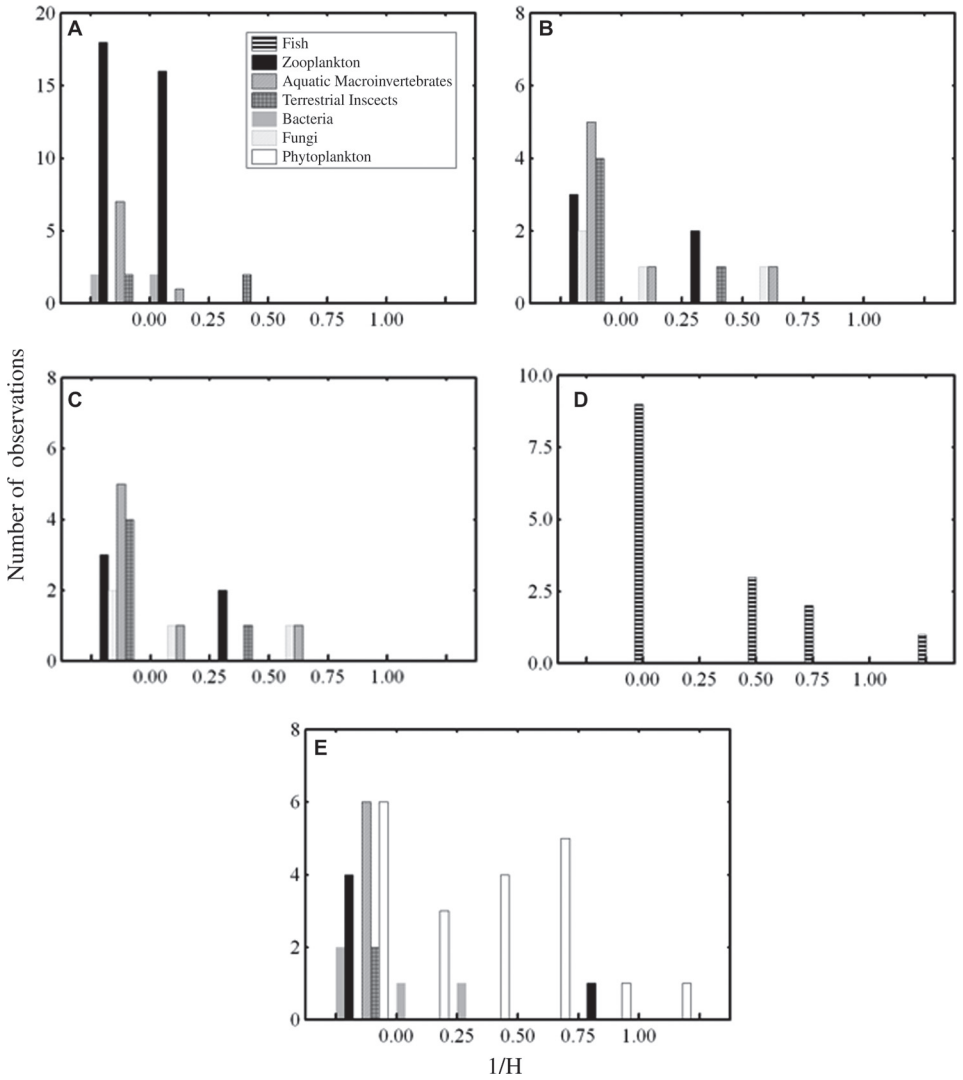

Figure 1. Histograms of 1/H versus the number of data sets in our quantitative literature analysis for (A) P:C, (B) N:C, (C) %P, (D) %N, and (E) N:P; four datasets (two macroinvertebrate studies, one P:C and one N:P; two zooplankton N:C) that gave negative 1/H were excluded from this figure. We classified the degree of homeostasis as: 1/H = 0 'strictly homeostatic', 0<1/H<0.25 'homeostatic', 0.25<1/H<0.5 'weakly homeostatic', 0.5<1/H<0.75 'weakly plastic', and 1/H >0.75 'plastic'.

macroinvertebrate, and zooplankton N:C were generally similar to resource N:C (Fig. 4). In contrast, terrestrial invertebrates consumed diets more different from their own composition (Fig. 4E). All groups generally exhibited strong N:C homeostasis (Fig. 1B). Excluding two species with negative $1/H_{N:C}$ values, zooplankton $1/H_{N:C}$ ranged from 0 to 0.33. Three of these five zooplankton species were strictly homeostatic (not including those with negative slopes). Five of seven aquatic macroinvertebrates were strictly homeostatic, and the fifth had a $1/H_{N:C}$ of 0.18. The snail *Radix ovata* was weakly plastic ($1/H_{N:C}$ = 0.60) in a low quantity treatment but strictly homeostatic in a high quantity treatment within the same experiment (Table A1). Four of five terrestrial invertebrates were stricly homeostatic, while the only bacterial species was homeostatic ($1/H_{N:C}$ = 0.16). Regulation of N:C homeostasis varied among the fungi with two being strictly homeostatic, one homeostatic ($1/H_{N:C}$ = 0.16, Fig 1B), and *Polyporus versicolor* being plastic (Table A1). No groups had mean $1/H_{N:C}$ significantly different from zero, and there were no significant differences among groups (Fig. 3).

N:P homeostasis

Datasets of $1/H_{N:P}$ were available for all broad groups except fungi and fish (Fig. 1E, 5). However, half of the datasets (20 out of 40) were from algae (mostly freshwater), biasing the comparison. Only five and two datasets for zooplankton and terrestrial insect respectively were found for N:P (Supplementary material Appendix 1 Table A1). Aquatic macroinvertebrates and terrestrial insects were more enriched in N than their diets (Fig. 5D–E). In contrast, zooplankton N:P was similar to the N:P of their diet, although variation in resource N:P was small for two-thirds of the datasets. Bacteria and algal N:P varied widely in relation to media N:P. Bacterial stoichiometry was generally different from media

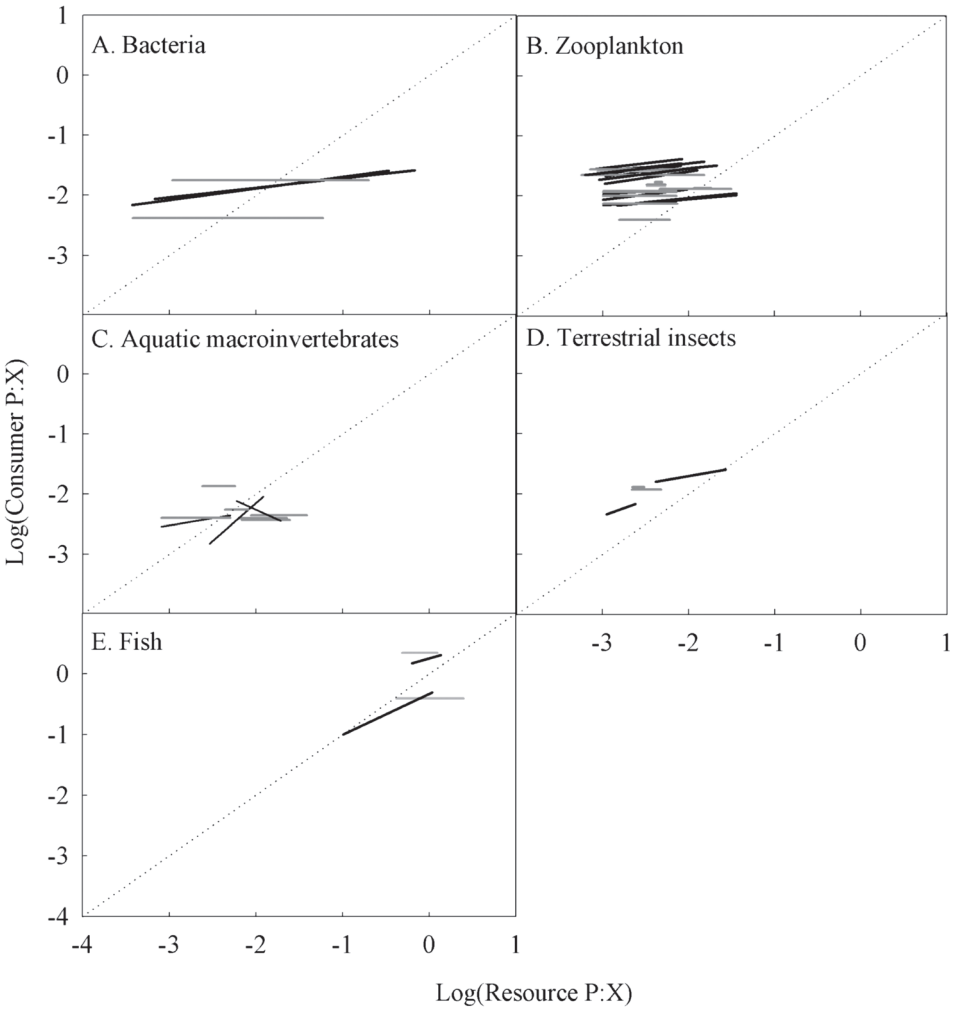

Figure 2. Regressions between consumer and resource P:X for (A) bacteria, (B) zooplankton, (C) aquatic macroinvetebrates, (D) terrestrial insects and (E) fish. X equals C (molar) for panel (A)–(D), and dry weight for panel (E). Black regression lines indicate least square regressions with $p<0.10$ (plastic), and grey lines indicate regressions with $p>0.10$ (strictly homeostatic). We considered species with insignificant ($p>0.1$) regression slopes as strictly homeostatic and their slope is displayed as zero ($1/H_{P:C} = 0$). The length of the displayed regression corresponds to the data range behind it. The dotted diagonal line shows the 1:1 relation.

N:P, regardless of whether the media N:P was high or low (Fig. 5B). Algal species exhibited a wide variety of responses (Fig. 5A). The N:P stoichiometry of some algal species were always markedly different from media N:P, similar to the bacteria. In contrast, the stoichoimetry of other algal species closely tracked media N:P. A few algal species were relatively more N rich than media N:P.

Strict homeostasis was observed for six out of the 20 algal datasets, and another seven had $1/H_{N:P}$ less than 0.5. Only one species (*Scenedesmus* sp.) exhibited no homeostasis ($1/H_{N:P} = 1$). The homeostasis of *Cyclotella meneghiniana* was negatively related to chemostat dilution rate (Supplementary material Appendix 1 Table A1). Among the algae, strong homeostasis appeared to be restricted to a few special cases. Out of the nine datasets with a $1/H_{N:P}$ below 0.25, three datasets were from studies with a high chemostat dilution rate (Shafik et al. 1997) while four others were haptophytes (Table A1).

The heterotrophs strongly regulated N:P homeostasis (Fig. 1E). Two of the four bacterial datasets were classified as strictly homeostatic ($1/H_{N:P} = 0$), while six of the seven aquatic macroinvertebrates were strictly homeostatic. The bivalve *Mytilus edulis* exhibited negative scaling between body N:P and resource N:P ($1/H_{N:P} = –0.35$). Four of the five zooplankton datasets were classified as strictly homeostatic, while the flagellate, *Paraphysomonas imperforata* was weakly plastic ($1/H_{N:P} = 0.77$).

Algae were the only group that had a mean $1/H_{N:P}$ significantly higher than zero (Fig. 3). The mean algal $1/H_{N:P}$ (0.53) was significantly steeper than that of aquatic macroinvertebrates (0.03), zooplankton (–0.16), and all heterotrophs combined (–0.03; Fig. 3). There were no other significant differences among the groups.

P and N homeostasis of fish

Datasets of $1/H_N$ and $1/H_P$ only included fish studies (Fig. 2E, 4F). Four %P datasets and 15 %N datasets were found; however, two species were represented three times each due to the use of multiple experimental conditions. The majority of these studies examined juvenile fish. In spite of the small

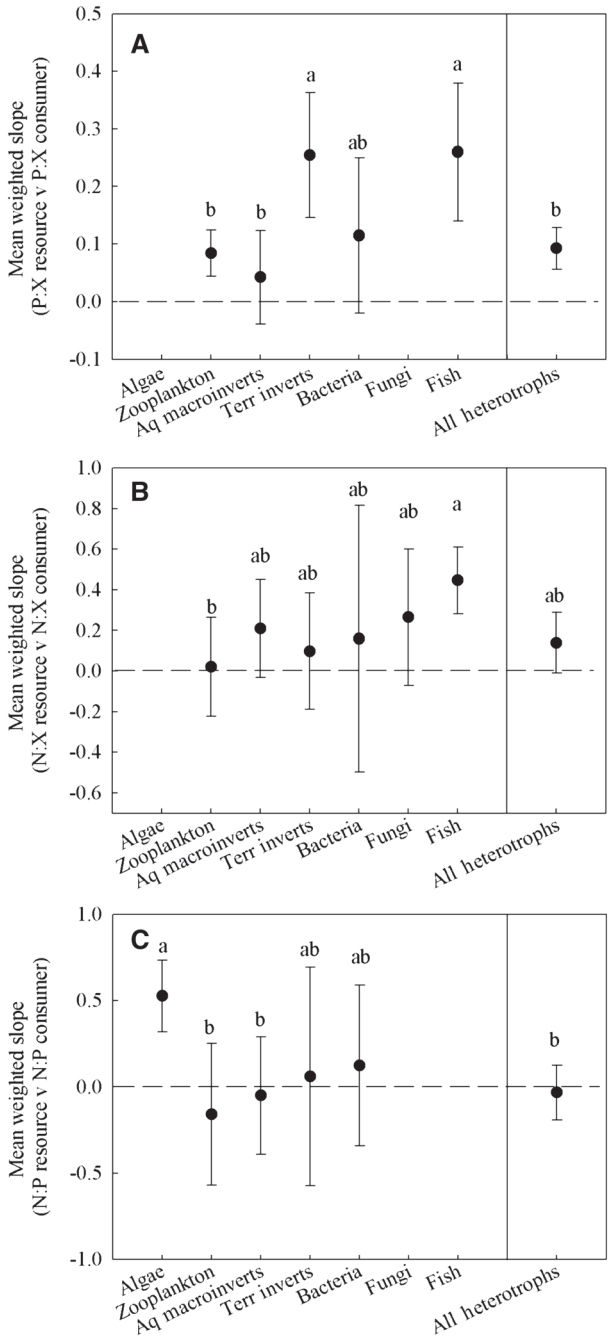

Figure 3. Mean weighted slopes and 95% confidence intervals from meta-analyses of resource versus consumer (A) P:X, (B) N:X, and (C) N:P regressions for algae, zooplankton, aquatic macroinvertebrates, terrestrial invertebrates, bacteria, fungi, fish, and all heterotrophs (except fish) combined. In panel (A) and (B), X equals C (molar) for all groups except fish where X equals dry weight. Regression slopes used to calculate the mean weighted slopes are given in Table A1. Different letters above bars represent significant differences.

sample size, together the %N and %P datasets include 13 families (Supplementary material Appendix 1 Table A1). In general, the N and P content of fish and diet were similar.

The degree to which fish regulated N and P homeostasis varied widely. Two of the four fish (*Oncorhynchus mykiss* and *Synechogobius hasta*) weakly regulated P homeostasis, while the remaining species were strictly homeostatic (Fig. 1C). Nine of the fifteen fish N datasets exhibited strict homeostasis (Fig. 1D). Of the remaining, four exhibited weak N homeostasis and the rest were plastic. N homeostasis did not vary between eel size classes (*Anguilla anguilla*, Supplementary material Appendix 1 Table A1) or with food abundance in rockfish (*Sebastes schlegeli*, Supplementary material Appendix 1 Table A1). Mean $1/H_P$ (0.26) and $1/H_N$ (0.45) were relatively high and significantly different from zero.

Are all cases with insignificant slopes examples of strict homeostasis?

To identify cases misclassified as strictly homeostatic due to especially noisy data, we compared the variation in homeostatic cases to the residual (i.e. background) variation among the plastic cases. These results suggest that most cases with insignificant regression slopes were correctly classified as strictly homeostatic (Fig. A1) and are not cases of especially noisy data. For nutrient ratios other than P:C, cases classified as strictly homeostatic had levels of variability similar to the residual variation among the plastic cases. In the P:C dataset, variation for the strictly homeostatic cases was skewed more to the right than the residual error, suggesting that some of these cases may be misidentified as strictly homeostatic. For instance, nine of the twelve datasets with a variance estimate greater than 0.1 (the largest occupied bin, Fig. A1) had insignificant slopes (five zooplankton datasets and two macroinvertebrates datasets). These datasets, potentially misclassified as strictly homeostatic, represent approximately one-third of the all P:C datasets classified as strictly homeostatic.

Discussion

Homeostasis across levels of organization

Much of the theoretical and experimental research in ecological stoichiometry is based on a simplifying assumption that autotrophs are stoichiometrically plastic while heterotrophs are strictly homeostatic (Sterner et al. 1992, Loladze et al. 2000). Studies conducted over the last decade indicate that heterotrophs may not be as strictly homeostatic as generally assumed (DeMott 2003, DeMott and Pape 2005, Fink and Von Elert 2006, Chrzanowski and Grover 2008). We conducted a quantitative review and a meta analyisis to (1) test these simplifying assumptions, (2) determine the variation in homeostatic regulation at different levels of organization, and (3) to investigate the potential drivers of variation.

At the broad autotroph versus heterotroph level these early simplifying assumptions appear to be adequate. As a group, heterotrophs exhibited strict N:P and N:C homeostasis and very strong P:C homeostasis. Heterotroph homeostasis was much stronger than autotroph homeostasis, although direct comparison is only possible for N:P (Fig. 1, 3). In the N:C and N:P datasets the heterotroph groups exhibited statistically similar mean slopes; however, comparisons at this course level of organization at times masked wide variation in the degree of homeostatic regulation (Fig. 3). For example, the mean

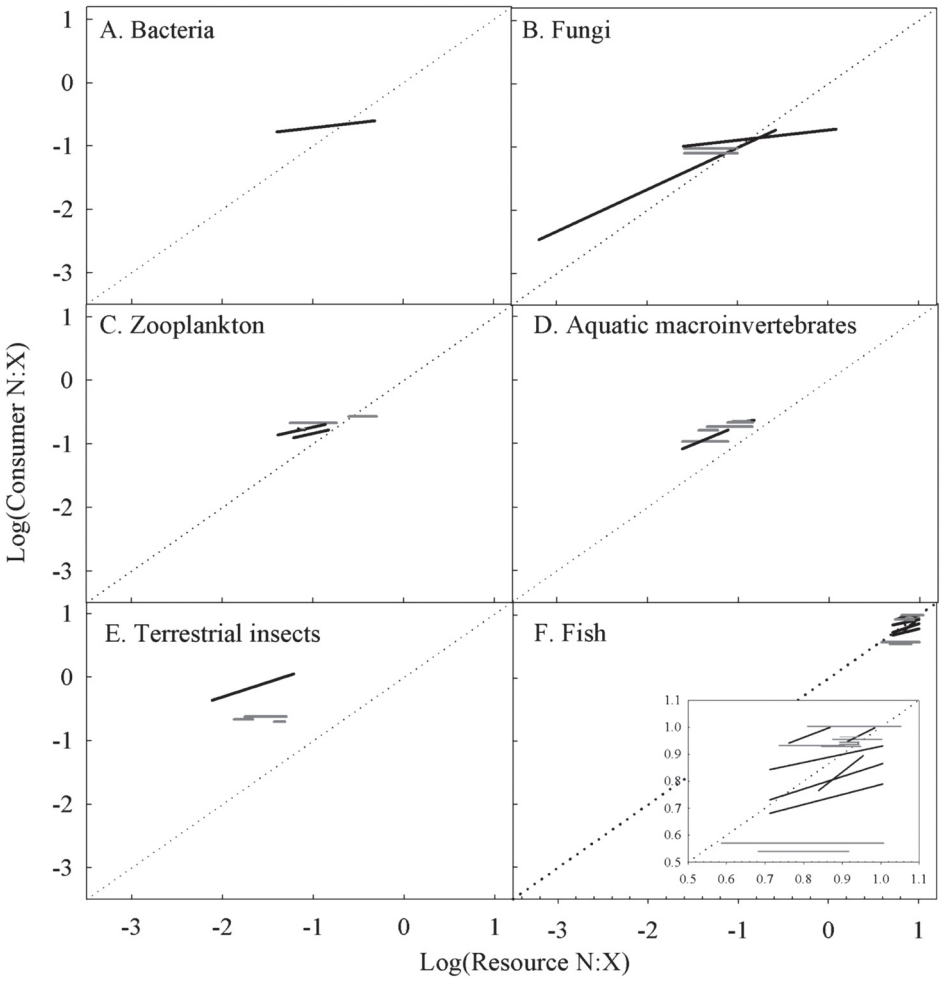

Figure 4. Regressions between consumer and resource N:X for (A) bacteria, (B) fungi, (C) zooplankton, (D) aquatic macroinvertebrates, (E) terrestrial insects and (F) fish. X equals C (molar) for panel (A)–(E), and dry weight for panel (F). Inset graph in panel (F) shows the data on a finer scale. Black regression lines indicate least square regressions with $p<0.10$ (plastic), and grey lines indicate regressions with $p>0.10$ (strictly homeostatic). We considered species with insignificant ($p>0.1$) regression slopes as strictly homeostatic and their slope is displayed as zero ($1/H_{N:C} = 0$). The length of the displayed regression corresponds to the data range behind it. The dotted diagonal line shows the 1:1 relation.

P:C homeostasis of terrestrial insects was much weaker than that of aquatic macroinvertebrates. Furthermore, we observed a wide variation in the degree of homeostasis among closely related groups and species, especially within the genus *Daphnia*. DeMott and Pape (2005) also observed wide variation in P homeostasis among *Daphnia* species in carefully controlled laboratory experiments. The degree of homeostasis also varied widely in several well-studied species. For example, clones of *D. pulicaria* vary widely in P homeostasis (Jeyasingh et al. 2009) and the $1/H_{N:P}$ of *Cyclotella meneghiniana* varied from 0 to 0.51 as a function of growth rate, which was experimentally controlled. Taken together, our results suggest that the assumptions outlined in the literature offer a generalized framework for predicting differences in nutrient homeostasis. Yet, these assumptions only provide rough boundaries. Nutrient homeostasis is neither a group nor species-level trait; instead, homeostasis varies widely as a function of both biology and environmental conditions.

To date, the influence of such factors on stoichiometric homeostasis has received scant attention, stunting our ability to generalize. Below, we highlight some factors that either appear to influence a species' homeostasis or potentially might do so. Growth rate could be one important determinate of nutrient homeostasis for algae. The N:P homeostasis of the diatom *Cyclotella* increased with growth rate (Shafik et al. 1997), as did the homeostasis of the green alga *Selenastrum minutum* (Elrifi and Turpin 1985). Algal homeostasis likely increases with growth rate due to a lack of luxury uptake and nutrient storage at high growth rates, when available nutrients are used to meet the high nitrogen and phosphorus demands of rapid growth (Elrifi and Turpin 1985). The influence of growth rate on algal homeostasis highlights an important contrast between phytoplankton and animal studies. In algal studies, primarily conducted in chemostats, growth rate is held constant while resource supply ratios are varied. In contrast, growth rate varies with resource stoichiometry in animal studies. It would

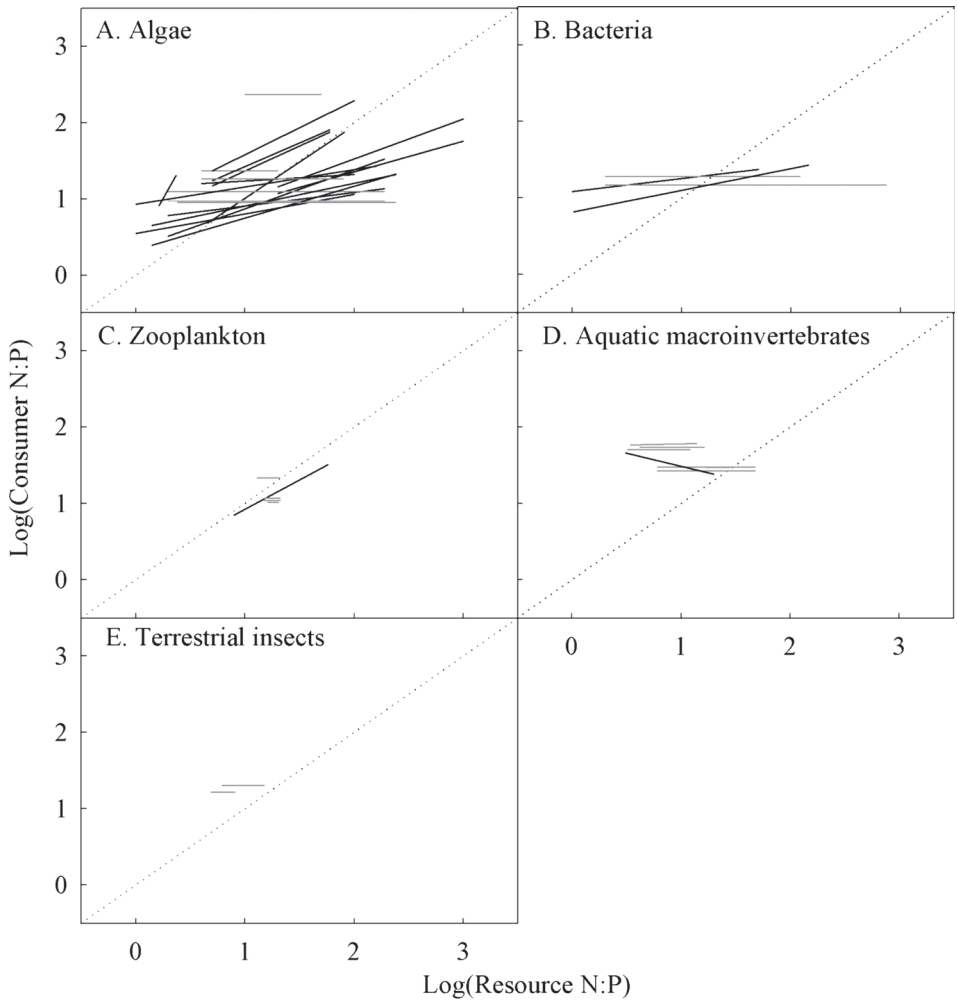

Figure 5. Regressions between consumer and resource N:P for (A) algae, (B) bacteria, (C) zooplankton, (D) aquatic macroinvertebrates, and (E) terrestrial insects. Black regression lines indicate least square regressions with $p<0.10$ (plastic), and grey lines indicate regressions with $p>0.10$ (strictly homeostatic). We considered species with insignificant ($p>0.1$) regression slopes as strictly homeostatic and their slope is displayed as zero ($1/H_{N:P} = 0$). The length of the displayed regression corresponds to the data range behind it. The dotted diagonal line shows the 1:1 relation.

be difficult to experimentally disentangle the influence of growth rate and resource stoichiometry on animal homeostasis. Yet, perhaps the same principles apply to both groups. In animals, rapid growth also requires a high nutrient content (Elser et al. 2003) while some animal studies associate both low and high nutrient stoichiometry with low growth rates (Acharya et al. 2004, Vrede et al. 2004, Ferrao-Filho et al. 2007). It is unclear if animals would exhibit weaker homeostasis, similar to autotrophs, if growth rate were experimentally maintained at low levels.

When resource nutrient content is constant, a variety of biological and environmental factors influence heterotroph nutrient content, including pH, temperature, and soluble calcium levels (McGrath and Quinn 2000, Chrzanowski and Grover 2008, Tan and Wang 2009). Currently, it is unclear whether these variables also influence a species' degree of stoichiometric homeostasis, yet for some organisms such an interaction is likely. For example, the yeast *Candida humicola* stored 10-fold more polyphosphate in a pH 5.5 medium compared to a pH 7.5 medium (McGrath and Quinn 2000). In particular, the influence of environmental factors on stoichiometric homeostasis deserves special attention in the context of global change.

We acknowledge that algae do not represent all primary producers. For example, homeostatic regulation may differ in perennial terrestrial plants, which have slower growth and develop tissues with potentially different nutrient ratios (Ågren 2008). Unfortunately, we could not find any plant data that met our criteria (particularly the criterion of whole-organism data). Therefore, it is difficult to make strong predictions on the homeostasis of such terrestrial primary producers. Similarly, whole-organism nutrient ratio data for larger-bodied animals (such as vertebrates) is not available from the existing literature. Certainly, more data is needed to evaluate the relations between consumer and resource homeostasis, especially in terrestrial ecosystems.

Limitations of the homeostatic parameter 1/H

The stoichiometric homeostasis coefficient, 1/H (Sterner and Elser 2002), indicates the degree to which an organism

maintains homeostasis and quantifies an important aspect of consumer physiology. Although 1/H has not been used extensively, it likely can be used to predict aspects of a species' role in population dynamics, food webs, and nutrient cycles. Yet, for both physiological and mathematical reasons 1/H should be used and interpreted with care.

Excluding cases of strict or no homeostasis, we categorized 1/H into several categories (Methods). These categories may over simplify the biology of stoichiometric homeostasis because the relationship between 1/H and the degree to which consumers regulate stoichiometric homeostasis is not linear but exponential. A species' degree of homeostatic regulation changes nearly five-fold as 1/H varies from 0.33 to one. In contrast, the degree of homeostatic regulation only changes 1.6-fold as 1/H varies from 0 to 0.2. Thus, weakly homeostatic species ($1/H > 0.33$) differ dramatically in their response to changes in resource stoichiometry, while strongly homeostatic species ($1/H < 0.2$) respond in a relatively similar manner to the same degree of variation in resource stoichiometry. Using 1/H instead of H diminishes these differences. It is unclear if these relatively small differences among strongly homeostatic species influence ecological interactions.

The parameter 1/H does not distinguish between strict homeostasis, when consumer stoichiometry is tightly constrained in spite of wide variation in resource stoichiometry, and cases where consumer stoichiometry is highly variable yet independent of resource stoichiometry. Furthermore, our statistical criteria for identifying strictly homeostatic species could lead to classification errors for cases with low power or especially noisy data. To examine whether some cases with insignificant slopes were misclassified as strictly homeostatic, we compared the residual variation in the datasets with significant regression fits (an estimate of background variation) to the variation in strictly homeostatic datasets. Although this approach is not definitive, most strictly homeostatic species appear to be correctly classified, with the exception of perhaps as many as one-third of the strictly homeostatic species in the P:C dataset. The misclassification of these cases may be due to the species' physiology (i.e. weak regulation of consumer stoichiometry), experimental design, or analytical error. Taken together, this analysis suggests that the approach we used accurately classifies most strictly homeostatic species. However, it is clear that caution must be exercised when determining how tightly species regulate stoichiometric homeostasis.

The homeostasis parameter 1/H is a useful tool that quantifies the stoichiometric homeostasis of consumers. Yet, it also simplifies the underlying physiology and biochemistry of homeostasis. An idealized scatterplot of consumer versus resource P:C, for example, would contain regions of both P and C limitation of the consumer. The biochemistry and physiology shaping homeostasis likely differs in these two regions. When P is limiting, variation in consumer P:C is a result of P scarcity which leads to a low RNA content and growth rate (Elser et al. 2003). In contrast, when C is limiting, variation in consumer P:C is likely shaped by P storage. These two physiological processes operate independently, potentially leading to nonlinearity in the relationship between consumer and resource stoichiometry. We did not observe any clear breakpoints in our datasets; however, these datasets were not designed to evaluate this hypothesis. In fact, the animal datasets only examined gradients of nutrient limitation and, therefore, do not clarify the influence of increasing carbon limitation on consumer stoichiometry. There is, however, ample evidence of consumer nutrient stoichiometry scaling with nutrient limitation (DeMott et al. 1998, DeMott 2003, Fink and Von Elert 2006) as well as nutrient storage under C limitation (P: Sterner and Schwalbach 2001, Frost and Elser 2002, Woods et al. 2002; N: Adams and Sterner 2000, Raubenheimer and Jones 2006). Whether one or both of these processes are utilized by a species will likely have a significant influence on the role species play in population dynamics, food webs, and nutrient cycles.

Estimates of the parameter 1/H might be misleading when the quality of a diet is not determined by its stoichiometry. For example, terrestrial insects generally consumed low nutrient diets relative to the other species in our dataset, suggesting nutrient limitation of these consumers. Yet, these consumers may not be nutrient limited. Terrestrial primary producers are generally high in relatively indigestible carbon-rich molecules (e.g. cellulose and lignin), compared to algae. Hence, terrestrial insects may often be limited by the availability of digestible carbon (Anderson et al. 2004) even though terrestrial autotrophs are considered as a low nutrient (P and N) diet relative to aquatic autotrophs (Elser et al. 2000a).

Implications and significance

Taken together, our results suggest that among all organisms nutrient homeostasis varies along a continuum from the most plastic algal species (i.e. body stoichiometry tracks resource stoichiometry) to strictly homeostatic heterotroph species. The observed continuum from weak to strong homeostasis likely reflects a gradient of strategic tradeoffs. For instance, stoichiometrically plastic organisms are likely better adapted to variable environments. These species can store elements when available in excess and use these stores later to supplement growth, instead of expending energy disposing of these materials (Sterner and Schwalbach 2001, Raubenheimer and Jones 2006). Furthermore, when consuming a nutrient poor diet, plastic species can increase their nutrient use efficiency by adding a lower concentration of nutrients to new tissue (Elser et al. 2003).

Yet, the benefits of strict homeostasis are not clear. Strict homeostasis may be more common among heterotrophs than autotrophs (as suggested by our analysis) because nutrient storage is energetically more costly for heterotrophs, relative to autotrophs. For example, a considerable part of the N and P stored in plants and algae can be nitrate and phosphate and their uptake and storage is mostly determined by their capacity to handle the ion balances (Ågren 2004). In contrast, N and P can only be stored as amino acids and proteins or as energy-rich polyphosphate in heterotrophs (Kornberg 1995, Raubenheimer and Jones 2006). Synthesis of these organic compounds in heterotrophs requires high amounts of C, both as a structural component as well as an energy source for the synthesis of the storage macromolecules (protein and polyphosphate). Surplus N and/or P for storage would

probably be only available in times of C (energy) limitation. The energetic costs of N and P storage might constrain the heterotrophic organisms' ability to store significant amounts of nutrients when C limited, which would in turn confine these species to a relatively constant body C:N and C:P ratio. Furthermore, autotroph plasticity of N and P also could imply that the extra N or P is allocated to useful tasks in the biomass (Ågren 2004), and in vertebrates bones might serve as P-storage compartments. Further experimental studies are required to understand the benefits of maintaining homeostasis for a broad variety of organisms.

Our results support the recent findings that the degree of homeostatic regulation varies among heterotroph species and clones. This variation in homeostasis among species appears to influence both food web dynamics and nutrient cycling. For instance, although most stoichiometrically explicit predator-prey models assume strict homeostasis of predator nutrient content (reviewed by Anderson et al. 2004), a few studies have explored interactions with weakly homeostatic predators. These studies suggest that weakly homeostatic predators are more resistant to extinction (due to nutrient-limitation) than strictly homeostatic predators (Grover 2003, Mulder 2007). The grazer's degree of homeostatic regulation can also influence the condition of its predator. For example, Malzahn et al. (2007) showed that when herbivores are not strictly homeostatic nutrient limitation can 'cascade' up the food web from algae to copepods to fish. It is unknown how a species' degree of homeostasis influences its competitive ability or nutrient recycling rates. Examining the influence of a species degree of homeostatic regulation on ecological and biogeochemical interactions may be a fruitful avenue for future research.

Conclusions

Overall, our analysis confirmed the generally assumed pattern of higher stoichiometric flexibility of autotrophs compared to heterotrophs. Nevertheless, not only autotrophs, but also many species of heterotrophs exhibit deviations from strict stoichiometric homeostasis (i.e. 1/H values different from zero). For both autotrophs and heterotrophs, the degree of homeostasis appeared to depend on external (environmental) and internal (e.g. physiological state and growth) factors. We suggest that the homeostasis parameter 1/H, a potentially useful predictive metric, has to be utilized with caution, as it likely over simplifies some important aspects of the response of organisms to elemental imbalances. Taken together, our findings provide a critical evaluation of stoichiometric homeostasis and thus will contribute to a better understanding of many food-web interactions, which are commonly driven by elemental imbalances between consumers and their resources.

To our knowledge, this analysis was based on the largest dataset of consumer homeostasis thus far compiled. Our comparison of groups of organisms along large-scale habitat types (e.g. terrestrial insects, aquatic macroinvertebrates, zooplankton) is a first, albeit coarse, search for relationships between the degree of homeostasis and the organisms' ecology. No other studies available to date address these questions. Although the scarcity of appropriate datasets for many taxa limits our ability to make broad generalizations, our analysis provides a valuable synthesis of the available literature and highlights the importance of future research needs with respect to stoichiometric homeostasis and its role in ecological processes.

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Supplementary material (available online as Appendix O18545 at www.oikos.ekol.lu.se/appendix). Appendix 1.