Herbivory versus omnivory: linking homeostasis and elemental imbalance in copepod development

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Organisms usually face and acquire food that is elementally imbalanced, and although they are affected by its quality, they do not reflect the elemental composition of their resources. We analysed whether elemental homeostasis during the ontogeny of freshwater calanoid copepods varied with herbivorous or omnivorous diet. *Boeckella gracilis* and *B. brevicaudata* are herbivorous species, whereas *Parabroteas sarsi* changes its feeding strategy throughout its ontogeny. We hypothesized that homeostatic investment would decrease when food matches consumer requirements in copepods that change their diet from herbivorous microphagy towards a predatory regime. Elemental analyses of each cohort and its food resource revealed a relation between trophic strategy and imbalance. A high level of elemental imbalance was found in the herbivorous species, which were strictly homeostatic during their whole life cycle. The shift in *P. sarsi* trophic strategy resulted in changes in both imbalance and homeostasis. When nutrient conditions are imbalanced, energy needs to be allocated to stoichiometric regulation in order to maintain a relatively tight homeostasis. Our findings provide an example of how variable feeding strategies can counter the imbalance in C:nutrient ratios that organism typically face.

KEYWORDS: ecological stoichiometry; homeostasis; elemental imbalance; ontogeny; zooplankton; copepod; food quality; herbivory; omnivory; predation

INTRODUCTION

Ecological stoichiometry is used to understand the balance of energy and chemical elements in ecological interactions, and in particular, it examines the trophic relationships linking the elemental physiology of organisms with their food web interactions and ecosystem function (Sterner and Elser, 2002). The transfer of energy (carbon, C) and nutrients (usually nitrogen, N, and phosphorus, P) may be limited by the specific needs of each organism, which then affect the quality of available resources for higher trophic levels (Elser *et al.*, 2003). Although consumers are affected by the quality

(C:nutrient ratio) of their food, they do not reflect the elemental composition of their available resources (Sterner *et al.*, 1995). However, the quantity of material is also important and handling of poor quality food is affected by the quantity available as well (Boersma and Kreutzer, 2002; Mitra and Flynn, 2007; Flynn, 2009). The degree to which organisms regulate their elemental composition is their stoichiometric homeostasis.

In aquatic environments, the high C:nutrient ratios of algae in response to resource limitation (Sterner, 1997) affect their quality as food for upper trophic levels (Hessen *et al.*, 2002). Algae have relatively variable C:P

and C:N ratios, which are functions of specific nutrient status and light availability (Sterner, 1997), whereas heterotrophs often have a more fixed stoichiometry because elements are bounded together in the biochemical constituents of their food (Frost *et al.*, 2005). Hence, herbivores may require multiple elements in different ratios than those provided by primary producers and thus face an elemental imbalance with their food (Elser *et al.*, 2003; Acharya *et al.*, 2004). Although studies on these imbalances in consumer–resource interactions have mainly focused on autotroph/herbivore dynamics (Acharya *et al.*, 2004; Andersen *et al.*, 2004), stoichiometric constraints may also affect interactions between species regardless of their trophic level (Moe *et al.*, 2005).

Selectivity in food resources may be a key process by which consumers maintain elemental or biochemical balance. Thus, omnivores that consume from more than one trophic level would increase the acquisition of the limiting nutrient, and a diverse prey spectrum would provide alternative pathways for obtaining a nutritionally balanced ration (Frost *et al.*, 2005). Nevertheless, imbalances may exceed the homeostatic capacity of consumers when they drop below the threshold elemental ratio (Urabe and Watanabe, 1992, 1993), and then changes in the somatic elemental ratios may deeply affect the functioning and fitness of organisms. Elucidating the degree of homeostasis in different taxa and the factors that explain regulatory variation would clarify the mechanisms linking food quality to the consumer traits that affect ecological interactions (Kay *et al.*, 2006).

Elemental requirements may vary with species and throughout their life cycle because changes in an organism's life history strategy (e.g. copepods) require adjustments in the organism's complement of cellular components (Villar-Argaiz *et al.*, 2002). Prey quality has a key effect on prey selection, consumption and trophic transfer efficiency (Jones and Flynn, 2005). In this sense, certain life stages may be more sensitive than others to variation in the stoichiometry of the resource. Copepods undergo strong ontogenetic changes that involve marked differences in body elemental ratios from nauplii to adults (Sterner and Schulz, 1998; Villar-Argaiz *et al.*, 2002). However, these imbalances depend on the quality of the food and could be dampened in copepods that shift from herbivores to predators during development. In this work, we aim to analyse elemental homeostasis (C:N:P ratios) in natural populations of three coexisting calanoid copepod species from Patagonia including two herbivores, *Boeckella gracilis* and *B. brevicaudata*, and *Parabroteas sarsi*, whose feeding strategy changes throughout its ontogeny from a herbivorous towards a predatory regime that includes copepods and cladocerans. We hypothesized that the investment in homeostasis would decrease when food matches consumer requirements in copepods that change their diet to a predatory regime. For this purpose, we conducted a field study to determine the elemental composition throughout development in a cohort of each copepod species, and analysed whether homeostatic regulation differs between copepods according to their trophic strategy.

METHOD

Study site and sampling

Copepods were sampled in Laguna Fantasma, a shallow, fishless vernal pond located at $41°07'$ S, $71°27'$ W and 780 m above sea level in North Patagonia, Argentina. The annual flooding period generally lasts 8–9 months from May to December (autumn to early summer). Maximum water levels occur in June or July when the surface area is 1.4 ha (170 m length and 80 m wide, approximately) and maximum depth is almost 2 m. During the hydroperiod, a single cohort of each copepod species develops from resting eggs to adulthood (Diéguez and Balseiro, 1998; Vega, 1999).

Sampling was carried out weekly during the entire hydroperiod from May to December 2008. Temperature, dissolved oxygen and conductivity were measured for each sampling date using an oxygen, conductivity and temperature meter (YSI 85 Yellow Spring Instruments, USA). Lake water was sampled at a depth of 50 cm with a 2 L limnological bottle. Zooplankton were collected with two plankton nets (200 μm and 55 μm mesh) and in five horizontal tows of 3 m length each. Samples were obtained in three different locations in the deep, central area of the lake. All samples were transferred to acid-washed polypropylene bottles and immediately carried to the laboratory in thermally insulated containers.

Laboratory

Immediately after each sampling, live copepods were separated for elemental analyses under a stereomicroscope. They were rinsed in MilliQ water then identified and classified by species and stages under a stereomicroscope in 5 mL Bogorov chambers. For *B. gracilis* and *P. sarsi*, we discriminated between nauplii, copepodites (CI, CII, CIII, CIV and CV) and adults (males, females and egg-bearing females). For *B. brevicaudata*, we were only able to analyse adults because nauplii and copepodites remained near the bottom regardless of the time of day, whereas adults were found in the water column.

Individuals of each species and instar were analysed for Carbon (C), Nitrogen (N) and Phosphorus (P). Nauplii ($\sim$200), copepodites (10–100, depending on size) and adults (8–50, depending on size) were separately placed in pre-combusted (2 h at 450°C) GF/F Whatman filters and analysed for C and N content on a Thermo Finnigan EA 1112 CN elemental analyser. Phosphorus analyses were carried out in 45 mL MilliQ water with the freshly separated copepods (numbers similar to those used for C and N) and then digested with persulfate at 1.5 atm (121°C) for 1 h, followed by molybdate reaction (Eaton *et al.*, 2005).

In order to discriminate between large and small fractions of food, we considered the small fraction as <80 μm and the large one as >80 μm (estimated as the difference between unfiltered lake water and the <80 μm sestonic fraction). Lake water samples were analysed for sestonic C:N:P ratio in the total (unfiltered) and <80 μm samples, where water was sieved through a clean plankton net (80 μm mesh net). A volume of 200 mL of each water fraction was filtered through pre-combusted (450°C, 2 h) GF/F Whatman filters and analysed for particulate C and N in a Thermo Finnigan EA 1112 CHN elemental analyser. Particulate phosphorus (PP) was calculated for each fraction as the difference between total phosphorus (TP) and total dissolved phosphorus (TDP). TP was measured directly from each fraction and TDP by filtering the water of each fraction through GF/F filters. Both fractions were digested with persulfate at 1.5 atm for 1 h, followed by molybdate reaction (Eaton *et al.*, 2005). Additionally, direct measures of C:N:P of large prey (cladocerans and copepods) were obtained in a manner similar to the copepod somatic elemental analyses.

Copepod diet

Gut contents of the three copepod species in the different instars were examined for diet analyses. The metasomal tube was removed by dissection and mounted on a slide in a drop of water. A drop of commercial bleach was introduced under the edge of the cover glass, and the remains of the dissolved gut contents were examined and identified by microscopy ($\times$400 and $\times$1000 magnification) (Green and Shiel, 1992; Modenutti, 1993). Since distinctive morphological features of the mandibles (especially cutting edges) and maxillipeds are known to be correlated with diet (Green and Shiel, 1999), we dissected the mandible and maxillipeds. The mouthparts were removed by dissection using fine needles under a stereomicroscope mounted in a drop of glycerine-water solution, and they were then analysed

under direct microscope with an image analysis system (Image Pro Plus, Media Cybernetic).

Data analysis

The homeostasis of copepods was analysed through linear regression analysis, and slopes were compared with ANCOVA. The logarithms of the consumer versus resource stoichiometry were plotted (log C:X$_{consumer}$ = a + b log C:X$_{food}$). If the consumer's nutrient content passively reflects the contents of the resources it consumed, then all points in the plot would lie on a line with slope 1 and intercept zero; departures from this line would be caused by differential nutrient processing of elements and thus indicate homeostatic regulation (Sterner and Elser, 2002).

To test the elemental imbalance, we measured the dissimilarity in relative supply of nutrients between the organisms and their resources. This measurement was done by taking the difference between the logarithms of food stoichiometry and the animal stoichiometry (Imbalance = log C:X$_{food}$ − log C:X$_{consumer}$). The elemental ratios of the resource (food) were considered according to copepod diet (see results). In the case of the herbivorous *B. gracilis*, the small fraction (<80 μm) of the seston was considered the food resource for the entire life cycle, whereas for *P. sarsi*, this fraction was considered as food for nauplii, CI and CII. The large fraction (>80 μm) was considered to be the food for CIII. A mixture of the large fraction of the seston, cladocerans and copepods in variable proportions, according to the diet results, was considered to be the food for the remaining stages.

Normality and homoscedasticity were checked prior to the corresponding analysis.

RESULTS

Development of calanoid copepod cohorts, mouthparts and diet

A single cohort of each species was developed during the sampling period. *Parabroteas sarsi* was present during the entire flooding period, and its life cycle was longer than the other species (Fig. 1A). Although up to three different stages coexisted on the sampling dates, one stage was always dominant over the others (Fig. 1B). A similar, but much less evident, pattern was observed for *B. gracilis*, where the development occurred in a shorter time causing the stages to overlap (Fig. 1C). Nauplii of both species were present from the beginning of the flooding period for almost 3 weeks; however, the dominance of
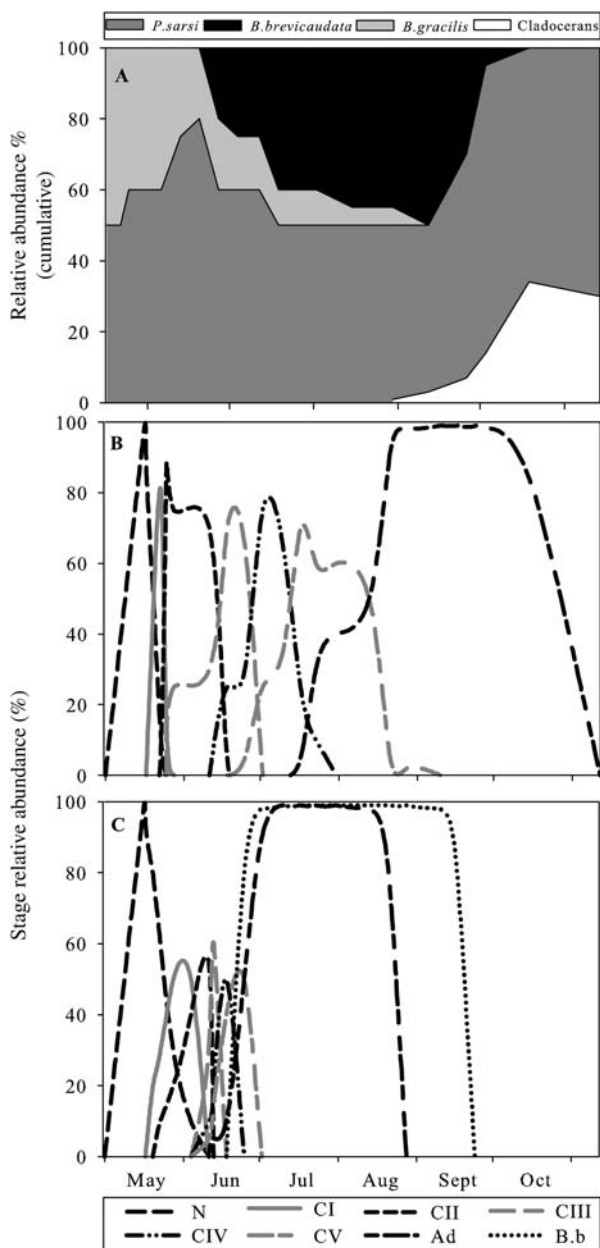
Fig. 1. (**A**) Species relative abundance during the sampling period. (**B** and **C**) Stages relative abundance for *Parabroteas* and *Boeckella*, respectively. (N) Nauplii, (CI) Copepodid I, (CII) Copepodid II, (CIII) Copepodid III, (CIV) Copepodid V, (Ad) Adults, (B.b) *Boeckella brevicaudata* adults only in (C).

this stage lasted for only 1 week. Hereafter, the juvenile (copepodite) succession occurred with different timing according to the species. *Boeckella gracilis* reached adulthood when the *P. sarsi* cohort was at copepodite IV stage (Fig. 1B and C). Although it was not possible to track the development of *B. brevicaudata*, adults of both *Boeckella* species appeared together (Fig. 1C), suggesting that both species have similar developmental times.

Boeckella gracilis and *P. sarsi* showed important differences in mouthpart sizes and morphologies (Fig. 2), indicating that these species exploit a different size fraction of the resource. *Boeckella gracilis* mandible edge ranged from 20 to 45 μm, and mandibles had small and homogeneous teeth and no internal tooth. On the other hand, the mandible of *P. sarsi* ranged from 57 to 290 μm and showed a pronounced internal tooth with medium-sized teeth along the edge. The first copepodite stage of *P. sarsi* had similar sizes of mouthparts to those of *B. gracilis* adults, suggesting that these stages have access to similar particle sizes. However, the CI of *P. sarsi* already exhibited the internal tooth in its mandible. Maxillipeds of both species also showed important differences in size (Fig. 2). *Parabroteas sarsi* maxillipeds continue growing until they reach almost 2 mm in length in adulthood, whereas this mouthpart in *B. gracilis* did not reach 500 μm (Fig. 2).

Parabroteas sarsi switched from microphagy to macrophagy and ingested prey of larger sizes as it moulted to later instars (Fig. 3). For nauplii and CI, the diet was based on algae smaller than 80 μm (including small diatoms, such as *Achnanthes* spp), and CII included some rotifers (*Trichocerca* spp and *Conochilus unicornis*). Animal items became more important than algae in CIII. Their importance increased during development, and copepods and cladocerans became the main prey item in stage CV and adults (Fig. 3).

Boeckella gracilis gut contents indicated that this species ingested small algae (<80 μm), such as *Achnanthes* spp, *Peridinium* sp, *Mallomonas* sp and *Strombomonas* sp. Therefore, the variations observed in the sizes of mouthparts during development did not contribute to substantial changes in diet.
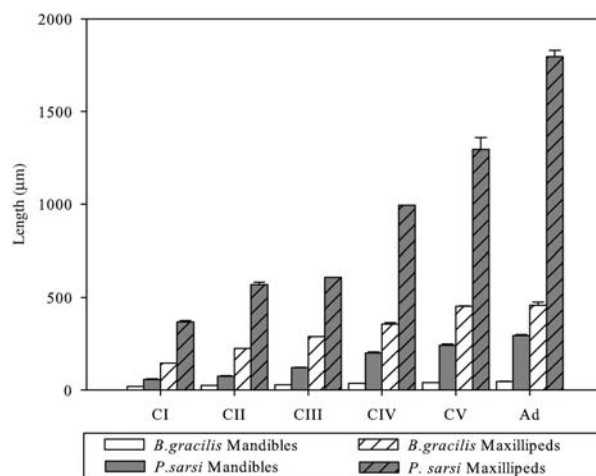
Fig. 2. Mandible (Md) and maxilliped (Mxp) sizes (error bars = 1 standard error) in *P. sarsi* and *B. gracilis* across the different life stages. Abbreviations as Fig. 1.

The first individuals of *B. brevicaudata* that were found in the water column were adults, and they were found on Day 40 after the pond began to be filled. This pattern occurred because nauplii and juveniles of this species remained near the bottom until the adult stage, when they moved up to the water column and began feeding on seston. The gut contents of *B. brevicaudata* adults showed that this species also has a herbivorous regime when they ingest similar items as *B. gracilis*.

Copepod elemental stoichiometry, imbalance and homeostasis

Although both copepod species showed differences in diet during their development, the trends in body

Fig. 3. Diet of *P sarsi* as a percentage of the total gut content in each life stage. Abbreviations as Fig. 1.

stoichiometry for C:P and C:N were similar in the two species (Fig. 4). The C:P was low during the early life stages until CIII. Thereafter, it began to increase and reached its highest values in the adult stage, resulting in the high P content of fast growing juveniles (Fig. 4A). In contrast, the maximum values for the C:N ratio in both species occurred in the earlier stages and decreased during development (Fig. 4B). During the early life stages, both species experienced a high stoichiometric imbalance with their resources (Fig. 5). In the first 2 months during which nauplii developed into copepodites III, the C:N and C:P ratios in seston <80 μm (the food resource for both species) were rather variable; C:N ranged from 7.7 to 28.8 and C:P from 86 to 400. Thereafter, the ratios became more stable with values of 13 for C:N and 175 for C:P (Fig. 6). However, during this second period, the diet of both species differed, as *P sarsi* shifted towards a predatory diet. This difference in diet resulted in differences in the elemental imbalance in each species. Throughout its life cycle, *B. gracilis* faced a high and variable imbalance with the resources (Fig. 5), whereas *P sarsi* became more balanced by eating a more variable diet towards adulthood (Fig. 5). Regarding stoichiometric homeostasis, homeostasis was strict in *B. gracilis* throughout its cycle (Fig. 7), with no changes in their herbivorous diet and a great elemental imbalance (Fig. 5). On the other hand, homeostasis was strict and elemental imbalance great in *P sarsi* while feeding mainly on phytoplankton (Fig. 8A–D). However, when it included copepods and cladocerans as prey, there was little or no imbalance, and its homeostatic capacity decreased, especially in the CIV, CV and early adult stages (Fig. 8E–I). At the end of the cycle, when ovigerous females appeared, there again appeared
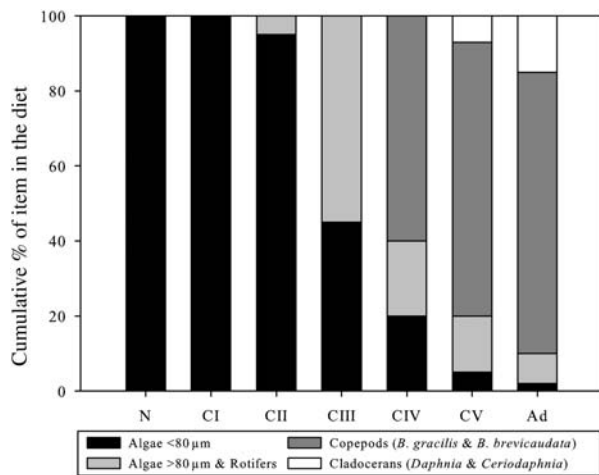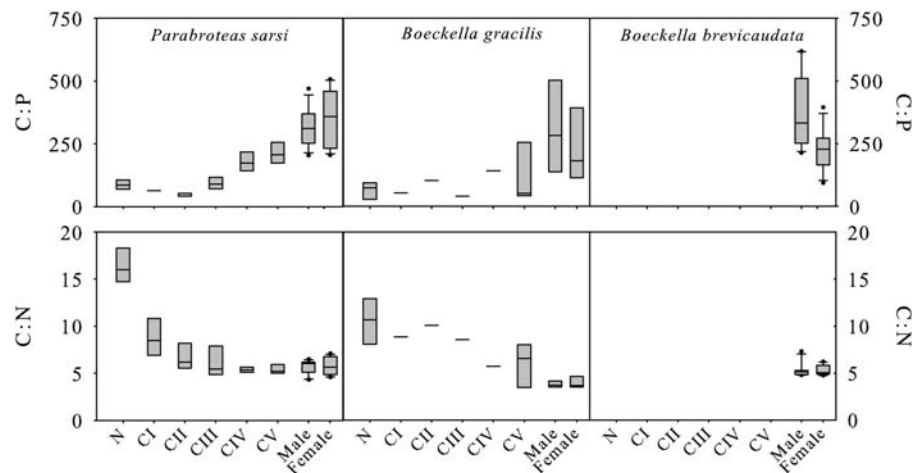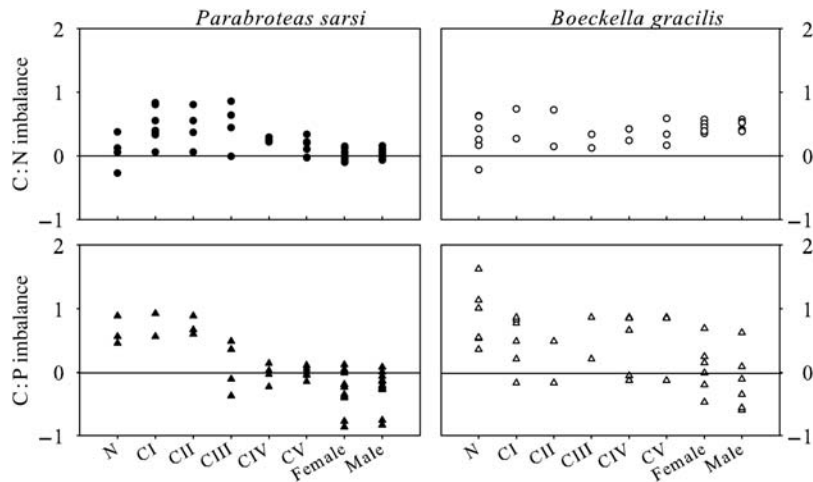

Fig. 4. Elemental atomic ratios in *P sarsi*, *B. gracilis* and *B. brevicaudata* life stages. (**A**) C:P and (**B**) C:N. Box and whiskers represent 10th, 25th, median, 75th and 90th percentiles. Abbreviations as Fig. 1.

Fig. 5. Elemental imbalance between consumers and food. C:N (atomic) is represented by circles and C:P (atomic) by triangles. Filled and opened figures represent *P. sarsi* and *B. gracilis*, respectively.
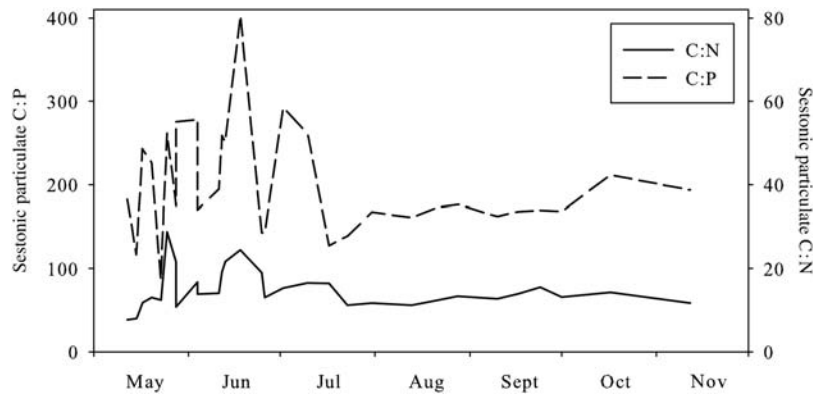
Fig. 6. Sestonic elemental atomic ratios (C:P and C:N) during the sampling period.

to be a P homeostasis (Fig. 8J), as body C:P varied independently of the ratios in prey. However, there was a delay in reaching N homeostasis (Fig. 8I). A strict homeostatic pattern was apparent in both species, while they experienced a high imbalance in the resources; however, *P. sarsi* changed to a non-homeostatic stage when it shifted its consumption towards the acquisition of a balanced diet.

DISCUSSION

Physiology varies with species and stages, especially between trophic strategies, and there is no single way in which consumers maintain their stoichiometry in the face of imbalanced resources (Sterner and Elser, 2002; Darchambeau *et al.*, 2003; He and Wang, 2008). *Boeckella gracilis* is a herbivorous copepod that ingests particles <80 μm during its entire life cycle. In

contrast, there was great variation in the diet of *P. sarsi* during its development, as its maximum size ingestion limit increased from <80 μm in the first life stages to ~1600 μm for the adults. This maximum prey size is consistent with the effective prey size refuge observed for this predator (Balseiro and Vega, 1994; Diéguez and Balseiro, 1998). The mouthparts and gut dissection also supported these data, as mandibles and maxillipeds showed an important change in size during development.

The C:N:P ratios were closely related to ontogeny in both species. Nauplii had low C:P (mean of all nauplii C:P ~80) and adults showed high values for this ratio (mean for adult stages C:P ~290). This ontogenetic trend is consistent with the growth rate hypothesis, which states that differences in C:N:P ratios are caused by differential allocations to RNA necessary to meet the protein synthesis demands for rapid rates of growth and development (Sterner and Elser, 2002), so that fast
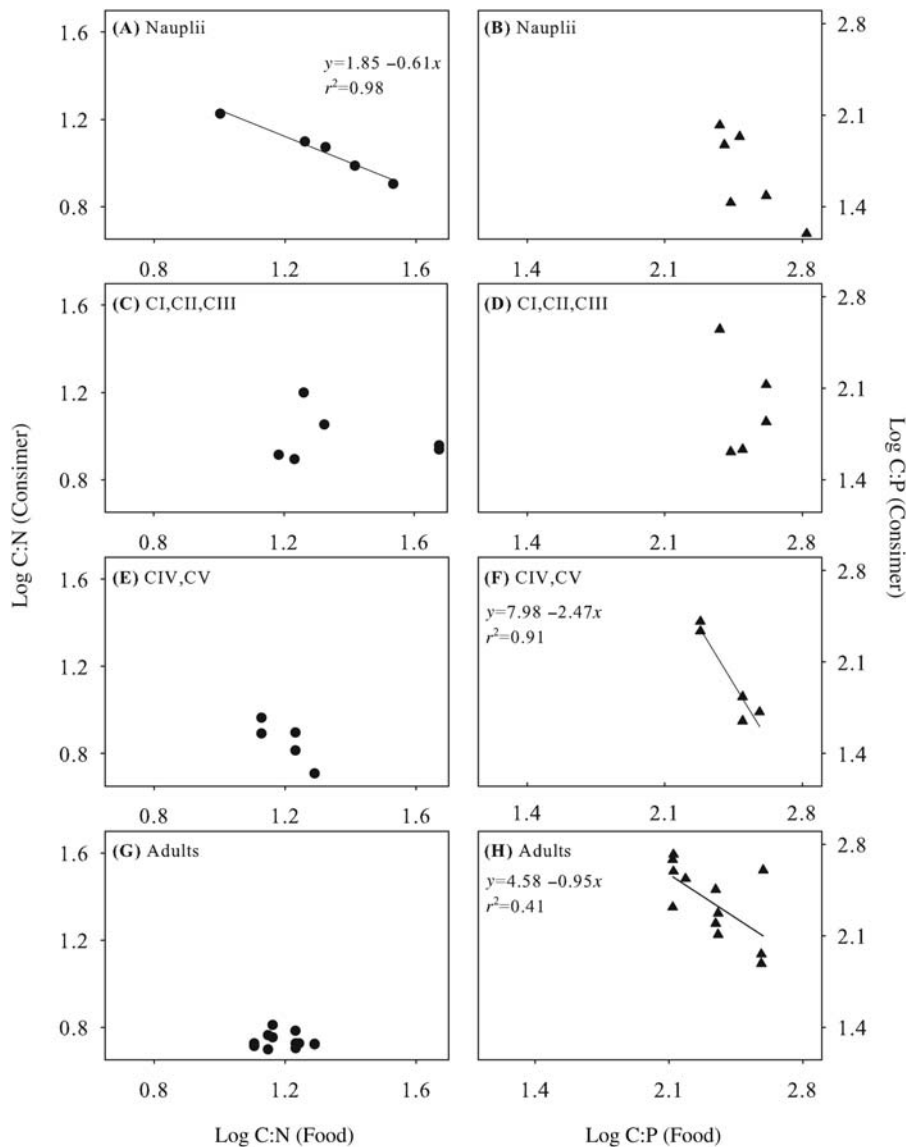
Fig. 7. Homeostatic regulation of elemental content in *B. gracilis* life stages. C:N atomic ratios are represented by circles and C:P (atomic) by triangles. Abbreviations as Fig. 1. The solid line is the best fit regression line.

growing consumers or stages must have a lower C:P ratio than slow growing ones. Nauplii and early copepodite stages are biochemically active periods with high growth rates that are characterized by a low protein:RNA ratio (Villar-Argaiz *et al.*, 2002), and these stages require more phosphorus per unit of body mass than adults, turning to be P-dependent stages. Consequently, these early stages will retain P and release more C, which promotes high environmental C:P ratios. Therefore, differences in physiology among copepod developmental stages would lead to differences in their elemental composition and, hence, in their roles in the ecosystem.

The dietary value of a prey type depends on its nutritional-status content, which has a powerful effect on prey selection, consumption and trophic transfer efficiency (Mitra and Flynn, 2005) and influences the outcome of resource limitation (Balseiro *et al.*, 1997; Moe *et al.*, 2005). The herbivorous *B. gracilis* has a narrow diet throughout its life cycle, and this food is usually poor in nutrient content. Consequently, this species experiences an elemental imbalance with its resources, resulting in strict stoichiometric and homeostatic control during its entire life cycle. In contrast, *P sarsi*'s stoichiometric homeostasis was not constant and depended greatly on its diet spectrum. Mouthpart sizes
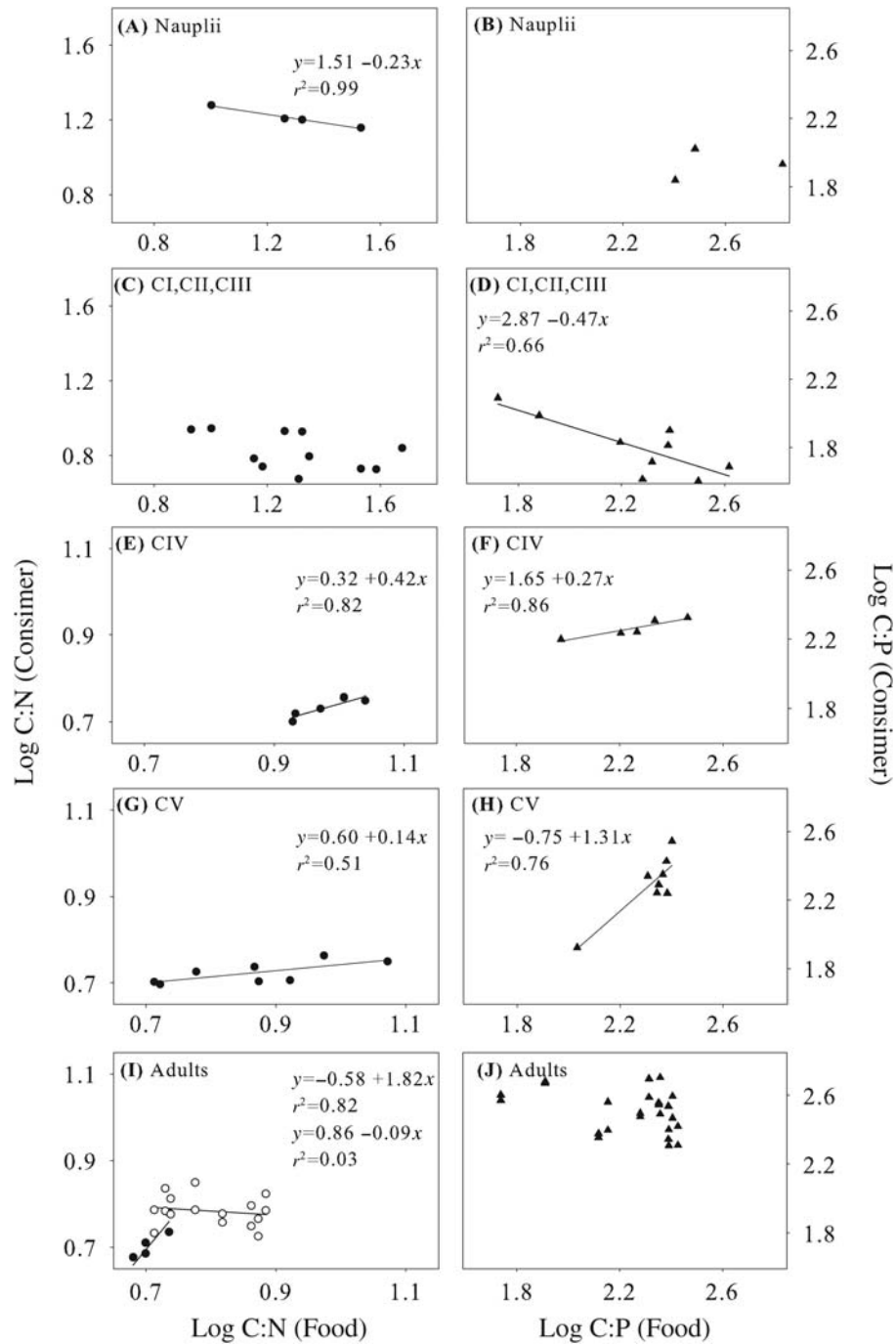
Fig. 8. Homeostatic regulation of elemental content in *P sarsi* life stages. C:N atomic ratios are represented by circles, C:P (atomic) by triangles and open figures represent early adults. Abbreviations as Fig. 1. The solid line is the best fit regression line.

and diet were similar between *B. gracilis* and *P. sarsi* during the naupliar stage and the first copepodite stage; during this time, *P. sarsi* overcame the elemental imbalance and invested greatly in stoichiometric homeostasis, which was found throughout the entire life cycle in *B. gracilis*. However, from CII and onwards, the prey range of *P. sarsi* extended towards a predacious omnivory, and the imbalance with the resources sharply decreased. Surprisingly, in contrast to the strict homeostatic pattern that was common when both species were herbivorous, the pattern became low or nonhomeostasis in *P. sarsi*. The elemental balance achieved

from the shift in trophic strategy allowed *P. sarsi* to invest energy that was formerly spent on overcoming the elemental imbalance to be used in other processes, while a complete and balanced nutrient ratio was bounded together in the food. For example, this energy could be used to counter UVR damage; recent evidence indicates that *P. sarsi* adults can invest in better performance against UVR due to higher antioxidant enzyme activities than the herbivorous copepod *B. gibbosa* (Souza *et al.*, 2010).

The maintenance of a relatively tight homeostasis in order to deal with imbalanced nutrient conditions involves energy-requiring strategies of stoichiometric regulation. Respiration is considered a major route of carbon loss by zooplankton (He and Wang, 2006b) and, hence, it would be a physiological strategy to cope with surplus C in their diet. Experimental studies showed that *Daphnia* feeding on high C:P diets had higher respiration rates than those fed on low C:P (Darchambeau *et al.*, 2003; He and Wang, 2006a; Jensen and Hessen, 2007; He and Wang, 2008). Since juveniles have higher ingestion rates and body-specific respiration rates than adults (He and Wang, 2008), we hypothesize that nauplii and early copepodites that face P-limited diets can regulate their homeostasis via CO_2 excretion through high respiration rates.

In contrast to herbivores, carnivores (here, *P. sarsi* adults) are generally confronted with lower concentrations of higher quality food (Boersma and Elser, 2006) and thus would be less likely to experience nutrient deficiency. While it is relatively common that the food C:P ratio is higher than expected thresholds for P limitation, little attention has been given to situations where imbalance is the other way round (Fig. 5, adults). Animal performance increased after low to moderate P enrichment, but it decreased with higher levels of P enrichment (Elser *et al.*, 2005). Plath and Boersma (Plath and Boersma, 2001) found reduced growth rates in *Daphnia* when fed low C:P food, reflecting an over-reduction in net C intake due to reduced feeding effort. High levels of P in the food decrease consumer growth, reproduction and survivorship; hence, the consumption of stoichiometrically over-rich food implies a cost for the consumer (Boersma and Elser, 2006). It seems that a stoichiometric imbalance, at least in terms of the C:P ratio, can impair consumers if the C:P ratio is higher or lower than the consumer's requirements. In our study, *P. sarsi* adults with a carnivorous diet acquired a surplus of P, and the imbalance turned to the direction of a nutrient surplus.

During the transition to maturity, there may be a strong trade-off between investment in somatic or reproductive tissue for both C and P (Faerovig and Hessen, 2003). With increasing age and decreased somatic growth, the conflict between allocation to somatic tissue and eggs will decrease, and older mothers could afford to spend not only more C but also more P on eggs. Carbon (as lipids) may fuel basic metabolism and, together with P and N, will shape juvenile growth. However, as somatic growth ceases, the somatic requirement for C versus P will increase. Carbon will still be needed for maintenance metabolism, but there will be a lower demand for P and N for protein synthesis; hence, more ingested P could be allocated to eggs at a lower cost for older individuals (Faerovig and Hessen, 2003). Food quality in terms of C:P during the reproductive stage may influence the allocation strategies for egg production. In our study, P-rich food may have increased the allocation of P for egg production, contributing to the growth and survival of the offspring and the maintenance of homeostasis. Otherwise, P-poor food may not severely affect the survival of mature individuals with low requirements for somatic tissues, but it could constrain egg production.

Stoichiometric models make several predictions about the behavioural and physiological flexibility of zooplankton. Our findings provide an example of how variable feeding strategies can counter the imbalance in C:nutrient ratios that organism typically face. Physiology is a nexus at which imbalances are translated into ecological phenomena, such as selective feeding and differential nutrient recycling. Thus, a stoichiometric perspective on physiology should explicitly link the metabolism of organisms with the elemental constraints imposed by the environment. We need to better understand the mechanisms of homeostatic control when nutrients are in deficit or in excess. Indeed, similar studies on additional species, as well as for other groups, would be important in understanding the importance of incorporating dietary heterogeneity in stoichiometric analyses.

FUNDING

REFERENCES

Acharya, K., Kyle, M. and Elser, J. J. (2004) Effects of stoichiometric dietary mixing on *Daphnia* growth and reproduction. *Oecologia*, **138**, 333–340.

Andersen, T., Elser, J. J. and Hessen, D. O. (2004) Stoichiometry and population dynamics. *Ecol. Lett.*, **7**, 884–900.

Balseiro, E. G. and Vega, M. (1994) Vulnerability of *Daphnia middendorffiana* to *Parabroteas sarsi* predation: the role of the tail spine. *J. Plankton Res.*, **16**, 783–793.

Balseiro, E. G., Modenutti, B. E. and Queimaliños, C. P. (1997) Nutrient recycling and shifts in N:P ratio by different zooplankton structures in a South Andes lake. *J. Plankton Res.*, **19**, 805–817.

Boersma, M. and Elser, J. J. (2006) Too much of a good thing: on stoichiometrically balanced diets and maximal growth. *Ecology*, **87**, 1325–1330.

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

Darchambeau, F., Faeovig, P. J. and Hessen, D. O. (2003) How Daphnia copes with excess carbon in its food. *Oecologia*, **136**, 336–346.

Diéguez, M. and Balseiro, E. (1998) Colony size in *Conochilus hippocrepis*: defensive adaptation to predator size. *Hydrobiologia*, **387**, 421–425.

Eaton, A. D., Clesceri, L. S., Rice, E. W. *et al.* (eds) (2005) *Standard Methods for the Examination of Water and Wastewater.* American Public Health Association, AWWA.

Elser, J. J., Acharya, K., Kyle, M. *et al.* (2003) Growth rate–stoichiometry couplings in diverse biota. *Ecol. Lett.*, **6**, 936–943.

Elser, J. J., Schampel, J. H., Kyle, M. *et al.* (2005) Response of grazing snails to phosphorus enrichment of modern stromatolitic microbial communities. *Freshwater Biol.*, **50**, 1826–1835.

Faerovig, P. J. and Hessen, D. O. (2003) Allocation strategies in crustacean stoichiometry: the potential role of phosphorus in the limitation of reproduction. *Freshwater Biol.*, **48**, 1782–1792.

Flynn, K. J. (2009) Food-density-dependent inefficiency in animals with a gut as a stabilizing mechanism in trophic dynamics. *Proc. R. Soc. Lond. B*, **276**, 1147–1152.

Frost, P. C., Evans-White, M. A., Finkel, Z. V. *et al.* (2005) Are you what you eat? Physiological constraints on organismal stoichiometry in an elementally imbalanced world. *Oikos*, **109**, 18–28.

Green, J. D. and Shiel, R. J. (1992) A dissection method for determining the gut contents of calanoid copepods. *Trans. R. Soc. S. Aust.*, **116**, 129–132.

Green, J. D. and Shiel, R. J. (1999) Mouthpart morphology of three calanoid copepods from Australian temporary pools: evidence for carnivory. *N Z J. Mar. Freshwater Res.*, **33**, 385–398.

He, X. and Wang, X. (2006a) Relative importance of inefficient feeding and consumer excretion to organic carbon flux from Daphnia. *Freshwater Biol.*, **51**, 1911–1923.

He, X. and Wang, X. (2006b) Releases of ingested phytoplankton carbon by Daphnia magna. *Freshwater Biol.*, **51**, 649–665.

He, X. and Wang, W. X. (2008) Stoichiometric regulation of carbon and phosphorus in P-deficient Daphnia magna. *Limnol. Oceanogr.*, **53**, 244–254.

Hessen, D. O., Faerovig, P. J. and Andersen, T. (2002) Light, nutrients, and P: C ratios in algae: grazer performance related to food quality and quantity. *Ecology*, **83**, 1886–1898.

Jensen, T. C. and Hessen, D. O. (2007) Does excess dietary carbon affect respiration of *Daphnia*? *Oecologia*, **152**, 191–200.

Jones, R. H. and Flynn, K. J. (2005) Nutritional status and diet composition affect the value of diatoms as copepod prey. *Science*, **307**, 1457–1459.

Kay, A. D., Rostampour, S. and Sterner, R. W. (2006) Ant stoichiometry: elemental homeostasis in stage-structured colonies. *Ecology*, **20**, 1037–1044.

Mitra, A. and Flynn, K. J. (2005) Predator–prey interactions: is 'ecological stoichiometry' sufficient when good food goes bad? *J. Plankton Res.*, **27**, 393–399.

Mitra, A. and Flynn, K. J. (2007) Importance of interactions between food quality, quantity, and gut transit time on consumer feeding, growth, and trophic dynamics. *Am. Nat.*, **169**, 632–646.

Modenutti, B. E. (1993) Summer population of *Hexarthra bulgarica* in a high altitude lake of South Andes. *Hydrobiologia*, **259**, 33–37.

Moe, S. J., Stelzer, R. S., Forman, M. R. *et al.* (2005) Recent advances in ecological stoichiometry: insights for population and community ecology. *Oikos*, **109**, 29–39.

Plath, K. and Boersma, M. (2001) Mineral limitation of zooplankton: stoichiometric constraints and optimal foraging. *Ecology*, **82**, 1260–1269.

Souza, M. S., Balseiro, E., Laspoumaderes, C. *et al.* (2010) Effect of ultraviolet radiation on acetylcholinesterase activity in freshwater copepods. *Photochem. Photobiol.*, **86**, 367–373.

Sterner, R. W. (1997) Modelling interactions of food quality and quantity in homeostatic consumers. *Freshwater Biol.*, **38**, 473–481.

Sterner, R. and Schulz, K. (1998) Zooplankton nutrition: recent progress and a reality check. *Aquat. Ecol.*, **32**, 261–279.

Sterner, R. W. and Elser, J. J. (2002) *Ecological Stoichiometry. The Biology of Elements from Molecules to the Biosphere.* Princeton University Press.

Sterner, R. W., Jones, C. G. and Lawton, J. H. (1995) Elemental stoichiometry of species in ecosystems. In Jones, C. and Lawton, J. H. (eds), *Linking Species and Ecosystems.* Chapman and Hall, 240–252.

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

Urabe, J. and Watanabe, Y. (1993) Implications of sestonic elemental ratio in zooplankton ecology: reply to the comment by Brett. *Limnol. Oceanogr.*, **38**, 1337–1340.

Vega, P. M. (1999) Life-state differences in the diet of *Parabroteas sarsi* (Daday) (Copepoda, Calanoida): A Field Study. *Limnologica*, **29**, 186–190.

Villar-Argaiz, M., Medina-Sanchez, J. M. and Carrillo, P. (2002) Linking life history strategies and ontogeny in crustacean zooplankton: implications for homeostasis. *Ecology*, **83**, 1899–1914.