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Consequences of inferring diet from feeding guilds when estimating and interpreting consumer–resource stoichiometry

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SUMMARY

1. Imbalances between the supply of elements from resources and their demand from consumers may constrain key ecological processes, such as growth and production. Most previous studies have estimated such stoichiometric imbalances between consumers and resources by inferring the diet of the former from functional classifications rather than by direct assessments of the diet. However, this does not allow for potentially plastic responses of consumers to a restricted supply of elemental resources.
2. Here, for three streams of very contrasting nutrient availability, we calculated elemental imbalances between consumers and resources using diets derived from empirical gut contents analysis and compared them with those inferred for the functional feeding guilds of the species concerned.
3. In almost every case, elemental imbalances (C:P and N:P) based on the realised diet differed significantly from those expected from the inferred diet, the former revealing greater alignment between the elemental composition of consumers and their resources, particularly for P.
4. Simply inferring the diet, as is commonly done, results in erroneous estimates of elemental imbalances and misleading conclusions about stoichiometric constraints on consumers.

Keywords: elemental constraints, elemental regulation, functional classification, gut contents, stoichiometric imbalances

Introduction

Ecological stoichiometry concerns the relative balance of key elements (carbon, nitrogen and phosphorus) between consumers and their food (Sterner & Elser, 2002). It has been proposed that consumers must maintain the ratio of these elements within a relatively narrow range (homeostasis). If consumers cannot sequester sufficient elements from their resources, key ecological processes, such as growth and production, could be limited (Sterner & Elser, 2002; Cross, Wallace & Rosemond, 2007; McGlynn, Fawcett & Clark, 2009). If we are to understand the importance of such imbalances, we must first identify where they occur.

A number of carefully controlled laboratory studies have investigated the response of consumers fed on resources of consistent type but varying stoichiometry (Hessen, 1990; Andersen & Hessen, 1991; Frost & Elser, 2002; Kendrick & Benstead, 2013). However, the potential stoichiometric constraints on consumers under natural conditions are less clear. The vast majority of previous studies of stoichiometric constraints in natural systems have compared elemental ratios of consumers and resources by inferring the diet of consumers based on classifications such as trophic levels (e.g. Elser & George, 1993; Elser & Hassett, 1994) or functional guilds (e.g. Cross *et al.*, 2003; Lauridsen *et al.*, 2012; Mehler *et al.*, 2013; but see Cross *et al.*, 2007). Such studies have

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reported large stoichiometric imbalances, particularly between detritivores and their food in both terrestrial and aquatic systems (Higashi, Abe & Burns, 1992; Cross *et al.*, 2003; Bowman, Chambers & Schindler, 2005; Small & Pringle, 2010; Lauridsen *et al.*, 2012).

However, assumptions that the diet consists inflexibly of a single resource type (Elser & George, 1993; Cross *et al.*, 2003; Bowman *et al.*, 2005; Lauridsen *et al.*, 2012) are unlikely to be realised in nature and do not allow for behavioural, or other, plastic responses of consumers to a restricted supply of elements from resources. Trophic levels are rarely well defined, and omnivory is a frequent characteristic of food webs (Polis & Strong, 1996; Coll & Guershon, 2002). Classifications such as the functional feeding groups of benthic freshwater invertebrates (FFG: Merrit & Cummins, 1996) strictly describe the mode of feeding rather than an actual diet (Cummins, 1973), although functional groupings are frequently used as a surrogate for diet (e.g. Elser *et al.*, 2000; Cross *et al.*, 2003; Bowman *et al.*, 2005; Mehler *et al.*, 2013). However, the organisms they are applied to are generally opportunistic feeders that exhibit large spatial and temporal variation in diet (Mihuc & Minshall, 1995; Mihuc, 1997; Ledger & Hildrew, 2000a,b).

Even if consumers are restricted to a single resource, it cannot be assumed that they ingest and assimilate the bulk resource unselectively: selective foraging and assimilation by both terrestrial and aquatic primary consumers are well-established phenomena (Arsuffi & Suberkropp, 1985; Huntly, 1991; Schatz & McCauley, 2007). Consumers may modify their diet where they cannot fulfil their elemental requirements and can switch diet depending on resource availability (e.g. *Daphnia* consumes terrestrial detritus when phytoplankton are scarce: Grey, Jones & Sleep, 2001; and the predatory Tanypodinae also switch to detritus when prey are scarce: Hildrew, Townsend & Hasham, 1985), so it could be expected that consumers will forage selectively for the elements required to satisfy metabolism. For instance, cattle engage in osteophagia, scavenging or carnivory when reared on low P soils, which suggests a behavioural response to reduced elemental availability (Wallisdevries, 1996).

Functional classifications (feeding group, trophic level, etc.) summarise traits and may act as good shorthand to understand many ecological phenomena (Cummins, 1973; Statzner *et al.*, 2001; Bremner, 2008; Baird *et al.*, 2011; Chown, 2012). Nevertheless, such functional classifications are likely to be of limited use when attempting to predict elemental imbalances between consumers and resources if they do not capture the realised diet with sufficient precision and accuracy. Although an accurate description of the diet in nature is often difficult, it may be necessary if we are to understand stoichiometric constraints on production and food-web dynamics.

Autotrophs are capable of luxury uptake and storage of non-limiting nutrients (Jaeger *et al.*, 1997; Sterner & Elser, 2002), and there are suggestions that bacteria (Scott, Cotner & LaPara, 2012) and aquatic hyphomycetes (Danger & Chauvet, 2013) may also have such capabilities. However, animal cells are believed in general to have very low variability in stored nutrients not immediately linked to cellular function (Miyashita & Miyazaki, 1992; Sterner & Elser, 2002; but see Woods *et al.*, 2002). Many studies support the notion of relatively strong consumer homeostasis, despite variation in the elemental composition of their food (Hessen, 1990; Andersen & Hessen, 1991; Sterner & Elser, 2002), although several recent studies have indicated that C : P in consumers can fluctuate more widely (Persson *et al.*, 2010; Small & Pringle, 2010; Tsoi, Hadwen & Fellows, 2011; Kendrick & Benstead, 2013) and may be influenced by environmental controls. The factors that lead to such plasticity in elemental ratios are not well established. It is possible that plasticity is an evolved response and is limited to certain taxa whose metabolism (or tissues) can be maintained under a range of elemental ratios. However, it is possible that food plays an important role and that those taxa that feed on resources varying temporally in elemental quality (or availability) respond plastically to variations in their resources (DeMott, Gulati & Siewertsen, 1998; Lauridsen *et al.*, 2012; Kendrick & Benstead, 2013). Clearly, we need to understand better the relationship between the elemental composition of consumers and their food.

Here, we exploited the detailed data available for three stream systems on elemental composition of basal resources and consumers, and on realised diets of consumers. The streams also had very different nutrient availabilities, enabling us to characterise consumer–resource stoichiometry over a wide range of conditions, characteristic of near-pristine to strongly enriched systems. The questions we addressed with these data were as follows: (A) How far does the elemental imbalances between consumers and resources, established from realised diet within these three systems, confirm expectations based on functional feeding guilds? If the elemental imbalances were as predicted based on such allocations (the typical approach used to date, e.g. Cross *et al.*, 2003; Bowman *et al.*, 2005; Mehler *et al.*, 2013), the task of modelling and predicting the role of elemental constraints on community productivity and other processes would be greatly simplified. Nevertheless, we anticipated deviations from this simple assumption and expected that elemental imbalances would be less pronounced if, for instance, foraging was related to the availability of elements in the food. (B) Are disparities between the elemental composition of inferred and realised diets predictable? We expected any selective feeding to be related to elemental availability and therefore expected a greater deviation from the inferred diet in nutrient-poor systems. (C) Is there any correlation between consumer tissues and the elemental composition of resources actually ingested? There is a general expectation that consumers will display a large degree of elemental regulation, if not strict homeostasis, and we hypothesised that correlation between consumer tissues and the elemental composition of resources ingested would be limited.

Methods

Site descriptions and water chemistry

Three sites were included in this study, from all of which comprehensive data on elemental composition of consumers and resources, and highly resolved data describing realised diets, were collected. The study sites were Tadnoll Brook, Dorset, U.K. (lat 50°41′N, long 2°19′W), and catchments 53 and 54 (hereafter C53 and C54) at the Coweeta Hydrologic Laboratory, Macon Co., North Carolina, U.S.A. (lat 35°02′N, long 83°27′W). These three sites have strongly contrasting nutrient availability; C53 is extremely nutrient poor, C54 was (experimentally) enriched in comparison with C53, and Tadnoll Brook is very nutrient rich (Table 1). Tadnoll Brook is a second-order tributary of the River Frome, draining a catchment of mixed geology (chalk, clay and sand). For detailed site description and chemistry methods, see Lauridsen *et al.* (2012). Coweeta Hydrologic Laboratory is a U.S. long-term ecological research (LTER) site comprising a network of small first- to third-order streams draining mixed hardwood forest with a dense understorey of *Rhododendron* (Swank & Crossley, 1988). During the study period, C53 and C54 had similar physical characteristics, but differed significantly in dissolved nitrogen and phosphorus concentrations as a result of an experimental nutrient enrichment of the latter (see Table 1; Cross *et al.*, 2007).

Dietary analysis of consumers (fish and invertebrates)

The diet of the dominant consumers in all three sites was established through the direct observation of gut contents. Fish were present in Tadnoll Brook, but absent from the Coweeta streams. In Tadnoll Brook, fish were caught by electric fishing. The guts of larger individuals [brown trout (*Salmo trutta*), body length >70 mm] were flushed using a small manual water pump, and the contents immediately preserved in 4% formalin. For smaller trout and other fish species, specimens were killed and frozen for subsequent dissection of the gut. In the laboratory, prey items were identified, linear dimensions measured and published length–mass regressions used to calculate the dry mass of individual prey items: for full details, see Gilljam *et al.* (2011). Gut contents from all fish species present were analysed.

The macroinvertebrates of Tadnoll Brook were sampled using a Surber sampler (0.06 m^2; mesh aperture 300 μm; see Lauridsen *et al.*, 2012 for details). To describe the main feeding links of the community, individuals of numerically dominant (comprising more than 0.5% of the total abundance) or trophically important (with an average individual dry mass larger than 0.3 mg) taxa were taken from the Surber samples and dissected for determination of gut contents. The length and diameter of each gut was measured to the nearest 0.1 mm. The contents of each individual gut were transferred to a separate microscope slide and fixed with Aquatex® (VWR International Ltd., Poole, U.K.). All slides were examined for animal prey at 100×

Table 1 Nutrient concentration in water and elemental composition of basal resources in Tadnoll Brook and Coweeta reference stream (C53) and enriched stream (C54). SRP, soluble reactive P; EPIL, epilithon; FPOM, fine particulate organic matter; CPOM, coarse particulate organic matter. Epilithon was collected from natural substrata in Tadnoll Brook, whereas it was collected from colonising tiles in Coweeta streams

			C : N			C : P			N : P		
Location	SRP (μg L^{-1})	N (μg L^{-1})	EPIL	FPOM	CPOM	EPIL	FPOM	CPOM	EPIL	FPOM	CPOM
C53	7	30†	8.7	34	82	1741	1015	4858	318	28	67
C54	46	380†	4.6	29	73	845	673	3063	201	23	39
Tadnoll	123	7000*	8.6	21	27	58	235	555	6.8	12	21

*total oxidisable N.

†$NH_4 + NO_3$.

magnification. Animal prey were then identified at 400× magnification, by comparison with reference slides, and linear dimensions measured to estimate the dry body mass of the original whole prey item from published length–mass regressions (Woodward *et al*., 2010; Gilljam *et al*., 2011). For slides containing animal tissue only, the total biomass within each consumer gut was determined as the sum of the biomass of identified prey items. Where there was no relationship between identifiable parts and prey dry body mass (i.e. the chaetae of Oligochaeta), the mean dry mass of all the identified prey items consumed by that particular predator taxon was used. Where no identifiable animal remains were found, any (unidentified) animal tissue present was allocated proportionally to the average (animal) diet of that predatory taxon.

Basal resources in guts were identified at 400× magnification and assigned to one of the following categories: CPOM (particle size >1 mm), FPOM (particle size >250 μm but <1 mm), diatoms, green algae, cyanobacteria, fungi, protozoa and animal tissue. An eyepiece graticule (1 × 1 cm divided into tenths i.e. 100 cells) was used to determine the areal proportion of each resource. The graticule (grid) was placed randomly in five different fields of view on each slide and the dominant food category in each of the 100 cells determined. For each gut, the average areal proportion of the resources consumed was calculated from the five fields of view.

The specific gravity of CPOM, FPOM and animal tissues was obtained by water displacement: dry mass [mg]: volume [mm^3] CPOM = 0.23 ± 0.003; FPOM = 0.20 ± 0.003; animal tissue = 0.23 ± 0.006. Specific gravities of photosynthetic microbes and fungi were obtained from Iversen (1974): photosynthetic microbes = 0.15 and fungi = 0.15.

For slides of the gut contents of omnivorous taxa containing both animal tissue and basal resources, the animal tissue was allocated to prey taxa from the remains observed at 100× magnification according to the relative biomass ingested (determined from measured identifiable remains).

To calculate the biomass of food items in guts with basal resources (i.e. other than strictly predatory taxa, which had only prey in their guts or only traces of non-animal material attributable to prey gut contents), it was assumed that the guts were cylindrical and full (Henderson, Hildrew & Townsend, 1990). The volume of the gut was estimated from the measured length and diameter, and the volume of the identified resource types calculated in accordance with the mean areal proportions obtained from the five fields of view.

In the Coweeta streams, invertebrates were collected in March 2002: individuals of each of the dominant taxa were collected from each stream for gut contents analysis. Invertebrate gut contents were filtered onto 0.45-μm metrical membrane filters (Gelman Sciences, Ann Arbor, MI, U.S.A.), and filters mounted on glass slides. Food particles from each slide were identified at 400× to one of the food categories described above, and their area measured (for full details see Cross *et al*., 2007). All predator guts contained prey only, and all traces of non-animal material found in predator guts were attributable to prey gut contents. The proportional area of all food types consumed was calculated for each individual and converted to dry mass using the specific gravity of the resource type in both the Coweeta and Tadnoll sites.

Elemental composition of consumers and resources

At both Tadnoll Brook and the Coweeta streams, additional material from the dominant consumers and basal resources was collected (see Table 1 for basal resources) for analysis of elemental composition, at the same time as quantitative sampling for the examination of gut contents. In-stream particulate organic material (CPOM and FPOM) was collected from the surface of depositional habitats. In Tadnoll Brook, epilithon was collected from the upper surface of stones (see Lauridsen *et al*., 2012 for detailed methods). In the Coweeta streams, epilithon was sampled from ceramic colonisation tiles that were left in both streams for 6 weeks in spring 2002: while it is possible that the addition of these artificial substrata may have influenced the sampled epilithic community, this approach is unlikely to alter the findings of this work. All samples were dried and homogenised, and part was analysed for elemental C and N with an elemental analyser calibrated with known quantities of urea. Phosphorus content of the remaining sample was determined spectrophotometrically, after initial combustion and acid digestion (see Cross *et al*., 2003 and Lauridsen *et al*., 2012 for detailed methods).

Elemental composition of inferred and realised diets

For the purposes of comparing consumers and resources using inferred food sources, macroinvertebrates were assigned to functional feeding groups (FFG: Moog, 1995; Merrit & Cummins, 1996; collector–filterers, collector–gatherers, shredders, scrapers, invertebrate predators and fish predators). Following standard protocols (e.g. Cross *et al*., 2003; Bowman *et al*., 2005), it was assumed that collector–filterers and collector–gatherers ate bulk

FPOM, shredders ingested bulk CPOM and scrapers bulk epilithon, while invertebrate predators were assumed to eat non-predatory invertebrates and predatory fish to eat all invertebrates. It is possible to allocate a diet comprising mixed resources to some taxa rather than following the FFG classification strictly (e.g. Small & Pringle, 2010). However, it was decided not to follow this approach as it is rarely used, and the direction and magnitude of such assumed deviation from the FFG classification requires prior site-specific knowledge of species-specific diet (e.g. based on observation of gut contents). The elemental composition of inferred diet was that of the resources (as determined above) of the FFG to which they were allocated.

The elemental composition of the realised diet was calculated from the relative proportions (by dry mass) of the different resources in the guts of the consumers and the elemental composition of these resources (as determined above). On the rare occasions when animal prey had been consumed but we lacked measured elemental composition for that taxon, data from a closely related taxon were used. No data were collected on the elemental composition of fungi and protozoa, so values obtained from the literature were used (fungi – Cross *et al.*, 2007; protozoa – Rothhaupt, 1995).

Reporting of CNP elemental imbalances and consumer groupings

Following Cross *et al.* (2003), elemental imbalances were calculated as the arithmetic difference in elemental ratios between the mean body composition of each species of consumer and the resources they consume. Although comparisons of bulk measures of the elemental quality of tissues and resources are a coarse measure of elemental imbalance, they enable comparison with previous findings.

To determine whether the method used to characterise diet affected the estimate of elemental imbalance, the difference between the elemental imbalance based on the realised diet and that based on the diet inferred from FFG was calculated for each species (i.e. elemental imbalances in the realised diet minus those in the inferred diet). Using individual species as replicates within FFG, *t*-tests (Bonferroni corrected to account for multiple comparisons) were used to determine whether the difference (i.e. elemental imbalances in the realised diet minus those in the inferred diet) was significantly different from zero: a significant effect would indicate that the method used to characterise diet affected the estimate of elemental imbalance.

In order to establish whether the method used to characterise the diet influenced the calculated elemental imbalance consistently across all FFG and across the three sites, a two-way ANOVA was undertaken. The influence of site, FFG and their interaction, on the difference in elemental imbalance (i.e. realised dietary elemental imbalance minus inferred dietary elemental imbalance), was tested using SAS (after testing for homogeneity of variance): a significant interaction between FFG and site would indicate that the influence of the method used to characterise diet on elemental imbalance was context dependent.

Comparison of the elemental composition of consumers and their diet

The relationship between consumers and diet in the context of homeostasis has conventionally been evaluated by calculating the regulation coefficient H (eta), which compares consumer stoichiometry (e.g. C : N, C : P and N : P) with resource stoichiometry using the formula (Sterner & Elser, 2002):

$$\log(y) = \log(\mathrm{c}) + \frac{\log(x)}{H}$$

where y is the consumer stoichiometry, x is the resource stoichiometry and c is a constant. Hence, where plotting the log elemental ratio of consumer diet (resource) against log elemental ratio of consumer results in a small slope, a high value of H is returned, indicating strong regulation. In this study, we apply the same method of analysis but compare across species within systems (i.e. we plotted log elemental ratio of diet against log elemental ratio of consumer for all species within the system) to derive a community-level measure of the relationship between diet and consumer tissue, rather than a measure of regulation within a species *per se*: we will call this $\hat{H}$ (eta hat). As Coweeta, C54 only differed from C53 as a consequence of experimental nutrient addition, altering resource quality rather than community composition (Cross *et al.*, 2003), for this analysis, these two streams were treated together. Tadnoll Brook was treated separately as it had a different community.

Results

Consumer diet

Consumers did not feed exclusively on the diet expected from their feeding mode at any site, except for predators in the Coweeta streams (Fig. 1). Although the gut contents of collector–gatherers and collector–filterers comprised mainly FPOM (63–83%), as inferred from their FFG, they also consumed substantial amounts of CPOM (13–31%) and some photosynthetic microbes (0.7–4.9%). Furthermore, animal tissue constituted 2.3–8.2% of the diet of collector–filterers (Fig. 1). The gut contents of scrapers mainly comprised FPOM (80–91%), while 4–16% consisted of photosynthetic microbes.

The dominant resource consumed by shredders was CPOM, which constituted 74–79% of the gut contents. Shredder diet also included 16–19% FPOM. In Tadnoll Brook, 10% of shredder diet comprised photosynthetic microbes, mostly filamentous green algae, whereas photosynthetic microbes comprised <0.5% of the diet in the Coweeta streams (C53 and C54).

In Tadnoll Brook, 76% of the matter consumed by invertebrate predators consisted of macroinvertebrate prey, although they also consumed a substantial amount of detritus (Fig. 1). In the Coweeta streams, the gut contents of invertebrate predators contained 100% animal prey.

Elemental imbalance

Elemental imbalances between consumers (body composition) and their diet based on gut contents (hereafter referred to as 'realised dietary imbalances') differed from those calculated using diet inferred from FFG (hereafter referred to as 'inferred dietary imbalances'). The differences between realised and inferred dietary imbalances varied significantly with FFG and, with the exception of C : P, site (Table 2). However, the interaction between FFG and site was significant for all three elemental ratios (Fig. 2), indicating that the implications of using an inferred diet to calculate imbalances for FFG were site specific.

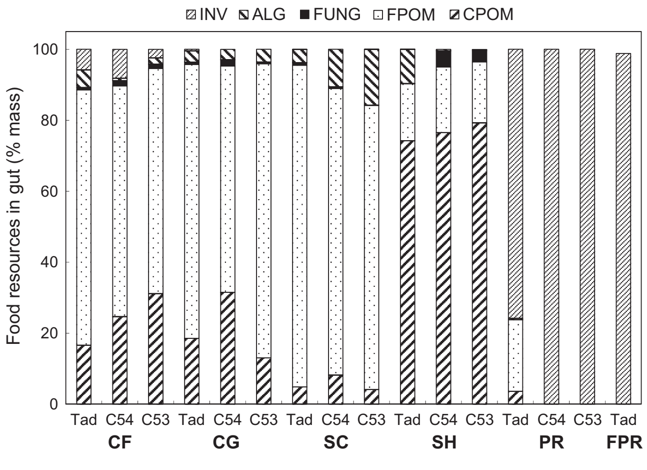

Fig. 1 Composition of diet of the various functional feeding groups in Tadnoll Brook and the Coweeta streams C53 and C54. Mean percentage (by dry mass) of different food resources consumed. CPOM, coarse particular organic matter; FPOM, fine particular matter; FUNG, fungi; ALG, photosynthetic microbes (including diatoms, green algae and cyanobacteria) and INV, macroinvertebrate prey; CF, collector–filterers; CG, collector–gatherers; PR, invertebrate predators; SC, scrapers; SH, shredders and FPR, fish predators.

Realised dietary imbalances in C : N existed for all primary consumer functional feeding groups (i.e. collector–filterers, collector–gatherers, scrapers and shredders). In Tadnoll Brook, the realised dietary imbalance of collector–gatherers was significantly smaller, and of scrapers significantly larger, than those inferred from FFG (Fig. 2a). In the two Coweeta streams, the realised dietary imbalances were smaller than those from inferred diet for shredders, but were larger for other FFG (Fig 2a); with the exception of collector–filterers in C54, all differences were significant. The realised dietary C : N imbalance calculated from the gut contents of fish in Tadnoll Brook closely matched that from inferred diet (Fig. 2a).

For all consumers, except collector–filterers in Tadnoll Brook and scrapers in C54, realised and inferred dietary C : P imbalances were significantly different (Fig. 2b). The realised dietary imbalances for shredders were smaller than those inferred in both Tadnoll Brook and the Coweeta streams (Fig. 2). For scrapers, realised dietary C : P imbalances were larger than inferred for Tadnoll Brook but smaller for C53, whereas there was no difference between realised and inferred imbalance for C54 (Fig. 2b).

The differences between inferred and realised dietary N : P imbalances were significant in every case, except for collector–filterers and collector–gatherers in Tadnoll Brook (Fig. 2c). In most cases, the numerical differences were small, with the exception of scrapers in the Coweeta streams, where the inferred dietary N : P imbalances were considerably lower than those based on the realised diet (Fig. 2c).

Table 2 Results of the two-way analysis of variance of the effect of method used to characterise the elemental imbalance. The influence of site, FFG and their interaction, on the difference in elemental imbalance (i.e. realised diet elemental imbalance minus inferred diet elemental imbalance)

	C : N	C : P	N : P
Site	≤0.0001	0.5701	≤0.0001
FFG	0.0002	≤0.0001	≤0.0001
Site*FFG	0.0001	≤0.0001	≤0.0001

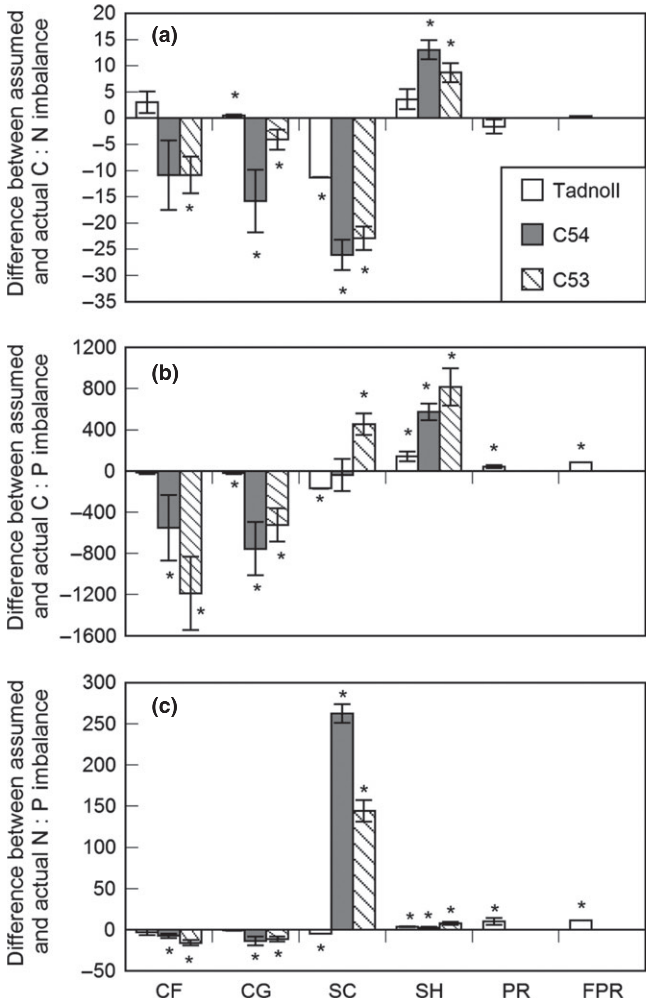

Fig. 2 Mean (±1SE) difference between inferred dietary imbalance and realised dietary imbalance of species within functional feeding groups (FFG) for Tadnoll Brook and the Coweeta streams C53 and C54. Inferred dietary imbalance is calculated from the diet predicted using FFG, and the realised dietary imbalance is calculated using gut contents analysis. (a) C : N (FFG $P < 0.0001$, site $P = 0.0002$, FFG*site $P = 0.0001$), (b) C : P (FFG $P < 0.0001$, site $P = 0.57$, FFG*site $P < 0.0001$) and (c) N : P (FFG $P < 0.0001$, site $P < 0.0001$, FFG*site $P < 0.0001$). The difference in imbalance is calculated by subtraction of the realised dietary imbalance from the inferred dietary imbalance (i.e. positive values indicate that the imbalance calculated using FFG is larger than that calculated using realised diet). Values significantly different to zero are indicated by an asterisk. Key for FFGs in legend to Fig. 1.

Elemental composition of diet and body

In Tadnoll Brook, relationships between the stoichiometry of consumers and of their diet inferred from FFG were not significant for C : P and N : P but significant, although of shallow slope, for C : N ($\hat{H} = 9.53$; Fig. 3a), suggesting little to no alignment of consumers with their diet. In the Coweeta streams, the relationships between the stoichiometry of consumers and of their diet inferred from FFG were all significant, but slopes were shallow, again suggesting only modest alignment of consumers with their diet (Fig. 3c).

For all elemental ratios at both sites, stronger alignment was found between consumer stoichiometry and their realised diet than when diet was inferred from FFG (Fig. 3a *cf* b & c *cf* d). For C : N, all relationships resulted in relatively high $\hat{H}$, indicating that consumers varied little with the C : N of their diet, irrespective of how it was characterised. However, marked differences between relationships established from realised and inferred diet were apparent for C : P in Tadnoll Brook and N : P in the Coweeta streams. In Tadnoll Brook, there was a strong alignment between C : P of the consumers and that of their realised diet ($\hat{H} = 1.26$), whereas no alignment was apparent when diet was inferred from FFG (Fig. 3a *cf.* b). In the Coweeta streams, a pronounced and highly significant alignment was found between the N : P of consumers and their realised diet ($\hat{H} = 0.8$; Fig. 3d), whereas a weaker alignment was found with the diet inferred from FFG ($\hat{H} = 3.1$; Fig. 3c).

Overall, for most FFG, we found significant differences between the elemental composition of realised and inferred diets, which had substantial implications for both calculated imbalances and the relationships between the stoichiometry of consumers and of their diet.

Discussion

Studies of ecological stoichiometry often make assumptions about the food consumed by different trophic guilds; however, we found such inferred diets were very different from the food actually consumed, while these differences also varied strongly across trophic guilds and sites. Furthermore, using the realised diet to determine elemental constraints on consumers, we have shown that their tissues were aligned to the P content of the resources actually exploited despite variation in nutrient availability at the study sites. This finding contrasts markedly with the elemental imbalances anticipated from a functional classification, the widespread use of which to infer diet could, therefore, lead to misinterpretations of relationships between the elemental composition of animals and their diet.

Although there remained elemental imbalances between consumers and their real resources, these imbalances were significantly different from those expected from inferred diets and, in particular, for elemental ratios involving phosphorus (i.e. C : P and

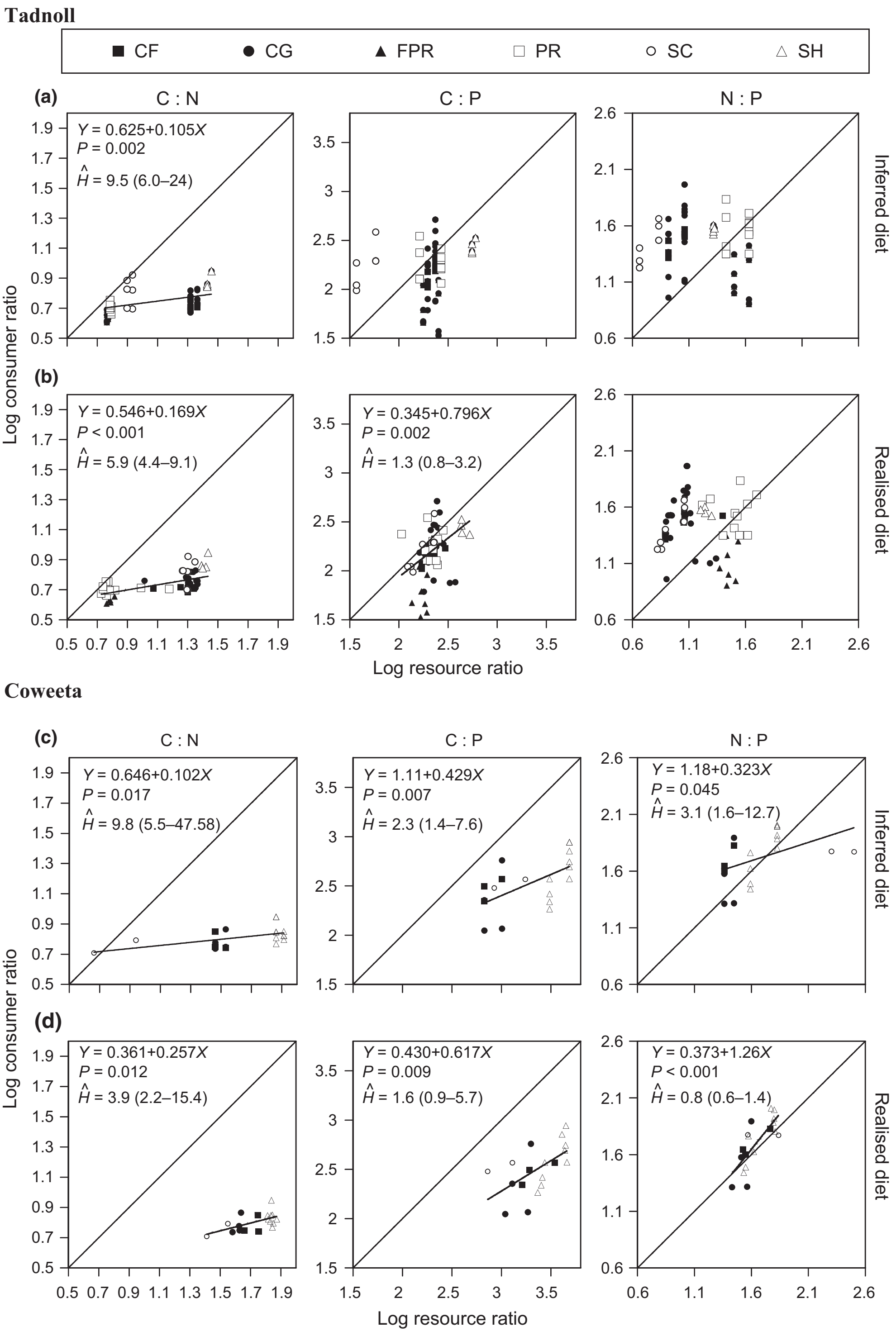

Fig. 3 Logarithmic stoichiometric ratio of realised and inferred diet versus consumer body tissue for all consumers. Each point represents the log elemental ratio of the diet of a consumer species versus the log elemental ratio of that consumer. Each functional feeding group (FFG) is represented by a unique symbol. Diet is inferred using FFG in (a) and (c) for Tadnoll Brook and Coweeta streams, respectively, whereas diet is determined from realised gut contents in (b) and (d) for Tadnoll Brook and Coweeta streams, respectively. Data are presented for C : N, C : P and N : P. On each plot, the linear regression was performed on the log-transformed data, and the equation for the relationship and the P-value is given. $\hat{H}$ (eta hat) is a measure of the association between diet and consumer tissues within the system, where $\hat{H} = 1/\text{slope}$: the $\hat{H}$-value is given for C : N, C : P and N : P with 95% confidence interval shown in brackets. The one-to-one line, indicating direct association, is also shown on the graphs. Non-significant relationships (i.e. slope not significantly different to zero, $\hat{H}$ infinity) not shown. Key for FFGs in legend to Fig. 1.

N : P). Notably, the functional group reported to have the largest imbalances in streams (i.e. shredders, assumed to feed solely on CPOM: Cross *et al.*, 2003; Bowman *et al.*, 2005; Lauridsen *et al.*, 2012) had lower real imbalances than expected in all three systems investigated. Conversely, primary consumers, typically assigned to high-quality food (i.e. scrapers), actually ingested considerable amounts of detritus and exhibited imbalances greater than expected from their functional classification, possibly reflecting a requirement for more carbon than is readily available from 'high-quality' food alone. Over- and underestimation of imbalances appears to be a particular issue for consumers with inferred diet of lowest and highest quality, regardless of nutrient availability in the water.

Nevertheless, the use of a realised diet did not simply result in modification of the extremes. In the nutrient-rich Tadnoll Brook, the imbalances calculated from the realised diet for functional groups feeding on detritus were lower than inferred, presumably as a consequence of detritivores ingesting resources of higher elemental quality than bulk detritus alone. In both Coweeta streams, however, detritivores that were supposedly eating FPOM had greater imbalances than inferred, as a result of extremely low-quality CPOM (i.e. wood and leaf particles) being included in their diet. Across all invertebrate functional groups, putative elemental imbalances calculated from inferred diet were significantly, and often substantially, different from imbalances calculated using the realised diet and were therefore misleading.

Even though every effort was made to standardise the methods and analysis used in the two studies (U.S.A. and U.K.), some methodological differences remained (i.e. different substratum used for analysis of epilithon and slight differences in the quantification of gut contents). These modest differences in methodology may have had some impact on the precise estimates of dietary composition and elemental imbalances, although it is unlikely to have influenced the overall conclusions.

While we inferred diets in a way commonly adopted in freshwater systems, alternative approaches could have produced different estimates of elemental imbalance. Nevertheless, our results indicate that the effect of deviation from realised diet is context dependent, and any approach using inferred diets is likely to result in misleading estimates of elemental imbalances.

Importantly, incorrect estimates of elemental imbalances between consumers and resources have consequences for our understanding of whether consumer stoichiometry reflects differences in the stoichiometry of their diets (and thus whether consumers are homeostatic or not). In all cases reported here, the use of an inferred diet led to weaker relationships with consumer elemental composition (higher $\hat{H}$) than when realised diet was adopted. This was particularly apparent for C : P in Tadnoll Brook and N : P in the Coweeta streams, where consumers were strongly aligned with the realised diet but not with the inferred diet. Thus, elemental constraints imposed on consumers by their diet may be less than previously assumed.

Although sites of contrasting nutrient availability were selected to determine whether stoichiometric imbalances estimated from real and inferred diets were greater in systems where nutrients were scarce, our results did not support this hypothesis. Differences between the elemental composition of inferred and realised diets occurred in all three sites, in a manner that was not straightforward. Although we investigated only a limited numbers of sites, due to the demanding data requirements and consequent logistic constraints, our results offer no indication that general, background nutrient availability affects selective foraging for elements from resources. It is likely that both the availability and quality of resources govern selection by consumers as they forage.

While the use of gut content analysis to determine the diet has distinct advantages over simple assumptions based on trophic guilds, it has some drawbacks. Resources are generally not homogeneous in their elemental composition, but vary in quality among patches or among components of the resource. Here, we matched observed gut contents to bulk measures of resource elemental quality and could not account for potential fine-scale selective feeding by consumers within resource types. Furthermore, gut contents analysis provides a snapshot of ingested material, whereas the elemental composition of the consumers reflects assimilation and excretion over longer time scales. True imbalances occur when there is a mismatch between the rate at which organisms can sequester elements from their resources (i.e. in the assimilate) and the rate of supply required to maintain metabolism (Frost *et al.*, 2005).

Although C : N ratios of consumers within both Tadnoll Brook and the Coweeta streams varied little with C : N of the diet, irrespective of how the latter was characterised, use of the realised diet indicated a stronger alignment of body C : P with dietary C : P in Tadnoll Brook and body N : P with dietary N : P in the Coweeta streams. It should be noted that our use of $\hat{H}$ does not provide a measure of homeostasis, rather it is a community-level measure of the alignment of consumers with their diet. This could be caused by consumers selecting a diet that matches their elemental composition or by possessing a degree of plasticity in their elemental composition such that they align with their diet. It has previously been reported, from both freshwater and terrestrial systems, that consumer C and N concentrations display much lower variability than P (Elser *et al*., 2000; Sterner & Elser, 2002; Cross *et al*., 2003), which may be linked to variations in RNA content at different points in the life cycle (Hessen & Lyche, 1991; Elser *et al*., 1996). This has led to the 'growth rate hypothesis' (Sterner & Elser, 2002), which states that differences in organismal C : N : P are caused by variation in the production of RNA necessary to meet the demands of protein synthesis during growth. The conventional interpretation of variation in C : P and N : P is that P content changes with growth and reproduction following the consumer's life cycle. The results presented here suggest that the P content of consumers is related to the elemental composition of their diet. Plasticity in the P content of consumers has been reported from Costa Rican streams (Small & Pringle, 2010), which, as in our sites (and many others), are dominated by allochthonous inputs: it is likely that growth and, consequently production, of the consumers in these detritus-driven freshwater ecosystems are at least partly regulated by the P concentration of their diet. This hypothesis is supported by the findings of Cross *et al.* (2007), where nutrient addition led to lower carbon to nutrient ratios of the basal resources which, in turn, corresponded with reduced C : P of consumers and increased secondary production of the system.

Our results indicate that a weak relationship between the C : N of consumers and diet was ubiquitous, whereas a strong relationship between C : P of consumers and their realised diet occurred regardless of whether animals were living in nutrient-rich or nutrient-poor systems. Contrasting results were found regarding the relationships between the N : P of realised diet and consumers among the different sites: no correlation was found in the nutrient-rich Tadnoll Brook, whereas a strong correlation existed at the two streams at Coweeta (including the experimentally enriched C54). The alignment between the elemental composition of consumers and their resources is interesting. It is possible that alignment could arise through plasticity in consumer elemental composition or selection (over the long or short term) of diet to suit their metabolic requirements. Irrespective of the mechanism, these findings suggest a strong association between consumers and certain aspects of the elemental quality of their diet, which differed between sites. Although speculative, it is possible that the difference in the relationship of N : P in body and diet between the nutrient-rich and nutrient-poor sites may indicate that consumers in Tadnoll Brook were limited by P, whereas those at Coweeta were colimited by N and P.

This study emphasises the need to recognise the potential role of feeding plasticity in food webs and to quantify imbalances more precisely than by assigning a putative diet using feeding mode. This is of concern because 'functional types' are often used to describe not just community structure but also to extrapolate to ecosystem processes and services, including nutrient cycling (Nowlin, Vanni & Yang, 2008; Van der Wal & Hessen, 2009). A proper appreciation of the constraints placed by elemental imbalances on productivity, and food-web morphology and dynamics, is fundamental. Clearly, a more accurate assessment of the realised harvesting of elements by consumers in a variety of systems over gradients of nutrient availability is a necessary first step.

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