

Allocation strategies in crustacean stoichiometry: the potential role of phosphorus in the limitation of reproduction

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SUMMARY

1. Elemental composition (carbon : nitrogen : phosphorus, C : N : P) was analysed in eggs and juveniles of two crustaceans, *Daphnia magna* (Cladocera) and the crayfish *Astacus astacus* (Decapoda). Stoichiometry was also analysed for the carapace, muscle tissue, hepatopancreas and gills of *Astacus*.
2. For both species the C : P ratio was significantly higher in eggs than juveniles, but there was a constant, homeostatic elemental ratio in eggs during embryogenesis (*Astacus*) and with different C : N : P in maternal food (*Daphnia*).
3. Differences in the stoichiometry of major tissue categories in *Astacus* suggest that there are distinct allocation strategies of elements to various somatic tissues as well as to reproduction versus somatic tissues overall.
4. There are strong ontogenetic shifts in the allocation of energy and elements in both species, as for crustaceans in general. During maturity there may be a trade-off with regard to the allocation of C, N or P to somatic or reproductive tissue, and poor food quality (high C : P in food) could pose other constraints on reproductive capacity than does food shortage (low C).
5. Egg production may be at least as sensitive to low P as is somatic growth and could result in a marked decrease in overall population growth rate more severe than would be expected from individual growth rate alone.

Keywords: *Astacus astacus*, C : P ratios, crustacean stoichiometry, *Daphnia magna*, ontogenetic shifts, P limitation

Introduction

There is growing evidence that grazers and detritivores with diets deficient in nitrogen (N) and phosphorus (P) are unable to utilise fully dietary carbon (C) in their diet for accumulation of body mass and in reproduction (Sterners & Hessen, 1994). This hinges on the rather tight regulation of elemental ratios in animal tissue. When C in food is in relative 'excess' to N or P, it will be disposed of either by defaecation, by excretion of organic C or by increased respiration,

unless it can be stored in C-rich compounds including starch or lipids. Urabe & Sterners (2001) also suggest that *Daphnia* may discard excess C as non-viable eggs. Hence a high specific P content and thus P demand of the consumer may impose reduced growth efficiency in terms of C when feeding on diets with a high C : P ratio as is typical for plant tissue and detritus. Growth and protein synthesis, however, are linked to the availability and tissue concentrations of N and P, and a particularly strong link from P to ribosomal RNA to growth rate has been identified (Elser *et al.*, 1996; Main, Dobberfuhl & Elser, 1997).

The potential role of P limitation has been particularly well explored in lakes, where the dominant grazer *Daphnia* quite commonly may suffer

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P limitation (Sterner, 1993; Elser *et al.*, 1996; Hessen & Faafeng, 2000). A wide range of thresholds for onset of P limitation have been suggested, ranging from C : P ratios (by atoms) of <100 (DeMott, Gulati & Siewertsen, 1998) to around 300 (Urabe & Sterner, 1996). The latter threshold is the most widely accepted, but the actual value will depend on factors like food quantity (Sterner & Robinson, 1994), assimilation efficiency (Hessen, 1992; Sterner *et al.*, 1993), somatic demands (DeMott *et al.*, 1998) and tissue allocation. At very low food concentration when somatic growth ceases, an increasing share of C will be used for maintenance metabolism, and the C : P threshold for the onset of P limitation will increase to infinity (i.e. no need of P for somatic growth). Under active growth, however, the need for P relative to C will be determined by the ability to extract P relative to C from the food, as well as by the grazers' own C : P ratio. As P in most invertebrates is intimately linked with growth rate due to the P content of RNA (McKee & Knowles, 1987; Hessen, 1990; Main *et al.*, 1997), fast-growing organisms should require more P and high access to P allows for high growth rate if food is abundant (Vrede, Persson & Aronsen, 2002). Commonly, the fastest growth is found in juveniles and, while there are limited data, both juvenile stages of copepods (Villar-Argaiz, Medina-Sánchez & Carrillo, 2002) and *Daphnia* neonates apparently have higher specific P content than adults (Hessen & Andersen, 1991; DeMott, 2003).

This extensive demand for P could have further implications for the allocation of elements or biochemical constituents to reproductive or other tissues. In general, data on elemental contents and ratios in zooplankton are based on whole animals, and this does not capture the potential demands for elements in different tissues. Further, the effect of food quality (C : P) on growth rate has typically been analysed for juveniles up to the onset of reproduction, but some studies have also documented reduced reproductive output due to food with low P (high C : P) (Sterner & Schulz, 1998; Urabe & Sterner, 2001). As somatic growth ceases, and most ingested carbon and other elements are allocated to reproduction, the population response to C : P ratio of the food will be determined by stoichiometric effects on reproductive capacity rather than on somatic growth. Under optimal conditions, about 80% of net C intake is typically allocated

to reproduction in adults (Lynch, Weider & Lampert, 1986; Andersen, 1997).

A number of studies has been devoted to the strategies for C allocation to egg production in *Daphnia* (Tessier *et al.*, 1983; Tessier & Consolatti, 1991; Lampert, 1993; Boersma, 1995) yet with somewhat contrasting findings. In general, there is a positive correlation between egg mass and size of mothers, and maximum mass per egg is commonly reported at intermediate food levels (Tessier & Consolatti, 1991; Boersma, 1995). Tessier & Consolatti (1991) also reported an increased C : N ratio in eggs with increased food abundance. There are few specific data on the allocation of P relative to N or C in *Daphnia* eggs, but Sterner & Schulz (1998) found C : N : P (by atoms) ranging from 192 : 16 : 1 to 310 : 15 : 1 in *Daphnia* eggs, which indicates a considerably higher C and/or lower P per unit dry weight (DW) compared with somatic tissue.

In this study we explore the specific content of C, N and P, and elemental ratios, in eggs and juveniles in *Daphnia magna* Straus and the crayfish *Astacus astacus* Linné. This should provide information on ontogenetic shifts in elemental demands, and thus the potential for ontogenetic stoichiometric bottlenecks, i.e. whether specific stages are more likely to be constrained by nutrient elements than others (cf. Villar-Argaiz & Sterner, 2002). We also compared the allocation of elements to somatic growth, eggs and carapace in both species. Both *Daphnia* and *Astacus* are freshwater crustaceans, belonging to different orders; the first is a cladoceran and the second a decapod. Nevertheless, we believe they face some of the same basic nutritional challenges, and they share the property of finite allocation of energy and matter to eggs during early embryogenesis without further exchange with maternal tissue. The advantage of including the crayfish is that it also allowed for a more detailed study on elemental allocation to major tissues like eggs, muscle, gill, hepatopancreas, shell and carapace.

Methods

Experimental design

Ten egg-carrying crayfish (*A. astacus*) were obtained from a local hatchery soon after extrusion of pleopod eggs, and kept in an aquarium. The mothers were fed

part-boiled potatoes and kept at low temperature (8–10 °C) for 2 months, when the temperature was gradually raised to 18 °C over the next month. Hatching occurred at 1500–1700 degree × days (average daily temperature multiplied by duration in days, cf. Hessen *et al.*, 1987). Juveniles were kept with their mothers for the two first moults and then separated to avoid cannibalism. During embryogenesis, subsamples of pleopod eggs were dissected off biweekly after the first 2 months of low temperature, and analysed for DW, C, N and P. At the end of the experiment, five adults were dissected and subsamples of abdominal muscle, hepatopancreas and carapace from the dorsal area of the postabdomen and tips of claws were analysed for C, N and P.

Cohorts of juvenile (<24 h) of *D. magna* were raised from a laboratory stock culture on a pure diet of the green algae *Selenastrum capricornutum* (clone NIVA CHL 10; Norwegian Institute of Water Research, Oslo, Norway). While the animals maintain a rather tight homeostatic regulation, at least compared with autotrophs (Hessen, 1990; Andersen & Hessen, 1991), it has nevertheless been demonstrated that the elemental ratios in their algal food may affect C : P ratios of *Daphnia* (Sterner, 1993; DeMott *et al.*, 1998). Hence, *Daphnia* were fed with three food qualities of algae raised from a two-stage culture system (Hessen, Færøvig & Andersen, 2002). The system consisted of six separate illuminated primary chambers with algae alone and six darkened secondary chambers with both algae and *Daphnia*. Modified COMBO medium (Kilham *et al.*, 1998), with final concentrations of 1, 5 and 50 µM P per litre, was pumped into the algal growth chamber at a dilution rate of 0.30 day⁻¹, yielding algal molar C : P ratios (ratio ± SD) of 420 ± 80, 270 ± 50 and 90 ± 15, respectively. Excess algae and medium entered the *Daphnia* growth chamber by a simple overflow tube. A 200 µm net prevented loss of animals through the outflow. Stock cultures of *Daphnia* mothers were raised under different food qualities from neonates, and hence any eventual maternal effect on the offspring could be taken account of. Cohorts were inoculated into growth chambers and raised at high food abundance (>2 mg C per litre) in all experiments. All experiments with *Daphnia* were carried out at 19.0 ± 0.4 °C in a temperature-controlled room.

To test whether mothers continue to allocate matter to eggs throughout embryogenesis eggs from both

Daphnia and *Astacus* were dissected from their respective mothers and incubated under aeration in the absence of the adult. *Daphnia* eggs were dissected off within 24 h of their being shed into the brood chamber. *Astacus* eggs were stripped off 2 months after fertilisation and extrusion of the egg sac. Previous experiments have verified that this can be carried out successfully for *Astacus* after some 600 degree days, which is approximately one-third of the number of degree days required for hatching (Hessen *et al.*, 1987). Both animals had a high hatching success (>90%), and there was no evidence of reduced viability in neonates, clearly suggesting a finite allocation of energy and matter in the ovaries with no further support during embryogenesis.

Analytical protocols

Algal quantity and composition was measured by filtering 100 mL samples onto preignited (500 °C, 2 h) GF/F-filters and measuring particulate C and N on an elemental analyser (Carlo-Erba CHN 1106, Milano, Italy). Particulate P was measured on corresponding filters placed in 15 mL of acidified (150 µL, 4 M H₂SO₄) distilled water. Total P and dissolved P were analysed correspondingly on unfiltered and GF/F filtered water samples. Samples for particulate, total and dissolved P analysis were added to peroxodisulphate (150 mg K₂S₂O₈), autoclaved (121 °C, 1 h) and analysed spectrophotometrically by the standard ammonium-molybdate method.

For crayfish, both DW and elements were analysed on subsamples from different organs in adult animals and on single eggs and juveniles. For *Daphnia*, DW and elements were analysed on groups of 10 to 20 eggs and juveniles. For both crayfish and *Daphnia* elements were analysed on samples after being washed in COMBO that lacked N and P, dried for 2 days at 60 °C and weighed on a microbalance (Mettler ME 30; Mettler-Toledo GmbH, Greifensee, Switzerland). Particulate C and N were analysed by following the procedure above. Total P samples were placed in 15 mL distilled water with 1.8 mL of a mixture of peroxodisulphate (30 g L⁻¹ K₂S₂O₈), boric acid (50 g L⁻¹ H₃BO₃) and sodium hydroxide (15 g L⁻¹ NaOH), and then autoclaved (121 °C, 1 h). Samples were centrifuged (2000 r.p.m., 10 min) before they were analysed by spectrophotometry.

Statistical analysis

Statistical tests were performed using JMP version 4.0.2 (SAS Institute, Inc., Cary, NC, USA). A two-way ANOVA was applied to test for differences in elemental ratios of *Daphnia* eggs and juveniles raised under different C : N : P ratios in food, and an eventual interaction between food quality and stage (egg–juvenile). One-way ANOVA was used to test for pairwise differences in elemental ratios of *Astacus* eggs over time (time t versus time $t + 1$) and in *Astacus* stage (egg versus juvenile). To test for differences in stoichiometry of major tissues of *Astacus*, including muscle, hepatopancreas, gills and two types of carapace, we compared each group of tissues by using non-parametric test (Kruskal–Wallis) as these samples were not normally distributed.

Results

The eggs of both species maintained a strong homeostasis in elemental composition irrespective of both developmental time and external conditions. No shift in DW, C, N or P content was detected over time for *Astacus* eggs. Similarly, neither *Daphnia* egg mass nor egg stoichiometry changed over time under different ambient P (and thus different C : N : P) in food (Fig. 2). This indicates that there were no major shifts of elemental ratios during embryogenesis. For *Daphnia* these findings also indicate that there was a rather constant allocation of elements to eggs, even with variable food quality.

The mean DW of *Astacus* eggs was about 5.5 mg DW per egg over the entire experimental period, and the specific contents of C, N and P were close to 3, 0.6 and 0.06 mg per egg, respectively, yielding atomic C : P and N : P ratios of 120 and 11 (Fig. 1). For *Daphnia*, the average egg weight was close to 5 µg DW, with corresponding contents of approximately 3 µg C, 0.6 µg N and 0.07 µg P per egg, yielding atomic C : P and N : P ratios of 120 and 20 (Fig. 2). Thus, while the C : P ratios of *Daphnia* and *Astacus* eggs were similar, the N : P ratio of *Daphnia* eggs was twice that of the crayfish.

For crayfish, there was a general loss of C and N during the transition from eggs to newborn (2 days), yielding significantly lower C : P and N : P ratios in juveniles than in eggs (Fig. 3, Table 1). This probably reflects the catabolism of yolk and adipose tissue

during the first 2 days, when the juveniles feed only on egg membrane that they shed. This is in contrast to the juvenile *Daphnia*, which after 2 days had already gained considerable body mass (Fig. 2). It is noteworthy, however, that the juveniles accumulated relatively more P than N and C, reflected in the significant decrease of the C : P ratio (d.f. = 20, F -ratio = 5.68, $P = 0.027$) from nearly 120 to <100. In contrast with crayfish neonates, *Daphnia* start feeding almost immediately after hatching, which was reflected in the higher growth rate and in terms accumulation of C, N and P in juveniles when fed high quality food (high P concentration). As the age of neonates could theoretically differ by as much as 24 h, there was a pronounced variability in these data. The specific P content of the juveniles was significantly higher at 50 µM P than at 5 µM P, which in turn was significantly higher than at 1 µM P. In contrast, for N and C there were significant differences only between the lowest and highest P concentration (Kruskal–Wallis test). Juveniles kept under different ambient P also had different C : P ratios (Tables 1 and 2).

Further information on elemental requirements and allocation strategies is available from the specific content of elements, as well as elemental ratios, in various major tissues in adult crayfish. The hepatopancreas constitutes a major fraction of body mass in crayfish. It had, as expected from the lipid-rich nature of such liver analogue organs, somewhat higher specific C content and lower N and P content than muscle tissue (Fig. 4). While the carapace contained far less of all elements per DW, it nevertheless constituted a large fraction of body mass and thus represents a drain of elements during moulting.

The hepatopancreas had an extremely high C : P ratio of >400 that differed significantly from the other tissues (Fig. 4), and also a high N : P ratio (almost 30), while elemental ratios in carapace and gill tissue ranged from 180 to 280 (C : P) and from 17 to 24 (C : N). Elemental ratio in muscle and shell tissue ranged from 140 to 160 (C : P). Abdominal muscle tissue had a very narrow range of C : P close to 145. Somewhat surprisingly, significant differences were recorded in elemental ratios between the carapace of the claws and that of the postabdomen. The importance of these data for *Daphnia* is not known, but we might expect that different tissue would have distinct elemental ratios in this species also. Existing data on *Daphnia* generally comprise the entire body including

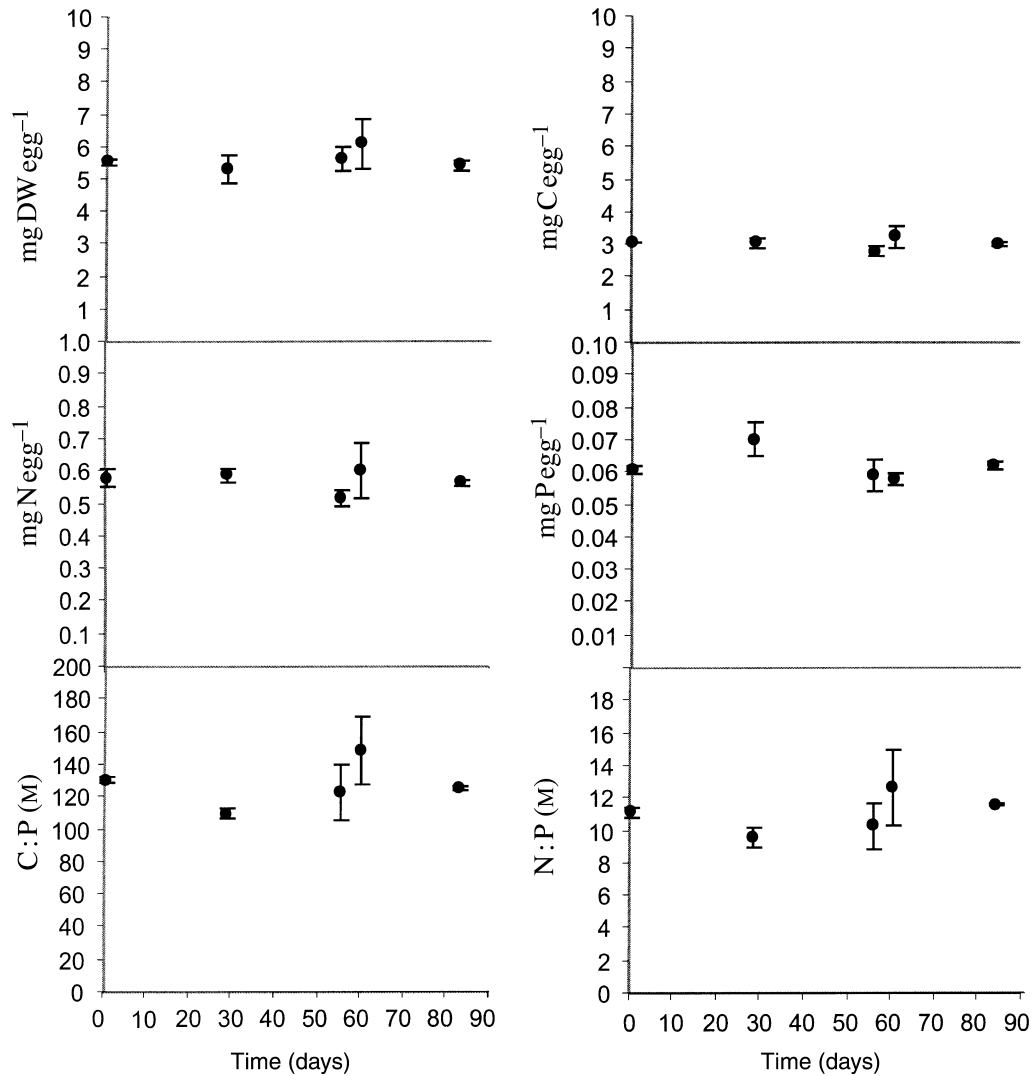


Fig. 1 Egg mass, elemental contents and elemental ratios (mean \pm SD) in eggs of *Astacus* during embryogenesis. Time 0 refers to the first sample of eggs that was taken approximately 2 months after egg extrusion.

the exoskeleton, however, and thus provide only integrated elemental ratios.

Discussion

Carbon allocation in crustaceans typically undergoes a marked shift at the onset of reproduction, when somatic growth ceases at the expense of reproduction (Andersen, 1997). Although the C : P or N : P ratio in eggs and juveniles was not strikingly different, there may nevertheless be a reallocation of elements into different macromolecules. Presumably the share of phospholipids is higher in eggs than in juveniles, where RNA probably accounts for a major share of P

(Vrede, Andersen & Hessen, 1999). For both *Daphnia* and *Astacus*, there was a significantly higher C : P ratio in eggs than juveniles, but the C : P ratio found for eggs in our study was nevertheless substantially lower than that reported by Sterner & Schulz (1998). These authors reported a C : N : P ratio of 192 : 16 : 1 for eggs of *D. magna* and 310 : 15 : 1 for *D. obtusa* Kurz. We have no obvious explanation for this difference. However, they found that C did not make up more than 34.6–41.4% of DW (38.2% in adults), while the share of P was substantially lower in eggs than adults. It is noteworthy that these two species displayed very different specific content of both C and P in their eggs. *Daphnia obtusa* also contained far less C

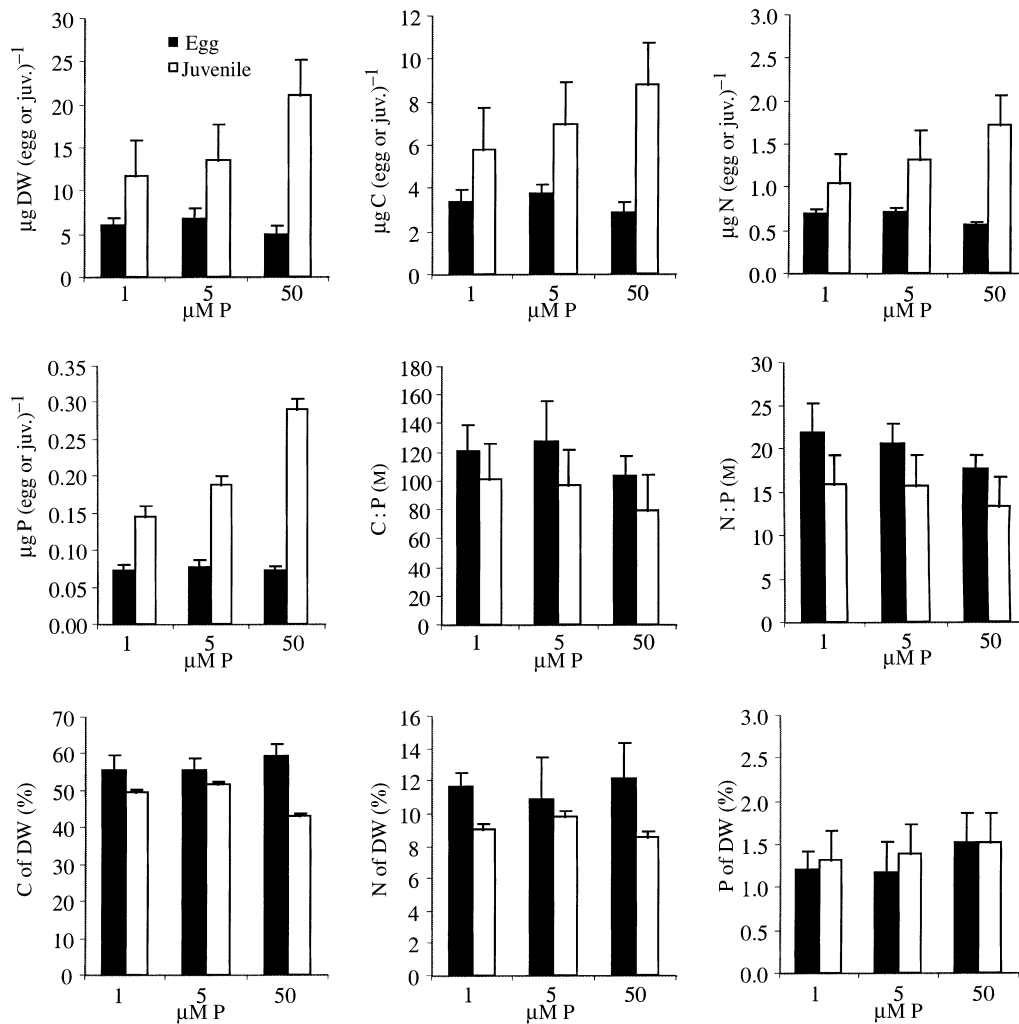


Fig. 2 Dry weight, elemental contents and elemental ratios (mean \pm SD) for eggs (black column) and <2-day-old juveniles (white column) of *Daphnia* raised under different ambient P (1, 5 and 50 $\mu\text{M P}$ per litre), yielding algal C : P ratios (atoms : atoms) of 90 ± 15 , 270 ± 50 and 420 ± 80 , respectively.

and N per unit DW, causing fairly similar elemental ratios in spite of the remarkably different specific content. The high C : P ratio of egg was thus mainly caused by the extremely low P content (0.56% P of DW for *D. magna* versus 0.29% in *D. obtusa*), which contrasts with the findings from our study (1.1% P in *Daphnia* eggs and 0.9% P in crayfish eggs). There may thus be substantial differences between species (or populations), as also suggested by the extremely different C : P ratio found for resting eggs (ephippia) in *D. obtusa* and *D. lumholtzi* Sars (2121 versus 349) in the same study (Sterner & Schulz, 1998). While the C : P ratios of eggs in general is higher than that of somatic tissue, the total demand for P in eggs may nevertheless be substantial. This is supported by

observations of reduced fecundity (Sterner, 1993; Urabe & Sterner, 2001) in *Daphnia* fed on P deficient food. Urabe & Sterner (2001) also recorded reduced egg viability under P deficiency.

At one level, there will thus be a P allocation strategy that reflects life stage and nutritional status. Juveniles will allocate all available P to somatic growth, while an increasing share of P will be spent on egg production upon maturity. The analysis of *Astacus* revealed differential elemental allocations between different internal structures. For instance, there was a substantially higher C : P ratio in the hepatopancreas than in other major tissues, suggesting that deviation from homeostasis in the entire animal could be a result of storage of adipose tissue,

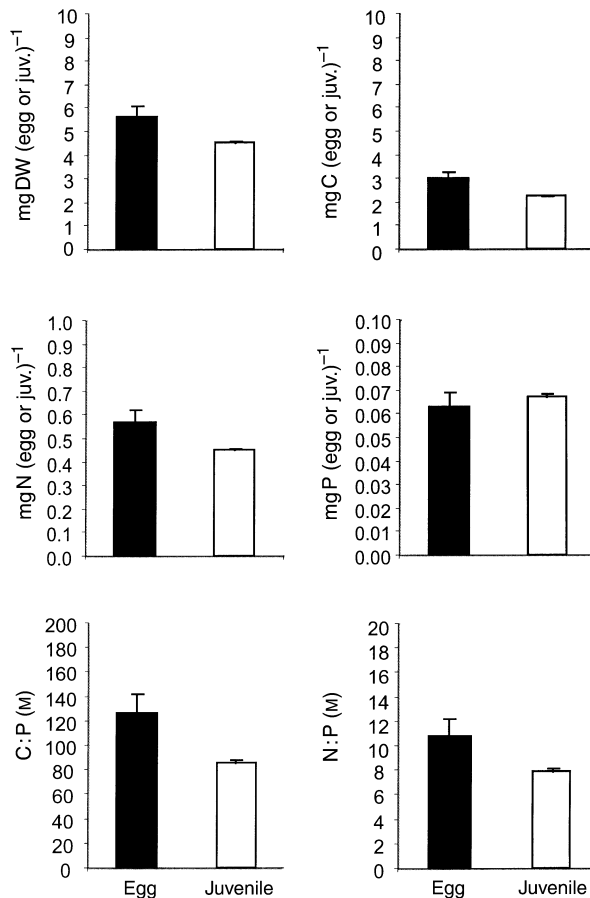


Fig. 3 Dry weight, elemental contents and elemental ratios (mean \pm SD) for eggs (black) and 2-day-old juveniles (open) of *Astacus*.

either in hepatopancreas, in reproductive tissue or subcuticular fat deposits. In specific organs or tissues like muscle, the elemental composition is more tightly

regulated. This could also be the case for smaller crustaceans like *Daphnia*.

The low specific content of C, N and P in carapace is due to a high content of Ca and other constituents (Rukke, 2002) and, while the carapace may constitute some 25% of total DW (Hessen *et al.*, 1987) and have a high C : P ratio, it nevertheless does not contain more than 10–12% of total body P, which is close to the values obtained for *Daphnia* (Vrede *et al.*, 1999). The potential for reclaiming P bound in the carapace prior to moulting is not agreed (Vrede *et al.*, 1999; Hessen & Rukke, 2000) but even with a total loss it would constitute a small fraction of the total P budget of both crayfish and *Daphnia*. However, this is not to say that the drain is unimportant. Lynch *et al.* (1986) and Andersen (1997) both demonstrated how a rather modest loss of C at each moult had major effects on accumulative body size over time.

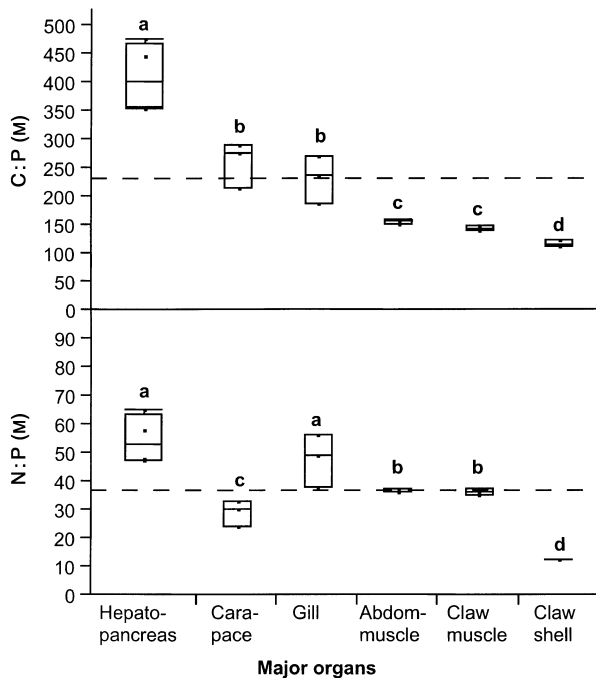
Growth rate constraints in *Daphnia* caused by strict P limitation in food have been demonstrated repeatedly (Sterner *et al.*, 1993; Urabe, Clasen & Sterner, 1997; DeMott *et al.*, 1998; Boersma, 2000). This is due to a skewed ingestion of C : P relative to the somatic demands, and in order to maintain homeostatic regulation, the grazer has to dispose of more of ingested C. Juvenile *Daphnia* tend to have a higher specific demand for P than adults (Hessen & Andersen, 1991; DeMott, 2003). DeMott (2003) recorded a drop in specific P content in *Daphnia* from 1.55 to 1.45% of DW at maturation (6 days), coincident with a drop in somatic growth rate. This could reflect the somewhat lower specific demand for P allocation to eggs relative to somatic tissue. It has been

Table 1 Summary of two-way ANOVA for elemental ratios of *Daphnia* eggs and juveniles raised under different food qualities C : P and N : P ratios (see text for further explanation)

Analysis of variance	d.f.	C : P				N : P			
		Sum of squares	Mean square	F ratio	P > F	Sum of squares	Mean square	F ratio	P > F
Model	2	4386	2193	7.9070	0.0029	122.5	61.3	8.0636	0.0027
Error	20	5546	277			151.9	7.6		
Total	22	9932				274.4			
Parameter estimates		C : P				N : P			
		Estimate	SE	t ratio	P > t	Estimate	SE	t ratio	P > t
Intercept		116.52	4.75	24.57	<0.0001	19.89	0.79	25.34	<0.0001
Stage (egg)		8.53	3.48	2.45	0.0236	-0.07	0.03	-2.93	0.0083
Food quality		-0.45	0.15	-2.98	0.0075	1.48	0.58	2.56	0.0187

Table 2 Summary of one-way ANOVA for elemental ratios (C : P and N : P) in *Astacus* eggs during embryogenesis and for *Astacus* egg versus juvenile

Analysis of variance	d.f.	C : P				N : P			
		Sum of squares	Mean square	F ratio	P > F	Sum of squares	Mean square	F ratio	P > F
Time	4	897	224	0.7642	0.57	4.33	1.08	0.4469	0.77
Error	11	3227	293			26.67	2.42		
Total	15	4124				31.00			
Stage	1	6095	6095	27.893	<0.0001	28.81	28.81	17.658	0.0005
Error	19	4152	219			31.00	1.63		
Total	20	10247				59.81			

**Fig. 4** C : P and N : P ratios for major tissue of adult *Astacus*. Extension of boxes represents the 25th and 75th quartiles. Different characters on top of each box express statistically significance difference ($P < 0.05$, Kruskal–Wallis rank test).

correspondingly demonstrated that P limitation by high C : P ratio in food may constrain egg production (Sternner *et al.*, 1993; Sternner & Schulz, 1998; DeMott, Gulati & Van Donk, 2001). While the *relative* demands for P relative to C will be slightly less when there is a shift in allocation from somatic tissue to eggs, there is still a high *absolute* demand for P in egg biomass as well.

As argued by Urabe & Sternner (2001) and DeMott (2003), newborn neonates may survive on the store of P obtained from the eggs, so that severe effects of low

P diets will be observed only when this initial pool of P is exhausted. This could also explain the observation by Boersma (2000), who found a stronger response to P enrichment in *Daphnia* from 3 to 6 days, than from 0 to 3 days. This could point to a key role of the specific P content in eggs for survival and growth early after hatching.

The relative proportions of C, N and P allocated to eggs in *Daphnia* differed significantly, yet not as substantially as found by DeMott *et al.* (1998). These authors reported a strong correlation between C : P of mothers and daughters, suggesting the possibility of a rather flexible investment of P to eggs. It should be noted, however that the range in C : P in the food was far higher in the experiments by DeMott *et al.* (up to 1000 in C : P) that that offered in our study (80–420), and this could explain the somewhat stronger effect in their study.

For crayfish, there is no corresponding data on direct P limitation, but we suspect that low specific P content in the food (high C : P ratio) could also be relevant for *Astacus*. For juvenile lobsters (*Homarus americanus*, H. Milne Edwards), Gallagher *et al.* (1978) reported both abnormal histology and moulting problems under P deficiency. Crayfish have a very low growth rate and, in Norwegian lakes, the onset of reproduction typically requires 5–6 years. While juveniles are mainly carnivorous, adults are truly omnivores, with a high fraction of low quality items like detritus in their diet (Hessen & Skurdal, 1985), and such ontogenetic shifts in diet could reflect the low C : P ratio (and higher P demands) of the juveniles, allowing for higher growth rates.

The allocation of energy and matter to eggs and offspring is a key parameter in any life history. Both for C and P, increased allocation to eggs will increase

the growth and survival of the offspring (Tessier *et al.*, 1983; DeMott, 2003). Juvenile survival is also increased when eggs contain a high share of maternal lipids (Tessier *et al.*, 1983). It is important to note that maternal allocation of lipids (high specific C) and P may have different costs and different consequences. While lipids may fuel basic metabolism, the juvenile growth rate will also depend on P (and N). The overall fitness consequences of these allocation strategies will depend on food availability (and food quality) for the early neonates (Tessier & Consolatti, 1989; Urabe & Sterner, 2001). Further, lipid accumulation may occur in adults when fed with low quality (high C : P) food (Sterner, 1998). Hence, it is reasonable to assume that there will be different allocation strategies for C and P to eggs along gradients of food quantity (C) and food quality (C : P) that will have different consequences for survival or growth rate in the juveniles. Tessier & Consolatti (1991) attributed differences in the ratio of mass to body length in *Daphnia* juveniles to their C : N ratios. Typically juvenile *Daphnia* produced at low food quantity had a lower mass, but were relatively long and had a low C : N ratios.

Egg stoichiometry could also affect metabolic processes in eggs. The specific respiration rate is generally very low in eggs (Glazier, 1991; Boersma, 1995), and is reflected in stable mass and stoichiometry during embryogenesis. In fact, we were unable to demonstrate any change in DW or elemental ratio in crayfish eggs during embryogenesis, suggesting strong retention of elements and an extremely low respiration rate. For obvious reasons an incubation period of several months, as found for temperate crayfish populations, would demand an efficient storage of elements. Boersma (1995) reported lower specific respiration rates in small embryos (under low food) than large embryos (at high food), which may be interpreted as minimising C losses. It may, however, also reflect egg stoichiometry, as higher C : P ratio could enhance respiratory losses.

While large female *Daphnia* generally produce large eggs, there is a considerable scatter in these data (cf. Boersma, 1995). This may in part reflect the nutritional status of the mothers, but may also reflect the fact that size is no more than a proxy of age. In general, old mothers may invest more in reproduction than younger ones (cf. Glazier, 1992). With increasing age and decreased somatic growth, the conflict between

allocation to somatic tissue and eggs would decrease, and older mothers could afford to spend not only more C, but also more P, on eggs. During the transition to maturity, there may be a strong trade-off between investment in somatic or reproductive tissue for both C and P. 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 can be allocated to eggs at a lower cost for older individuals. These allocation strategies may also be influenced by food quality in terms of C : P. When C : P in the food is high, this may not severely affect the survival and maintenance of mature individuals with low requirements for somatic tissues, but it could indeed constrain egg production.

Under the range of elemental ratios applied, we were unable to demonstrate any effect of food stoichiometry on *Daphnia* egg stoichiometry. Under more severe P limitation, however, low food quality might result in an increased C : P in eggs due to decreased specific P contents (cf. DeMott *et al.*, 1998), and it is likely that such an effect will have strong bearings on individual fitness and thus population dynamics. In conclusion, there are marked ontogenetic shifts in P demand for both species, and juveniles could thus represent a population bottleneck with regard to sensitivity to P limitation. In addition, egg production may be at least as sensitive to low P as is somatic growth rate, and both may add up to a greater decrease in population growth rate under P deficiency than would be expected from the response in individual growth rate alone.

Acknowledgment

This work was financed by grants from the Norwegian Research Council, grant numbers 123860/410 and 147220/432 to D. Hessen.

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(Manuscript accepted 1 July 2003)