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Does the stoichiometric carbon:phosphorus knife edge apply for predaceous copepods?

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Abstract Recent work has indicated that stoichiometric food quality in terms of the carbon:phosphorus (C:P) ratio affects consumers whether the imbalance involves a deficit or an excess of nutrients; hence, organisms exist on a "stoichiometric knife edge". While previous studies have focused primarily on autotroph–herbivore trophic transfer, nutritional imbalances may also affect the interactions between species at higher trophic levels. Since the foods of carnivores are normally stoichiometrically similar to the body compositions of those carnivores, they may be more severely affected than herbivores if imbalances become pronounced. We analysed the response of the predatory copepod *Parabroteas sarsi* to monospecific diet treatments consisting of high and low C:P prey items. These dietary treatments strongly affected the predator's elemental composition and growth, although prey selection, excretion, egestion, and respiration rates were not affected. We suggest that, due to their low threshold elemental ratio and a narrow C:P stoichiometric knife edge, these predators are highly vulnerable to stoichiometric imbalances, whether an excess or a deficit of nutrients is involved. Our results demonstrating this high sensitivity to prey C:P ratio show that the stoichiometric knife edge may apply to not only herbivores but also higher trophic levels. Thus, predators such as *P. sarsi*, with a much narrower range of food quality, may also be strongly affected by fluctuations in the quality of their prey, with negative consequences for their secondary production.

Keywords Predator · Food quality · Growth rate · Homeostasis · Phosphorus

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Introduction

Nutritional imbalances between consumers and their resources are the rule rather than the exception at the bottom of food webs. Primary producers usually reflect the nutrient ratios of their surrounding environment (weak stoichiometric homeostasis), and can achieve quite divergent C:N:P (carbon:nitrogen:phosphorus) ratios when limited by nutrients (Sterner and Elser 2002). In contrast, consumers exhibit a greater degree of regulation of their body C:N:P ratios (stoichiometric homeostasis) (Sterner and Elser 2002). Hence, herbivores may often experience C:nutrient ratios in their food that are considerably higher than those in their own biomass and are consequently forced to use mechanisms to compensate for the low quality of the food (Darchambeau et al. 2003). These mechanisms include adjustments to ingestion (Plath and Boersma 2001), respiration (Darchambeau et al. 2003), digestion (Sabat et al. 1999), or excretion (He and Wang 2006, 2008). Whatever the strategy, adjusting to excess C is not cost-free and is generally accompanied by a decrease in growth or reproduction (Boersma 2000; Boersma and Kreutzer 2002).

While much previous work has emphasised the impacts of poor food quality when C:P ratios are high (food is low in P), recent work has suggested that stoichiometric imbalance can also affect consumers when imbalances tend toward an excess of nutrients. This has led to the proposal that herbivores exist on a "stoichiometric C:P knife edge" in which their growth rate is depressed at either high or low food C:P ratios, leading to an optimal intermediate food elemental ratio (Plath and Boersma 2001; Boersma and Elser 2006). Boersma and Elser (2006) suggested that herbivores and detritivores might be extremely vulnerable to the effects of high nutrient content, as they normally feed on high C:P (low-quality) food and have relatively low body contents of nutrients. On the other hand, carnivores, which eat other animals and thus process foods that are stoichiometrically similar to their own body compositions, might not necessarily have developed a sophisticated suite of post-ingestion homeostatic strategies to cope with elemental mismatches. Hence, their response to imbalanced resources—to either a deficit or an excess of nutrients—might be different from that of herbivores, and could even be more sensitive to changes in the quality of their food.

Elemental mismatches at the base of the food web can have different effects depending on the consumer species and on the magnitude that an imbalance must exhibit before the elemental composition of the primary consumer is changed (incomplete homeostasis) (Balseiro et al. 2008; Persson et al. 2010). However, due to interspecific variations in the C:P ratios of consumers, different species can react differently to elemental imbalances. Species with an elevated P content may be more susceptible to low-P food and vice versa, all else being equal (Andersen and Hessen 1991; Sterner and Elser 2002). Therefore, elemental imbalances could be sufficiently large to change the species composition of the herbivorous zooplankton, with a decline in P-rich species occurring as the C:P ratio of their phytoplankton food increases (DeMott and Gulati 1999; Hall et al. 2004; Laspoumaderes et al. 2013). Such a change in species composition at the herbivore level would then represent a shift in the stoichiometric quality of the food available to higher trophic levels.

Studies analysing nutrient transfer higher in the food web (e.g. the herbivore–predator link) have shown that P limitation of secondary consumers may indeed occur in freshwater systems (Boersma et al. 2008), and that the efficiency of energy transfer through food chains can be mediated by stoichiometric effects on both herbivore efficiency and carnivore efficiency (Dickman et al. 2008). Secondary consumers can suffer nutritional imbalances as a consequence of weak stoichiometric homeostasis in their prey (Malzahn et al. 2007; Boersma et al. 2009; Schoo et al. 2010). In addition, changes in the relative availability of stoichiometrically contrasting prey can be common in predator–prey interactions and would also imply a change in the elemental ratios of predators' resources. Indeed, prey nutrient composition can strongly affect a variety of predator life-history traits (Raubenheimer et al. 2007; Jensen et al. 2012). Much of this previous work has highlighted the regulatory abilities of predators in relation to macronutrients such as lipid and protein, as well as the impacts of nutrient balance between carbohydrates and nitrogen across trophic levels in terrestrial environments (Wilder et al. 2010; Hewson-Hughes et al. 2011). Fewer studies have examined nutritional balancing for key elements such as P, and none to our knowledge have considered the possibility of an optimal dietary P content for an aquatic predator under ecologically realistic conditions.

In a previous study analysing natural populations of the calanoid copepod *Parabroteas sarsi* (Daday), we found variable homeostatic responses (regarding C:P ratios) as a result of different feeding strategies throughout ontogeny (Laspoumaderes et al. 2010). *P. sarsi* switches from microphagy to macrophagy as it moults to later instars. In its third copepodid developmental stage (CIII), it ingests algae and some rotifers. However, in copepodid stages CIV and CV, animal food items predominate. In these stages, *P. sarsi* shows an incomplete homeostatic capacity, as its elemental composition depends greatly on the elemental composition of available resources. By the end of its ontogeny, cladocerans and other copepod species become the main prey items and stronger P stability is observed (Laspoumaderes et al. 2010). While analysing C:N ratios, we found that nitrogen was not a limiting element in the development of *P. sarsi*, nor in the development of *B. gracilis* (included in the same study). In addition, in another study (Laspoumaderes et al. 2013) we found that changes in seston C:P led to shifts in the relative abundance of cladocerans vs. copepods in local habitats. So, for the study reported in the present paper, we focused on dietary C:P as a key food quality parameter to test the stoichiometric C:P knife-edge hypothesis proposed by Boersma and Elser (2006) on a predator. Based on the hypothesis that the feeding mode (herbivory vs. carnivory) determines the strength of homeostatic regulation, we predicted that, during the predatory stages of the copepod, there would be a loss of stoichiometric regulation and increased susceptibility to stoichiometric imbalances towards an excess or deficit of nutrients. To test this prediction, we performed laboratory experiments that compared body elemental ratios and growth rates of the predatory stages of the calanoid copepod *Parabroteas sarsi* when fed prey of different C:P ratios, and we analysed the mechanisms used to compensate for these imbalances, analysing prey choice, respiration rates, and excretion and egestion of nutrients.

Methods

Experimental design

We performed laboratory experiments with young pre-reproductive females (predatory stage) of the copepod *Parabroteas sarsi* fed on adults of the herbivorous copepod *Boeckella gracilis* (Daday) and the cladoceran *Daphnia commutata* (Ekman). These organisms were all collected from Laguna Fantasma, northwestern Patagonia, Argentina (41°05′S, 71°27′W, 780 m.a.s.l. and 2.5 m maximum depth). Populations of *P. sarsi* in Laguna Fantasma present a single cohort. Nauplii appear as soon the pond begins to fill during the autumn rains that, depending on annual conditions, generally occur between April and late May. After 3 months, the first females of *P. sarsi* appear in late July or August (Laspoumaderes et al. 2010). During the year of these experiments, the lake filled during late May. Ovigerous females appear 2 months later than the first females, so *P. sarsi* spends almost 2 months as non-ovigerous females. This means that, during that period, all females are nulliparous. Moreover, non-ovigerous females that coexist with ovigerous ones are also nulliparous, since they develop only one brood that remains attached to the female until the lake dries out in early summer. These eggs hatch in the following autumn when the lake fills again.

Adult females are the most commonly studied group when measuring copepod growth (Hirst and McKinnon 2001). If the aim is to make accurate estimates of growth in adult copepods, then changes in body weight are of fundamental importance (Hirst and McKinnon 2001). Individual carbon biomass continues to increase beyond moulting to the adult stage, as females need to put on weight over the period from reproductive immaturity to sexual maturity (Marshall and Orr 1972; McKinnon 1996). Thus, growth should be expressed as output of reproductive material, as well as changes in body weight. Moreover, Bullejos et al. (2014) showed that the RNA:DNA ratio in adult females of *Mixodiaptomus laciniatus* varied with food quality, indicating that these females were still synthesizing biomass although no further moulting was taking place. As a consequence, when measuring adult copepod growth, changes in body weight are of fundamental importance, and egg production should be added once reproductive maturity has been reached (Marshall and Orr 1972; McKinnon 1996; Hirst and McKinnon 2001; Swalethorp et al. 2011). In nature, *P. sarsi* has a lifespan of 8 months, with females remaining for about 4 months (Laspoumaderes et al. 2010). All our experiments lasted from 2 weeks to a month and we used recently moulted females, taking into account that *P. sarsi* females start producing eggs 2 months after moulting. For this purpose, we collected animals at the CV copepodid stage and used the females that moulted within 48 h after sampling to measure growth rates during the immature period.

Both prey items that were offered to *P. sarsi* in the experiments, *B. gracilis* and *D. commutata*, are common prey of *P. sarsi* in this pond (Vega 1999; Laspoumaderes et al. 2010). Zooplankton samples were collected during September and October with a plankton net (200-µm mesh) by making at least three independent horizontal tows (replicates) in the central pelagic area of the pond. *B. gracilis* and *D. commutata* were then maintained in the laboratory for at least 10 days in filtered lake water in incubators at 15 °C and a 12:12 h light:dark cycle. Organisms were fed every 2 days with *Chlamydomonas reinhardtii* (Dangeard). Algal batch cultures in the exponential growth phase were maintained in COMBO (Kilham et al. 1998) at 90 µmol photon m^{-2} s^{-1} and a 12:12 h light:dark regime (C:P atomic ratio ≈200). All experiments with *P. sarsi* were carried out in the same conditions of light and temperature in which prey were maintained in the laboratory. During the experiment, no deaths of *P. sarsi* occurred in the experimental systems. Initial experimental conditions (C:P of each species) were assessed by separating the three species under a stereomicroscope and analysing them for the individual biomass (IB) of C and P (µmol of C or P per individual, IB_C and IB_P, respectively) of each species for each replicate. For all carbon analyses, individuals (for exact numbers of each species see below) were separately placed onto pre-combusted (2 h at 450 °C) GF/F Whatman filters and analysed in a Thermo Finnigan EA 1112 CN elemental analyser (Thermo Scientific, Milano, Italy). Phosphorus analyses were performed by placing individuals (for exact numbers of each species see below) in 45 mL MilliQ water (previously rinsed in Milli-Q water) by digestion with potassium persulfate at 125 °C at 1.5 atm for 1 h. Phosphorus concentrations were analysed with the ascorbate-reduction molybdenum method (APHA 2005). The actual concentrations of particulate P (PP) and particulate C (PC) in the *C. reinhardtii* cultures were monitored by routine analysis. On the basis of these measurements, we collected and added algae to reach a concentration of 70–80 µmol C L^{-1} to feed the zooplankton (a C concentration similar to that of Laguna Fantasma).

Feeding rates and prey selection

Prior to starting the experiments, we carried out two different measurements. To determine if *P. sarsi* eats both prey taxa effectively and if there is any prey selection, we placed 1 *P. sarsi* adult in a 250-mL flask to feed on a mix of 51.5 µg (SE 6.5, $n = 5$) of *D. commutata* and 52.8 µg (SE 5.9, $n = 5$) of *B. gracilis* for 3 h. After that period we removed the predator and analysed the number of each prey eaten by *P. sarsi* as the difference between the prey offered

and the remaining prey. We repeated the selectivity experiment after *P. sarsi* was fed with only one prey item for several days (see the sections describing "Experiments 1, 2 and 3" below).

To establish the amount of food for the experiments, we determined the feeding rates of *P. sarsi* by placing 1 adult in a 250-mL beaker to feed on a known biomass (same as previous) of *D. commutata* or *B. gracilis* for 24 h in 8 replicates. After that period we removed the predator and analysed the amount of prey consumed as the difference between the number of prey offered and the number remaining.

Experiment 1

In the first experiment, we analysed the effect that prey with different C:P ratios might have on predator C:P ratio and growth. For this purpose, we fed *P. sarsi* with two monospecific dietary treatments differing in C:P. We used 24 beakers (500 mL) filled with filtered lake water (GF/F Whatman filters precombusted at 450 °C, 2 h) and placed 8 *P. sarsi* in each. Half of these beakers were supplied every 2 days with 250–280 (C ~550 µg) *B. gracilis* each (high C:P treatment ~140) and the other half with 120–160 (C ~550 µg) *D. commutata* each (low C:P treatment ~60). During each feeding occasion we checked that there were prey remaining in the flasks to ensure that the cultures never ran out of food, and continued with the same feeding regime.

The experiment lasted 1 month. At the beginning of the experiment (initial conditions) and every 5 days, the C and P contents of the predators and prey were analysed (2–3 replicates each). The predators in 2 beakers from each treatment (2 samples of 2 individuals for C analysis and 2 samples of 1–2 for P) and 2 samples of *D. commutata* (20–25 individuals for C analysis and 10–15 for P analysis) and *B. gracilis* (30–35 for C and 25–30 for P) from the cultures were taken for the analyses. Live copepods and cladocerans were suspended in Milli-Q water for 3 h to clean the individuals and empty their guts and were later separated for elemental analyses under a stereomicroscope.

Experiment 2

In a second experiment, we measured the P excretion and egestion of *P. sarsi* after feeding on monospecific diets as used in "Experiment 1". The experimental arrangement was similar to that of the previous experiment, but samples were only taken at the beginning and the end of the experiment. This experiment was conducted in 10 beakers (500 mL), half of which were supplied with *B. gracilis* and the other half with *D. commutata*, with 8 *P. sarsi* each. The experiment lasted 2 weeks because, as determined by "Experiment 1", this length of time was sufficient to obtain differences in the C:P somatic ratio of *P. sarsi*. We measured P excretion and egestion after 2 weeks of feeding on each diet. To perform this determination, we pooled the predators within a treatment and put 3 *P. sarsi* in individual 125-mL beakers (3 replicates per treatment) filled with Milli-Q water. After 4 h, the predators were removed with a clean pipette and the water was filtered through acid-washed and pre-combusted (450 °C, 2 h) GF/F Whatman filters. The filters were analysed for PP and the filtered water for total dissolved phosphorus (TDP) using three replicates. Both samples were digested and analysed for P following APHA (2005). Individual C and P biomass analyses of *P. sarsi* and the prey were performed in four replicates following the same procedure and number of individuals as used in "Experiment 1".

Experiment 3

A third experiment was conducted to measure P and C egestion through faeces and P and C excretion through respiration of the predator fed with monospecific prey. To measure P and C excretion and egestion, we also used the same methods as in "Experiment 2". After 2 weeks of feeding on each diet, we pooled the individuals within each treatment and put 3 *P. sarsi* in individual 125-mL beakers (7 replicates per treatment) filled with Milli-Q water. After 4 h, the predators were removed with a clean pipette, and the water was filtered through acid-washed and pre-combusted (450 °C, 2 h) GF/F Whatman filters. The filters were analysed for particulate P in 3 replicates and particulate carbon (PC) in 4 replicates (analysed on filters in a Thermo Finnigan EA 1112 CN elemental analyser) and the filtered water for TDP (3 replicates for each treatment), following the same procedure as in "Experiment 2". Individual C and P biomass analyses of *P. sarsi* and the prey were performed using the same procedure and number of individuals as in "Experiment 2". We determined the respiration rates (R) of *P. sarsi* from both feeding treatments before (natural field diet) and after 2 weeks of monodiet feeding. Respiration was assessed as oxygen consumption over time for all treatments (5–8 replicates per treatment). To measure respiration rates, we placed 1 *P. sarsi* in a 20-mL flask in Milli-Q water in the dark, stoppered to ensure that no air bubbles were present. We measured dissolved oxygen at the initial time and every hour thereafter with an optical, fluorescence-based (Presens®) DO meter that allowed the oxygen concentrations to be determined from the outside without opening the flask. We calculated weight-specific respiration rates by standardising oxygen consumption rates to the C (µg) of each *P. sarsi* in each treatment. By standardising O_2 consumption to each individual's C mass, we obtained the O_2 consumed per µg C biomass [ng O_2 (µg C)$^{-1}$ h^{-1}] of an

individual, thus correcting for size dependence of respiration rates. Respiration rates were estimated as the slope of a least-squares regression of O_2 concentration vs. time.

Experiment 4

A fourth experiment was carried out to test the possibility that the results of our previous experiments were due to differences in aspects of prey quality other than the C:P ratio that can occur among groups as copepods and cladocerans (e.g. differences in fatty acid composition). Following the same procedure as in "Experiment 2" and "Experiment 3", we carried out the experiment using 3 different prey with 6 replicates each. We used young *D. commutata* (the same size as adult *B. gracilis* to avoid any size differences among prey) as low C:P cladoceran prey items, *B. gracilis* adults as high C:P copepod prey items, and added a new prey species—CI copepodids of *Boeckella poppei* (Mrázek) (C:P ~97, and the same size as adult *B. gracilis*), which is a low C:P copepod. This copepod is also a common prey of *P. sarsi* in temporary ponds of Patagonia.

Threshold elemental ratio (TER)

To estimate the C:P ratio above which *P. sarsi* growth is P-limited (its threshold elemental ratio, or TER), we carried out experiments in 6 beakers (250 mL) with filtered lake water and 25 *B. gracilis* as prey. We introduced an individual *P. sarsi* into each beaker to feed on the prey for 2 h, and then removed the predators and placed each in individual 20-mL flasks filled with Milli-Q water for 5 h and measured respiration rates. After 5 h, we removed the predators and measured P and C egestion and excretion as PP, TDP and PC in the remaining water, following the same procedure as in "Experiment 3". We also measured dissolved carbon excretion with a Shimatzu TOC analyser. Both predators and prey were analysed for C and P contents. From the 6 replicates, 3 of the beakers were used for all C variables (C of *P. sarsi*, C excretion and egestion) and the other 3 for all P variables (P of *P. sarsi*, P excretion and egestion), while ingestion and respiration rates were measured in the 6 replicates.

Using these data, we calculated the $TER_{C:P}$ of *P. sarsi* following Frost et al. (2006),

$$TER_{C:P} = \frac{A_P}{\frac{I_C A_C - R_C}{I_C}} \times \frac{Q_C}{Q_P}.$$

Here, A_P and A_C are the assimilation efficiencies of P and C, respectively (dimensionless) [A_{PC} = (ingestion – egestion)/ingestion], I_C (µmol C µmol C^{-1} day^{-1}) is the mass-specific ingestion rate above a saturating food level, R_C (µmol C µmol C^{-1} day^{-1}) is the mass-specific respiration rate, the volume of O_2 consumed during respiration was converted into µmol C respired using a respiratory quotient of 1.0 (Lampert and Bohrer 1984; Frost et al. 2006), and Q_C (µmol C µg DM^{-1} and Q_P (µmol P µg DM^{-1}) are the proportions of C and P relative to animal dry mass.

Data analysis

Prey selection experiments were analysed with a one-way ANOVA for each experimental treatment and with a two-way ANOVA to test for the effect of the experimental treatment on prey selection by *P. sarsi*. For the first experiment, comparisons between both treatments involving the C:P ratio, as well as comparisons of the individual biomasses of C and P of *P. sarsi* and its food, were performed with an ANCOVA. In cases where the interaction term was nonsignificant ($P > 0.05$), indicating parallel slopes, we proceeded with an ANCOVA to evaluate the differences between treatments. In cases with significant interactions ($P < 0.05$), we demonstrated that the slopes were different and proceeded to compare them. In subsequent experiments, comparisons between treatments involving the C:P ratios and the individual biomasses of C and P of *P. sarsi* were performed with one-way ANOVA.

The growth rates (GRs) of *P. sarsi* were estimated as follows:

$$GR(day^{-1}) = \frac{\ln(C_{(f)}) - \ln(C_{(i)})}{time(days)},$$

where $C_{(f)}$ and $C_{(i)}$ are the final and initial IB_C values, respectively, expressed in µmol C ind^{-1}. Although adult copepods do not need to grow to moult to a new instar, females still grow in order to synthesise new biomass to produce offspring (Swalethorp et al. 2011). In our study, we used young pre-reproductive females, so we were able to use GR as changes in body C as a proxy for secondary production of females (Marshall and Orr 1972; McKinnon 1996; Hirst and McKinnon 2001). To investigate the effects of different food qualities on the predators' GR, GR was plotted vs. the C:P ratio of the food. The plot included data from all experimental feeding treatments, and the regression line was estimated from the mean values of the food versus the mean values of growth. The model was selected with the Akaike information criterion (AIC). We determined a set of candidate models and selected the one with the minimum AIC value.

The degree of homeostasis (Sterner and Elser 2002) of *P. sarsi* was characterised using the present laboratory data and field data from Laspoumaderes et al. (2010) as

$$H = \frac{\log(x)}{(\log(y) - \log(c))},$$

where x is the prey's elemental (C:P) ratio, y is *P. sarsi*''s C:P ratio, and c is a constant. It follows from this formula that $1/H$ is the slope of the standardised major axis regression (SMA) of $\log_{10}(y)$ on $\log_{10}(x)$, and should take values between zero and one (Sterner and Elser 2002; Persson et al. 2010). SMA is more adequate than ordinary least squares regression when the statistic of primary interest is the slope of the regression and data are log–log transformed (Warton et al. 2006). All regressions were classified according to their slope ($1/H$) following Persson et al. (2010) as follows: $0 < 1/H < 0.25$ is homeostatic, $0.25 < 1/H < 0.5$ is weakly homeostatic, $0.5 < 1/H < 0.75$ is weakly plastic, and $1/H > 0.75$ is plastic.

To assess elemental imbalance, we measured the dissimilarity between the $TER_{C:P}$ of *P. sarsi* and the C:P ratio of its prey. Imbalances calculated in this manner provide a more meaningful index of the elemental imbalance between a consumer and its food resource than simple arithmetic differences between body and food elemental compositions (Frost et al. 2006).

In the third experiment, respiration rates were calculated using a linear regression of oxygen consumption standardized to body size (in µg C) vs. time, and comparisons were performed with an ANCOVA. We first tested all individuals (replicates) in each treatment for homogeneity of slopes. The slopes for all treatments were then compared with each other. For the analysis of the growth and excretion and egestion rates of phosphorus, we pooled the data from the different experiments to increase the number of observations and the sensitivity of the statistical tests and applied a two-way ANOVA, discarding the effect of the individual experiment as a factor.

The normality and homoscedasticity of the data were confirmed for all tests. Statistical analyses were done with Sigma Plot 12.0. SMA analyses were done with RMA: Software for Reduced Major Axis Regression, Java version (Bohonak and Linde 2004), and AIC with R software.

Results

Feeding rates and prey selection

In the feeding rate experiment, individual *P. sarsi* consumed 21.6 µg (SE 0.6, $n = 8$) of *D. commutata* and 21.1 µg (SE 1.1, $n = 8$) *B. gracilis*. This result shows that feeding *P. sarsi* in all the monodiet experiments with 550 µg C of each prey every 2 days was enough to ensure that *P. sarsi* never ran out of food.

The prey selection experiment showed no selectivity by *P. sarsi*—neither at the beginning nor at the end of the experiments—in all treatments. *P. sarsi* ingested the same amount of each prey 5.12 µg h^{-1} (*SE* 0.38, $n = 30$) of *B. gracilis* and 5.24 µg h^{-1} (SE 0.56, $n = 30$) of *D. commutata* ($F_{1,59} = 0.010$, $P = 0.92$), regardless of the previous feeding treatment ($F_{3,59} = 0.857$, $P = 0.47$).

Prey elemental composition

Prey (*B. gracilis* and *D. commutata*) were successfully maintained on *C. reinhardtii* in the experimental cultures in the laboratory during the experiments. Although both prey were supplied with the same food (C:P ~200), they maintained distinct C:P ratios (Fig. 1a), with the C:P ratio of *B. gracilis* significantly higher than that of *D. commutata* (means: 140 vs. 60, Fig. 1a; $F_{1,23} = 82.36$, $P < 0.001$). The different C:P ratios of *D. commutata* and *B. gracilis* were stable throughout the experiment and showed no prey species × time interaction during the experiment (ANCOVA, $F_{1,22} = 0.60$, $P = 0.45$). This result indicates that the two treatments maintained a consistent difference in the prey C:P ratio supplied to *P. sarsi*. For experiment 4, we added CI of *B. poppei* as another copepod prey with a C:P ratio [97.3 (SE 2.3, $n = 5$)] that was intermediate between those of *B. gracilis* and *D. commutata*.

Elemental composition of the predator

The C:P ratio of the predator changed according to the C:P of the prey offered. Although *P. sarsi* began with the same C:P ratio in each experiment (Table 1), by the end of the experiments the groups of *P. sarsi* that were fed only with *B. gracilis* (high C:P in our experiments) had higher C:P ratios than those fed only with *D. commutata* (low C:P prey) (Table 1, see experiment 1 in Fig. 1b). In experiment 4, we added a new copepod prey with an elemental composition (C:P ~97) that was between those of *D. commutata* (C:P ~60) and *B. gracilis* (C:P ~140). In this case, by the end of the experiment, the C:P ratio of *P. sarsi* was found to be intermediate between the values obtained in the *B. gracilis* and in the *D. commutata* treatments, with significant differences among all of them (in all cases, ANOVA $P < 0.025$) (Table 1).

The observed variation in the C:P ratio of *P. sarsi* among treatments was due to changes in individual C biomass (IB_C, µmol C ind^{-1}), and not in the individual P biomass (IB_P, µmol P ind^{-1}) since the IB_P did not change among treatments. By the end of all of the experiments, the IB_C of *P. sarsi* was higher in the treatments fed with *B. gracilis* than in the *D. commutata* treatments (Table 1) (see "Experiment 1" in Fig. 1c). Furthermore, when we added *B. poppei* as an intermediate C:P prey in experiment 4, *P. sarsi* showed an intermediate IB_C between those fed *B. gracilis* and those fed *D. commutata* (Table 1). However, IB_P did not differ between treatments in any of the experiments (see "Experiment 1" in Fig. 1d) (Table 1).

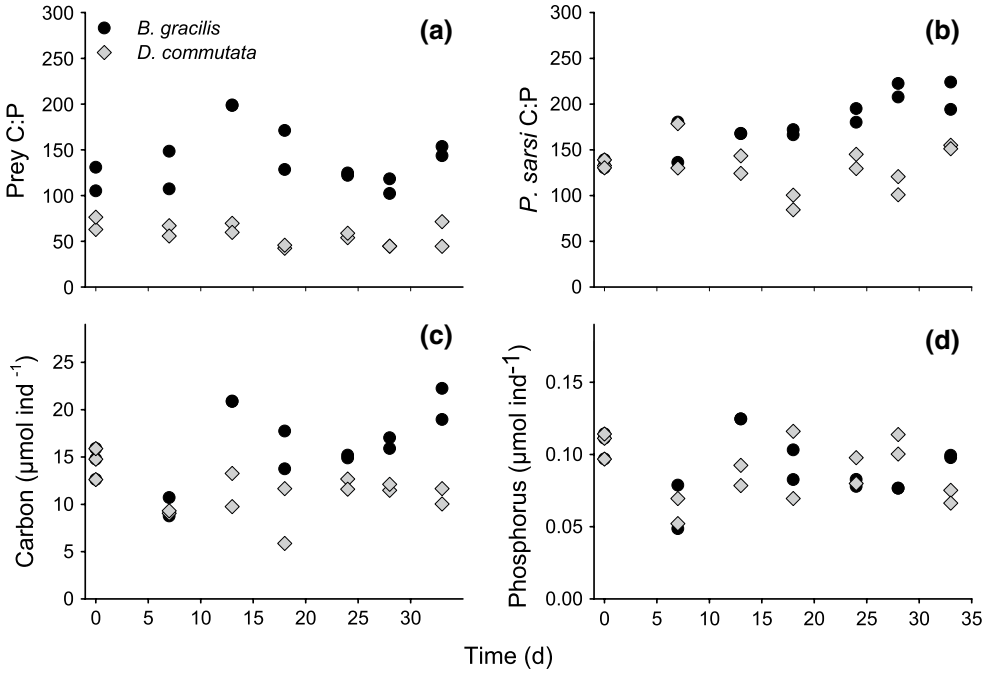

Fig. 1 Elemental compositions of the predator and prey during experiment 1. C:P atomic ratios of **a** the prey and **b** the predator. Absolute: **c** carbon and **d** phosphorus contents in µmol ind^{-1} of the predator *P. sarsi*. *Black* and *grey* patterns indicate that the *P. sarsi* were fed with *B. gracilis* or with *D. commutata*, respectively ($n = 2$)

Homeostasis and imbalance

We found that *P. sarsi* has weak homeostatic regulation, as its C:P ratio changed consistently with the C:P ratio of its prey. The degree of elemental homeostasis for *P. sarsi* was determined as the slope of the regression between the $\log_{10}$-transformed C:P of the predator and prey (*B. gracilis* and *D. commutata*). We obtained a significant relationship with a slope of $1/H = 0.49$ (95 % CI 0.34–0.63; H ~2; Fig. 2a) ($r^2 = 0.52$, $df = 24$, $P < 0.001$). These results suggest that *P. sarsi* is weakly homeostatic during this adult stage, and contrast with previously published results that seemed to show that *P. sarsi* was strictly homeostatic during adulthood (Laspoumaderes et al. 2010). However, on that occasion, the C:P range of the food supplied was not enough to show weak homeostatic regulation of *P. sarsi*. However, when combining the present homeostasis results with those from Laspoumaderes et al. (2010), we observe that the homeostatic regulation of *P. sarsi* is even weaker than that indicated in our experiment, as the recalculated regression line (laboratory and field data pooled) has a slope of $1/H = 0.74$ (95 % CI 0.60–0.88; $H = 1.35$) ($r^2 = 0.64$, $df = 42$, $P < 0.001$) (Fig. 2b), indicating that the homeostatic regulation of *P. sarsi* is in the range of weakly plastic and plastic according to the criteria of Persson et al. (2010).

Consideration of the stoichiometric imbalance between *P. sarsi* and its food showed that the diet based on *D. commutata* caused an elemental imbalance involving an excess of P in the food. In this sense, the P-rich diet (*D. commutata*) produced a stronger elemental imbalance of *P. sarsi* with its prey (−109.4 (SE 3.1, $n = 14$)) than the low-P diet consisting of *B. gracilis* (−27.0 (SE 8.5, $n = 14$)) ($H_{(1)} = 20.28$, $P < 0.001$).

Excretion and egestion

Prey elemental ratios did not affect P excretion or egestion rates of *P. sarsi*. Excretion and egestion did not differ between treatments in experiment 2 (TDP $F_{1,4} = 0.14$, $P = 0.73$; PP $F_{1,4} = -0.369$, $P = 0.58$) nor in experiment 3 (TDP ($F_{1,4} = 2.76$, $P = 0.17$), PP ($F_{1,4} = 2.318$, $P = 0.21$). When the P excretion and egestion data for experiments 2 and 3 were pooled to increase the sensitivity of the statistical analyses, they confirmed that prey elemental ratios did not affect the excretion and egestion of P by *P. sarsi*, as no significant differences were detected [TDP ($F_{1,11} = 2.685$, $P = 0.14$), PP ($F_{1,11} = 2.218$, $P = 0.18$)]. The overall mean TDP excretion was 0.77 nmol P ind^{-1} h^{-1} (SE 0.06, $n = 6$) and PP egestion was 2.8 nmol P ind^{-1} h^{-1} (SE 0.3, $n = 6$) (Fig. 3a).

Prey elemental ratios did not affect C egestion of *P. sarsi.* When *P. sarsi* was fed *D. commutata*, C egestion was 0.136 µmol C ind^{-1} h^{-1} (SE 0.022, $n = 4$), and when it was fed *B. gracilis*, C egestion was 0.143 µmol C ind^{-1} h^{-1}

Table 1 Elemental compositions (C:P, C and P) and growth rates of *P. sarsi* for the initial condition and after the monodiet treatments for the four experiments

	Initial condition			Experimental treatment (prey)									Statistics		
Exp ID				*B. gracilis*			*D. commutata*			*B. poppei*					
C:P	Ratio	SE	*n*	Ratio	SE	*m*	Ratio	SE	*n*	Ratio	SE	*n*	*df*	*F*	*P*
1	137.9	2.7	3	208.9	14.9	2	153.2	1.9	2						
1′				2.37	0.31	15	−0.19	0.55	15				1,26	16.3	***
2	138.3	8.6	3	166.0	4.0	4	128.1	8.7	4				1,6	15.68	**
3	192.4	10.3	3	218.1	5.2	3	186.4	8.7	4				1,5	8.04	*
4	267.4	36.7	6	272.7	7.4	6	197.5	12.0	6	231.4	8.9	6	2,15	15.23	***
Carbon(IB_C)	µmol ind^{-1}	SE	*n*	µmol ind^{-1}	SE	*n*	µmol ind^{-1}	SE	*n*	µmol ind^{-1}	SE	*n*			
1	14.21	0.96	3	20.59	1.65	2	10.85	0.82	2						
1′				0.16	0.08	15	−0.059	0.06	15				1,26	5.28	*
2	16.07	1.52	3	17.21	0.72	4	14.34	0.78	4				1,6	6.86	*
3	22.63	0.46	3	24.85	0.73	4	20.36	1.12	5				1,7	10.39	*
4	16.34	1.61	6	19.16	0.98	6	13.30	0.20	6	15.94	0.43	6	2,15	21.96	***
Phosphorus(IB_P)	µmol ind^{-1}	SE	*n*	µmol ind^{-1}	SE	*n*	µmol ind^{-1}	SE	*n*	µmol ind^{-1}	SE	*n*			
1	0.107	0.009	3	0.098	0.001	2	0.071	0.005	2						
1′				3.9×10^{-4}	4.8×10^{-4}	15	2.7×10^{-4}	4.7×10^{-4}	15				1,26	0.03	n.s.
2	0.116	0.007	3	0.103	0.005	5	0.114	0.008	4				1,7	1.45	n.s.
3	0.118	0.007	3	0.117	0.005	3	0.105	0.002	4				1,5	5.24	n.s.
4	0.058	0.002	6	0.070	0.004	6	0.069	0.005	6	0.069	0.003	6	2,15	0.05	n.s.
Growth rate				day^{-1}	SE	*n*	day^{-1}	SE	*n*	day^{-1}	SE	*n*			
1′				0.011	0.003	2	−0.009	0.002	2				1,2	33.94	*
2				0.005	0.003	4	−0.008	0.004	4				1,6	6.43	*
3				0.006	0.001	4	−0.007	0.003	5				1,7	9.67	*
4				0.008	0.002	6	−0.012	0.001	6	−0.001	0.002	6	2,15	27.56	***

The last column shows statistics for the comparisons among treatments within each experiment

ns nonsignificant *P* values, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

For experiment 1, the 1′ rows show the values of the regression line (slope, SE and *n* of the regression) of C:P, C, P and GR of *P. sarsi* during experiment 1 and the ANCOVA results in the statistics column

All other values are means with the standard error (SE), the number of replicates (*n*), and statistics are ANOVA

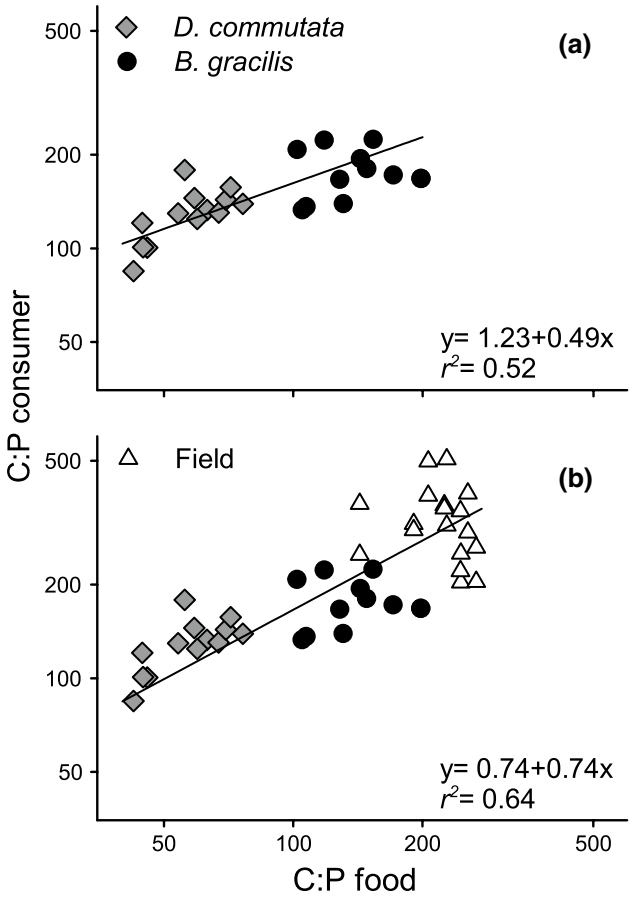

Fig. 2 Homeostatic regulation of the elemental content of *P. sarsi* during experiment 1: **a** experimental data obtained in experiment 1 ($n = 24$); **b** experimental data from experiment 1 along with field data from Laspoumaderes et al. (2010) added ($n = 18$). Regression lines represent the standardised major axis regression (SMA). All axes have logarithmic scales

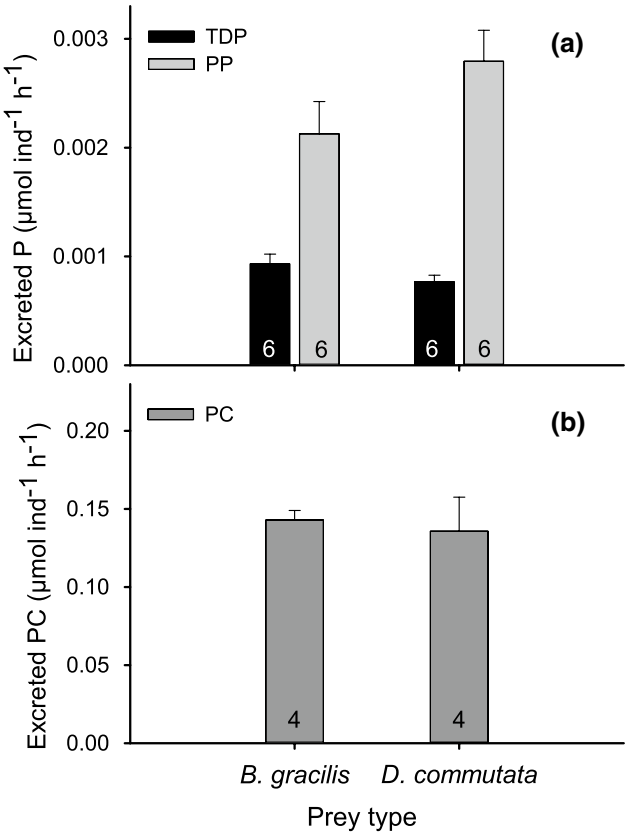

Fig. 3 Excretion by *P. sarsi* in both feeding treatments after data from experiments 2 and 3 were pooled together. **a** *Black bars* are the total dissolved phosphorus (TDP) and *grey bars* are **a** the particulate phosphorus (PP) or **b** particulate carbon (PC, only for experiment 3) ±1 SE. Sample sizes are shown *inside bars*

(SE 0.006, $n = 4$), with no significant differences observed among treatments ($F_{1,6} = 0.101$, $P = 0.76$) (Fig. 3b).

Respiration

Prey elemental composition did not affect mass specific respiration rates of *P. sarsi*. When feeding on *D. commutata*, the respiration rate of *P. sarsi* was 4.77 ng O_2 h^{-1} µg C^{-1} ($r^2 = 0.77$, $df = 38$, $P < 0.001$), whereas it was 5.15 ng O_2 h^{-1} µg C^{-1} ($r^2 = 0.82$, $df = 27$, $P < 0.001$) ($F_{1,65} = 0.35$, $P = 0.55$) when *P. sarsi* was fed *B. gracilis*.

Growth rates

Prey elemental composition significantly affected the growth rate of *P. sarsi* ($P < 0.05$, Table 1). Growth rates were higher and positive when *P. sarsi* fed on high C:P *B. gracilis* and negative when they fed on low C:P *D. commutata* (Table 1). In experiment 4, where food of intermediate quality (C:P ratio) was added by feeding one experimental treatment with *B. poppei* copepodites, the growth rate of *P. sarsi* was found to be intermediate between the treatments fed with *B. gracilis* and *D. commutata* (Table 1).

When the growth data from the four experiments were analysed together, we observed that we could discard the effects of the individual experiments as a factor, since similar treatments in different experiments gave similar results (ANOVA, $F_{3,31} = 0.39$, $P = 0.76$). This result allowed us to pool all of the experiments to increase the sensitivity of the statistical analyses. The pooled growth rates of *P. sarsi* differed between treatments (all cases $P < 0.05$). Growth rates of *P. sarsi* were related to prey C:P (*B. gracilis* C:P = 140, GR = 0.008 day^{-1}, SE 0.002; *B. poppei* C:P = 97, GR = −0.001 day^{-1}, SE 0.002, and *D. commutata* C:P = 60, GR = −0.009 day^{-1}, SE 0.002). Our analysis of the variation of the GR of *P. sarsi* as a function of the elemental ratio (C:P) of the food based on data from experiments 1–4 revealed a hump-shaped relationship. A

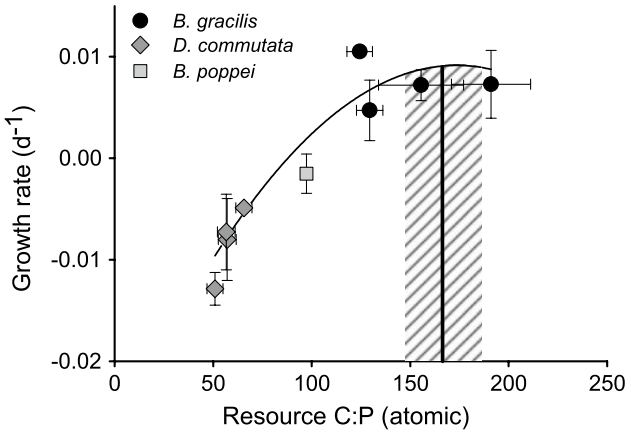

Fig. 4 Growth rates of *P. sarsi* as a function of resource C:P (atomic) ratio in the experiments. Each data point is the mean of all replicates in a particular experiment and treatment ±1 SE. The *solid line* represents the stoichiometric knife edge fitted by least-squares regression to a quadratic polynomial function [$f(x) = -0.028 + 0.0004x - 1.25\times10^{-6}x^2$; $r^2 = 0.91$, $df = 8$, $P = 0.0007$]. The *dashed area* shows the calculated mean $TER_{C:P}$ ±1 SE ($n = 3$)

quadratic polynomial function [$f(x) = -0.028 + 0.0004x - 1.25\times10^{-6}x^2$; $r^2 = 0.91$, $df = 9$, $P = 0.0007$; Fig. 4] fitted better (lower AIC) than a linear function or a rise to a maximum function. Moreover, the threshold elemental ratio of *P. sarsi* (estimated in a separate experiment) revealed a strong correspondence with the shape of this observed hump-shaped curve, as the $TER_{C:P}$ was 166 (SE 19.5, $n = 3$), coinciding with the food C:P that produced the highest growth rates (Fig. 4).

Discussion

In this study, we found that feeding a predator with a stoichiometrically imbalanced diet significantly affects the predator's elemental composition and growth, and that this occurs when the food has a C:P ratio either above or below the predator's threshold elemental ratio.

Low growth rates for *P. sarsi* females can be expected, as growth tends to decrease with increasing body size (Richardson and Verheye 1999; Hirst and McKinnon 2001). In particular, growth rates <0.095 day^{-1} are common in many adults with body mass >10 µg C ind^{-1} (Hirst and Lampitt 1998; Richardson and Verheye 1999) and are not uncommon in temperate (Hay et al. 1991; Kiørboe and Nielsen 1994) and oligotrophic environments (McKinnon 1996; Calbet and Agustí 1999). The total length of *P. sarsi* in Laguna Fantasma is ~5 mm and its body weight is ~150 µg C ind^{-1}, so growth rates ranging from 0.008 to −0.009 day^{-1} in optimal to poor food quality conditions seem to be reasonable.

Predators, unlike herbivores, may encounter imbalanced diets in both directions: either a deficit of P (Malzahn et al. 2007) or an excess (Laspoumaderes et al. 2010). Because herbivores most commonly face imbalanced resources involving an excess of C and a deficiency in P in their diets, their homeostatic strategies will most likely involve strategies for conserving P while discharging excess C through an increased C excretion rate via the faeces (He and Wang 2006, 2008) or through an increased respiration rate (Darchambeau et al. 2003). In our experiments, however, the predator *P. sarsi* suffered a stoichiometric imbalance due to an excess of P relative to C. A decrease in performance as C:P decreased below a given threshold has also been observed in herbivorous *Daphnia* (Plath and Boersma 2001) and in a review of published studies involving several different animal taxa (Boersma and Elser 2006). In our study, we fed a predator with prey of different C:P ratios and analysed its excretion and egestion, looking for the ways that this predator addresses the excess P in its diet. Contrary to our expectations, none of the excretion experiments showed any differences among the experimental treatments. Hence, we were not able to gain insight into the strategy that the predator uses when facing imbalanced resources except to note that large shifts in P excretion or respiration do not seem to be involved. The differences may be too small and fall within the experimental error. We infer that the lack of clear signs of stoichiometric regulation in *P. sarsi* is consistent with the hypothesis that stoichiometric regulation depends on the animal's dietary regime (e.g. predator vs herbivore) (Laspoumaderes et al. 2010). Most studies that observed strong responses of excretion or egestion rates to differing food qualities have been carried out with herbivores exposed to food with higher C:P ratios than used in our study (He and Wang 2006, 2008; Schoo et al. 2012b; Morehouse et al. 2013). The imbalances that we reached in our experiment with the different prey types may not have been large enough to induce physiological responses aimed at saving or disposing of elements in deficit or in excess. However, such imbalances did have substantial effects on growth rate, indicating that the effects of imbalanced food nevertheless bring about important ecological consequences.

Given compositional differences among prey items in nutritional quality, the nutritional state of the consumer is known to determine how much of a given food is eaten, selectively feeding on nutrients in deficit to reach a nutritional target (Raubenheimer and Simpson 1993; Simpson and Raubenheimer 1996; Simpson et al. 2010). Hence, it should be possible to predict which food a consumer would eat and which one the consumer should avoid if we know the nutrient needs of the animal and the nutritional composition of the foods available (Simpson and Raubenheimer 2012). Surprisingly, in contrast to what this reasoning

predicts and to previous findings for other predator arthropods (Mayntz et al. 2005; Raubenheimer et al. 2007; Schmidt et al. 2012), *P. sarsi* did not appear to have any prey selection strategy as a response to the imbalanced diets that were affecting its growth. At the beginning of the experiments, when *P. sarsi* from the natural environment were offered both copepod and cladoceran prey, they consumed both prey equally with no signs of prey selection. The predator might actually prefer the copepod prey but, as copepods are harder to catch than the low C:P *Daphnia*, the net ingestion rates were equal. Likewise, at the end of the monodiet experiments (experiments 1, 2, 3), when the elemental composition and growth rate of *P. sarsi* were already affected by the composition of its food, both prey were again eaten equally. Hence, we did not observe quality-dependent foraging decisions by *P. sarsi* when facing imbalanced resources. If the predator persists in eating foods that do not satisfy its nutrient needs, we should wonder about the long-term consequences (Simpson and Raubenheimer 2012), which may involve changes in instar duration (Raubenheimer and Simpson; Mayntz et al. 2003; Raubenheimer and Simpson 2003) or a reduction in overall growth.

Our data especially supports the left arm of the stoichiometric knife edge (low C:P), because the prey we offered to *P. sarsi* had low or medium C:P. The right arm of the knife edge (high C:P) has been thoroughly studied in herbivores (Sterner and Elser 2002) and predators (Malzahn et al. 2007; Boersma et al. 2008; Schoo et al. 2012a), with abundant data supporting a general effect of low-P (high C:P) food on consumer growth (Hessen et al. 2013). Thus, in our study, we focused on the left arm of the knife edge in the case of a predator. Regardless of the lack of high C:P prey in our experiments, the model that fits best to our lab data is a humped-shaped function, indicating that this predatory copepod lives on a stoichiometric knife edge (Fig. 4). Indeed, if we add field data on growth rates of *P. sarsi* in relation to food C:P that include prey not tested in the laboratory experiments (e.g. high C:P *Conochilus hippocrepis*), the right arm is fitted very well with a quadratic polynomial function (see Fig. S1a–c in the Electronic supplementary material, ESM). Originally, the stoichiometric C:P knife edge hypothesis was proposed for herbivores, which have food elemental ratios that vary by more than an order of magnitude (Plath and Boersma 2001; Elser et al. 2005). Our study indicates that this predatory copepod is sensitive to much narrower changes in the elemental ratio of its food; indeed, small changes (C:P in the range 50–190) were sufficiently great to encompass the entire left side and the beginning of the right side of the stoichiometric knife edge of *P. sarsi'*. Growth rates of *P. sarsi* were positive under optimal conditions (C:P from ~100 to ~200) but negative when the food had a low C:P (<100). Due to the low $TER_{C:P}$ value and the narrow range of food qualities that includes the whole variation in growth rates, we expect that *P. sarsi* could face ecological consequences if the relative availability of high C:P prey and low C:P prey items changed. Stoichiometrically imbalanced prey that affect female growth would in turn reduce reproduction, because females need to put on mass to reach maturity in order produce offspring.

Since other biochemical constituents of prey besides the C:P ratio may also determine prey quality (Brett and Muller-Navarra 1997; Elser et al. 2005; Brett et al. 2009), in the last experiment we added another low C:P prey item, in this case a copepod, to determine if the negative effect of the low C:P *D. commutata* on predator growth may have been due to a food quality parameter other than C:P, such as a deficiency in fatty acid composition or digestibility. The new prey C:P was intermediate between the C:P ratios of *D. commutata* and *B. gracilis*, and the growth rate of *P. sarsi* fed this new prey was also intermediate between the growth rates observed for the other two food treatments (Fig. 4). These results support an interpretation that the observed pattern was mainly due to differences in prey C:P ratios rather than a correlated parameter.

Most studies on stoichiometric effects propagating across multiple trophic levels in food webs have been performed with predators on monophagous diets (i.e. Kagata and Ohgushi 2007; Malzahn et al. 2007; Boersma et al. 2009). Our study documents changes in a predator's growth rate as well as in its elemental composition as a result of diets consisting of prey with different C:P ratios. While general patterns in the response of secondary consumers to stoichiometric changes in primary producers remain elusive (Boersma et al. 2008), our data contribute to the growing literature that assesses the potential for stoichiometric propagation of bottom-up food quality effects through the food web. Thus, an analysis of the effects of stoichiometric food quality on secondary consumers (predators) should consider a scenario in which prey change their relative abundances as a result of nutrient imbalances in the producer–herbivore interface (Tao and Hunter 2012; Laspoumaderes et al. 2013). The predator will encounter a prey community with an altered species composition and will, therefore, face a new range of elemental ratios in the available prey. That is, the nutritional target can move away from the intake target (Raubenheimer and Simpson 1993) due to changes in relative prey abundances, which may then affect the predator's growth, as observed in our experiments. In a previous study, we documented changes in the relative abundances of two dominant herbivorous species (from *D. commutata* to *B. gracilipes*) due to changes in the stoichiometric food quality of the algae resulting from changes in the ecosystem light:nutrient balance (Laspoumaderes et al. 2013). In the present study, we found that the performance

of the predator *P. sarsi* was affected by the availability of stoichiometrically contrasting prey. Taken together, these results suggest that environmental conditions such as changes in the light:nutrient balance can affect higher trophic levels by changing the relative abundances of prey of different elemental compositions. This indirect effect would not be mediated by a change in the elemental composition of a specific herbivore but instead by a change in the herbivore assemblage caused by changes in producer quality. Overall, our study shows that the stoichiometric knife edge hypothesis may apply to not only herbivores but also higher trophic levels, as they have much narrower ranges of suitable food quality, implying that they might be strongly affected by fluctuations in the relative abundances of stoichiometrically contrasting herbivores.

Author contribution statement CL, BM, JE, and EB developed the idea. EB and CL designed the experiments. CL carried out the experiments, CL, BM, EB and JE analysed the data and CL wrote the manuscript. JE, BM, and EB made substantial contribution to the manuscript.

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