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Relationship between N:P ratio and growth rate during the life cycle of calanoid copepods: An *in situ* measurement

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The mean nitrogen and phosphorus contents of the calanoid copepod Mixodiaptomus laciniatus Lilljeborg were measured to the stage level throughout the ice-free period of a high mountain lake. Our results indicate large intraspecific variations in calanoid elemental composition. While mean N as dry weight increased from 3.0 ± 1.3 in nauplii to 6.0 ± 2.1 in copepodites, mean P content showed the opposite trend, varying intraspecifically from 0.98 ± 0.26% in nauplii, to 0.87 ± 0.21% in copepodites and 0.51 ± 0.16% in adults. Thus, the mean N:P ratio increased ontogenetically from 3.3 in nauplii to 13.3 in copepodites and 24.6 in adults. Two ontogenetic parameters, the growth rate and body size, were associated with zooplankton stoichiometry. Among all 11 copepod stages, growth rate was positively related to %P and negatively related to %N and N:P ratio. A two-part analysis of these relationships, before and after metamorphosis, showed that the nauplii growth rate explained nearly all the variance in naupliar P content. A high P content in nauplii may reflect a high content of RNA, translating into rapid growth rates. Overall, these results tend to support the hypothesis linking specific growth rate with P content for copepods, but these results also suggest that the validity of this hypothesis is robustness when differences in the life history of copepods as a consequence of metamorphosis are accounted for. We suggest that the intra-stage variation in P content is associated with peaks of intensive metabolic activity during the process of molting in copepods, and we emphasize the importance of new empirical evidence to examine this hypothesis further.

INTRODUCTION

Recent studies have hypothesized that differences in elemental composition of zooplankton (N:P ratio) are related to differences in specific growth rate, as increased growth rate requires greater ribosomal RNA, which should result in increased %P and decreased N:P ratio (Main *et al.*, 1997). This hypothesis is based on empirical evidence showing pronounced interspecific differences between the major crustacean groups. Thus, cladocerans are characterized by N:P ratios between 12 and 18 whereas calanoid copepods have N:P ratios exceeding 30 (Andersen and Hessen, 1991; Hessen and Lyche, 1991). Differences in body %P, but not in %N, among zooplankton taxa are responsible for these ratios. In fact, the %N in zooplankton is nearly constant [range 8–12%; (Andersen and Hessen, 1991; Hessen and Lyche, 1991; Gismervik, 1997; Walve and Larsson, 1999)], while the %P in zooplankton is quite variable. Among cladocerans, *Daphnia* are particularly rich in P, with a percentage as dry weight of ~1.2, but this content can be as high as 2.5% in *Scapholebris mucronata* (Main *et al.*, 1997). Calanoid copepods, on the other hand, can have P contents of ~0.5, between two- and fivefold less than in cladocerans.

Some evidence suggests that additional P in high P zooplankters is used for carrying higher levels of RNA (Hessen, 1990; Sterner, 1995), a key biomolecule in the anabolic synthesis for an animal's biomass production. This idea is consistent with the life cycle of cladocerans having successive parthenogenetic reproduction cycles

before sexual maturity, short generation times and continuous somatic growth. In contrast, high N:P ratio organisms such as calanoid copepods are characterized by low RNA levels, sexual reproduction cycles, long generation times and a slow somatic growth in a series of stepwise increments, each occurring at ecdysis to the next instar, including a major metamorphosis process. Differences in P content and N:P ratio between both zooplankton taxa perhaps reveal the evolutionary pressure behind zooplankton nutrient content (Sterner and Schulz, 1998).

The relationship between growth rate and elemental body stoichiometry has been recently tested for cladoceran species (Main *et al.*, 1997). These authors have found that the body N:P ratio in cladocerans is negatively related to the specific growth rate, in a pattern primarily reflecting changes in %P. However, before the P growth rate hypothesis is proclaimed to be a general hypothesis for all zooplankters, more research should be conducted on zooplankton taxa other than daphnids. As pointed out by Main *et al.* (Main *et al.*, 1997), the growth rate hypothesis can also be tested by considering intraspecific variation, as many taxa undergo substantial shifts in specific growth rate during ontogeny. Although their results tend to support the growth rate hypothesis as an explanation for differences in body N:P ratio in zooplankton, their experiments are not definitive, as the growth rate proved negatively related to N:P for *Daphnia lumholtzi*, but unrelated for *D. obtusa* and *D. magna*. Because there is no *a priori* reason to assume the generality of the growth rate hypothesis for all other zooplankton species, and because several researchers have documented large differences in the P content during the life history of copepods (Carrillo *et al.*, 1996a; Schulz, 1996), this hypothesis needs to be tested for these organisms. If the relationship between P and copepod development is confirmed, this will provide a good measure of the generality of the phosphorus stoichiometry hypothesis and a major unexplored feature of the ecology of copepods (Sterner and Schulz, 1998).

In the present study, we seek to establish the relationship between the elemental composition (N and P content) and growth rate in a calanoid copepod, *Mixodiaptomus laciniatus*, and to relate P content variations to metamorphosis and molting processes.

METHOD

The study was performed in a small oligotrophic lake, La Caldera, located in the Sierra Nevada Mountains (southern Spain) at 3050 m above sea level in a glacial cirque. The ice-free period generally lasts for 5 months from July to early November. During the ice-free season, the lake does not stratify, and water temperatures usually range from 4 to 12°C by the end of August. The quantity of available food for the zooplankton assemblage is frequently below 100 μg C l^{-1} (Carrillo *et al.*, 1996b). A dissolved inorganic nitrogen:total phosphorus (DIN:TP ratio) of >12 throughout the ice-free period indicates strong P limitation (Morris and Lewis, 1988). The sestonic N:P ratio in 1997 varied between the lowest values of 2.1 and 4.1 after thaw, and the highest values of 37.3 by mid-summer, averaging N:P = 11.0 over all (Villar-Argaiz, 1999). Similarly, the sestonic C:P ratio was low during ice-out (C:P <100), increased markedly to a maximum value of 320 by mid-summer, and remained relatively constant (C:P between 110 and 160) towards the end of the season. For further biotic and abiotic characterization of the system see Medina-Sánchez *et al.* (Medina-Sánchez *et al.*, 1999) and Villar-Argaiz *et al.* (Villar-Argaiz *et al.*, 2001).

Sampling

Field samples were taken approximately every 10 days, over the entire ice-free period, from late June to early November of 1997. Zooplankton abundance was sampled using a Van Dorn sampler at four depths (0.5, 5, 8 and 10.5 m) at a central station (Z_{max}= 14 m).

The abundance of zooplankton was determined by sieving 12 l, from each of the above depths, through a net (40 μm mesh size) and immediately preserving samples in 4% formaldehyde. All samples were counted under an inverted microscope at 25× magnification. For each sample, 20 individuals of every stage present were measured by image analysis (Leica, Quantimet 500). The biomass of *M. laciniatus* was estimated from a length–weight (dry weight, *DW*) relationship developed specifically for this species in this study (nauplii $DW(\mu g) = -0.26 + 0.003 * L(\mu m)$, $r^2 = 0.97$, $P < 0.001$; copepodites ($DW(\mu g) = -4.98 + 0.01 * L(\mu m)$; $r^2 = 0.92$, $P < 0.001$). The *DW* of adults (males, females and females carrying eggs) was estimated from average values of direct weights for males, females and females carrying eggs at each sampling. The biomass of other zooplankton taxa was estimated using length–mass relationships from Botrell *et al.* (Botrell *et al.*, 1976).

The elemental composition of the calanoid copepod *M. laciniatus* was measured on animals taken in qualitative vertical hauls (40 μm mesh net). Samples were brought cold and dark to the laboratory within 2 h.

Chemical analyses

Stage-specific dry weight, C, N and P of *M. laciniatus* samples were determined in the laboratory. For this purpose, live individuals of different stages (nauplii NII–NVI, copepodites CI–CV and adults) were identified and measured with the aid of an inverted microscope.

Among adults, males, females and females carrying eggs were distinguished. Individuals were washed in GF/F filtered lake water and sorted live to stage level in specific Petri dishes. Six replicates of the most abundant stages, containing at least 40 nauplii or 15 copepodites and adults, were obtained for each sample. Three randomly selected replicates were placed onto pre-weighed carbon and nitrogen-free tin capsules, dried in an oven at 60° C for 24 h and re-weighed with a Mettler UMT2 microbalance (± 0.1 μg) to determine dry weight. The mean weight per individual was calculated as *DW*/number of animals per capsule. Samples were stored in a dessicator until carbon and nitrogen analysis with a CNH-Perkin Elmer 2400 elemental analyzer (thermal-conductivity detector). For P determination (three replicates), the animals were immediately placed into acid-washed vials and determined as soluble reactive phosphorus (acid molybdate technique) after digestion with a mixture of potassium persulfate and boric acid at 120ºC for 30 min, and sonication for 15 min at 50 Hz (Ultransons P Selecta). Blanks and standards were carried out for these procedures (American Public Health Association, 1992). The coefficient of variation (standard deviation/average ×100) of the elemental content for a given stage and date never exceeded 5% for C, 7% for N and 10% for P.

Determination of population growth rates

In situ stage-specific growth rates (r) for *M. laciniatus* (assuming that all individuals at each stage were growing exponentially) was calculated as:

$$r = [\ln DW_{(i+1)} - \ln DW_{(i)}]/D \quad (1)$$

where $DW_{(i)}$ and $DW_{(i+1)}$ were the mean dry weight for two consecutive stages of *M. laciniatus*, and D was the stage duration. The time between the occurrence of two stages (stage duration, D) was determined following Rey and Capblancq (Rey and Capblancq, 1975) recommendation for *M. laciniatus* in Lake Port-Bielh (French Pyrenees):

$$D_i = 1/N_i \sum n_i \quad (2)$$

where N_i was the mean *M. laciniatus* abundance for each stage calculated from the mortality curve (see equation 4 below) and n_i was the sum of all individuals for each stage during the time (in days) present in the lake.

From the seasonal series of *M. laciniatus* abundance data, the mortality curve was calculated as the decrease in the total number of organisms, after individuals at nauplii stage NI disappeared (mortality rates not biased by newborn individuals). If we assume a constant mortality over time, then the deaths become proportional to the number of individuals present (N) and an instantaneous mortality rate (m) is calculated as:

$$dN/dt = -m * N \quad (3)$$

The equation that describes the population loss is

$$N_i = 25.3 * \mathrm{EXP}(-0.006 * t) \quad (4)$$

where N_i is the mean number of individuals present for each stage, m is the mortality rate and t is the period at which individuals of stage i were present in the lake.

Accurate estimates of copepod mortality are difficult to make and are virtually non-existent in the literature. However, it is known that the mortality rate is a stage-dependent process, and that most copepod mortality occurs during the naupliar stage, when these animals are most vulnerable to starvation (Mullin and Brooks, 1970; Paffenhöfer, 1976; Borchers and Hutchings, 1986). Plagányi *et al.*, based on the lipid reserved during the ontogenetic development of copepods, estimated the starvation tolerance of nauplii to be half that of the copepodite stages (Plagányi *et al.*, 1999). Consequently, and for comparative purposes in calculating nauplii growth rates, we also assumed the mortality rate for nauplii to be twice ($m = 0.012$) that found for copepodite and adult stages.

The copepod univoltine reproductive cycle in La Caldera represents a unique opportunity to calculate the growth of a copepod cohort throughout the ice-free period under natural conditions. However, when growth rate data are to be contrasted with elemental content of the copepod, care must be taken to ensure all data are simultaneously obtained. In this respect, conclusions of similar analyses may differ depending on whether growth rate is obtained by theoretical models [e.g. (Hirst and Sheader, 1997)] or by extrapolating the stage duration for the same species under different conditions of the lake (Cruz-Pizarro, 1981; Villar-Argaiz *et al.*, 2000).

Statistical analyses

The relationship between copepod elemental composition and ontogenetic parameters such as body size and growth rate were analysed by linear regression models (Statistica 5.1, StatSoft 97). All regressions were computed using quasi-Newton iterations.

Differences in elemental composition for copepod stages grouped into nauplii, copepodites and adults were performed via Kruskal–Wallis one-way ANOVA, with the elemental composition (%C, %N and %P) as the dependent variable and copepod stage as the grouping variable. All pairwise multiple comparisons were performed by the Mann–Whitney U-test.

RESULTS

The calanoid copepod *M. laciniatus* dominated the zooplankton community throughout the ice-free period, comprising 89% in mean biomass. Rotifers (*Hexarthra bulgarica*) and cladocerans (*Daphnia pulicaria*) were scarcely represented. The evolution in number of *M. laciniatus* individuals is shown in Figure 1. The life cycle of this calanoid copepod included an intensive hatching of eggs as the thaw approached, followed by the development of a single cohort during the ice-free period where nauplii (120–390 μm in size) were present until day 239 (end of August), when they became replaced by copepodites. The appearance at this time (days 184 to 199) of copepodites stage V and large adults (between 200 and 300 μm larger than late period adults; Table I), including ovigerous females, provides evidence of an overwintering strategy of late copepodite stages. Mean individual size of zooplankton was largest in late October, when the assemblage comprised mainly copepodite stages III and IV (680 ± 52 μm and 800 ± 17 μm, respectively; Table I), with only a few reaching adulthood before the end of the ice-free period.

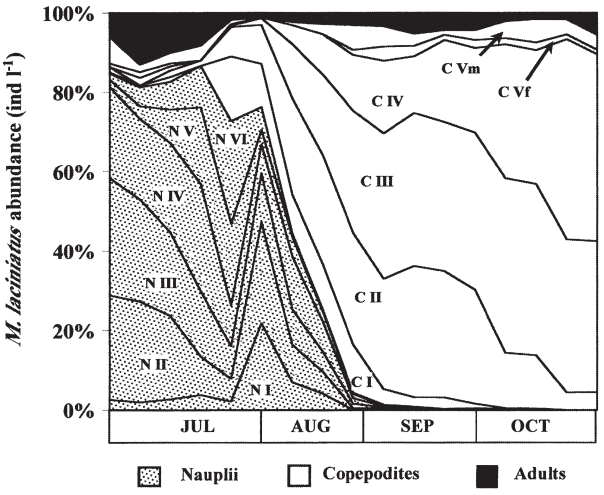

Fig. 1. Ice-free period variations in the abundance (in percentage) of *Mixodiaptomus laciniatus* developmental stages.

N:P body stoichiometry

Nitrogen content as dry weight increased substantially from 1.4% ± 1.1 in nauplii (mean ± SD) to 6.0% ± 2.1 in copepodites and 5.3% ± 0.1 in adults (Table I). Thus, the %N varied significantly (about sixfold) between nauplii and copepodites ($P < 0.001$; Mann-Whitney *U*-test), but no significant differences in nitrogen content were found between copepodites and adults ($P = 0.31$).

Although the P content per individual increased with ontogeny (Figure 2), the %P as *DW* offered a different pattern. Thus, the observed increase in the %P across nauplii stages was followed by a sharp decline in copepodite stage I and a progressive decrease from copepodite stage II onwards (Table I). Differences in the %P among copepod developmental stages were statistically significant ($P = 0.04$; Kruskal–Wallis test). Phosphorus content decreased about twofold from nauplii (0.98 ± 0.26; mean ± SD) to adults (0.51% ± 0.16) and over all stages, the mean P content was 0.84% ± 0.29. Within stages, variability in P content was more pronounced in nauplii than in copepodite and adult stages, as indicated by the higher standard deviation (Table I).

The intraspecific variation in N:P was well related to

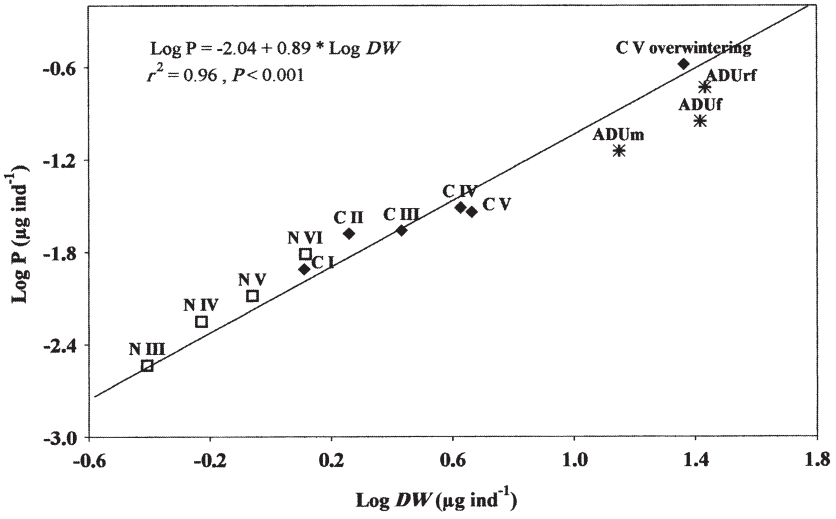

Fig. 2. Phosphorus content plotted against dry weight. The line represents the best fit from least-squares regression. (NI–NVI) nauplii, (CI–CV; CV overwintering; CVm-male; CVf-female) copepodites, and (ADUm-adult male; ADUf-adult female; ADUrf-adult reproductive female) adults.

Table I: Size, dry weight, nitrogen (N) and phosphorus (P) as a percentage of dry weight, N:P ratio, growth rate (r), and growth rate assuming double nauplii mortality (r′) for Mixodiaptomus laciniatus stages

M. laciniatus stages	Size (μm ind^{-1})	Dry weight (μg ind^{-1})	%N	%P	N:P	*r*	*r′* (nauplii mortality ×2)
N II	120–160	0.27 ± 0.03 (5)	0.71			0.52	0.29
N III	170–210	0.39 ± 0.01 (7)	0.73 ± 0.32 (4)	0.75 ± 0.15 (3)	2.23 ± 1.36 (3)	0.65	0.42
N IV	220–270	0.59 ± 0.06 (11)	0.92 ± 0.15 (7)	0.96 ± 0.27 (5)	2.40 ± 0.78 (5)	0.79	0.51
N V	280–320	0.87 ± 0.08 (9)	1.34 ± 0.32 (4)	0.95 ± 0.32 (3)	3.22 ± 0.85 (3)	1.01	0.59
N VI	330–390	1.30 ± 0.19 (10)	3.28 ± 0.10 (4)	1.19 ± 0.17 (4)	5.01 ± 0.94 (4)	1.34	0.97
C I	375–500	1.29 ± 0.22 (11)	4.37 ± 1.82 (8)	0.95 ± 0.03 (2)	8.18 ± 2.78 (2)	0.58	0.58
C II	525–650	1.81 ± 0.56 (13)	5.84 ± 1.36 (8)	1.11 ± 0.15 (2)	11.15 ± 1.48 (2)	0.10	0.10
C III	675–725	2.70 ± 0.93 (17)	5.57 ± 2.12 (14)	0.81 ± 0.17 (8)	15.21 ± 6.31 (8)	0.04	0.04
C IV	750–825	3.91 ± 0.76 (10)	5.54 ± 1.47 (10)	0.73 ± 0.06 (6)	16.42 ± 6.61 (6)	0.09	0.09
C V	850–925	4.60 ± 0.62 (4)	5.30 ± 0.86 (4)	0.63 ± 0.07 (2)	17.57 ± 2.28 (2)	0.13	0.13
C V overwintering female	1000–1275	23.03 ± 1.41 (2)	4.40 ± 0.79 (2)	1.14 ± 0.11 (2)	9.44 ± 1.70 (2)		
ADULT male	925–	14.07 ± 0.33 (9)	5.22 ± 1.89 (9)	0.51 ± 0.12 (3)	24.76 ± 6.49 (3)	0.003	0.003
ADULT female	925–	26.07 ± 2.02 (6)	5.27 ± 0.74 (5)	0.43 ± 0.18 (3)	26.16 ± 7.16 (3)	0.003	0.003
ADULT female with eggs	925–	26.98 ± 0.08 (3)	5.54 ± 1.24 (3)	0.69 (1)	20.87 (1)		

Values are means ± 1 SD of *n* replicates in parenthesis.

the ontogenetic development of *M. laciniatus* (Table I). The nauplii N:P ratio varied between 2.2 and 5.0 from the first to the last naupliar stage, with a mean nauplii value of 3.0 ± 1.3. In contrast, copepodites and adult stages showed much higher mean N:P ratios of 13.3 ± 5.6 and 24.6 ± 5.7, respectively. Differences in the body N:P ratio among copepod developmental stages were all significant ($P < 0.001$; Kruskal–Wallis test).

When we represent the %P against dry weight, we find an intra-stage variation in the P content, with a tendency towards a greater P content as the individuals become heavier (Figure 3) and hence, hypothetically closer to a molt or ecdysis event in almost all stages. This may suggest some fundamental links between molting and P content in copepods, a tendency particularly accentuated before metamorphosis for the naupliar stages NIV, NV and NVI.

Growth rate, body size and elemental content

Due to the significant negative relationship found between growth rate and body size (see solid line in Figure 4) and the different slopes for naupliar and copepodite/adult stages (see dotted line in Figure 4), the influence of both ontogenetic parameters (body size and growth rates) on changes in elemental content was explored for all copepod stages (see Table II) and separately for nauplii and copepodites/adults by fitting simple linear models (see Figure 5).

When the data for all copepod stages were considered together, the %N and N:P were negatively correlated with specific growth rate ($P = 0.010$ and $P = 0.002$, respectively), but there was a significant positive relationship between the %P and growth rate ($P = 0.025$) (Table II). In contrast, the %P was negatively correlated with specific dry weight ($P = 0.009$), but no relationship between %N and dry weight was found (Table II). When the growth rate for nauplii was calculated, assuming a mortality rate twice that calculated from the mortality curve (see Method), these relationships did not diverge greatly from those initially found; however, the statistical significance in the relationship %N versus the growth rate decreased, while the statistical significance in the relationship between the %P and growth rate was strengthened (Table II).

When the data for copepod stages were considered separately, i.e. before metamorphosis (nauplii) and after

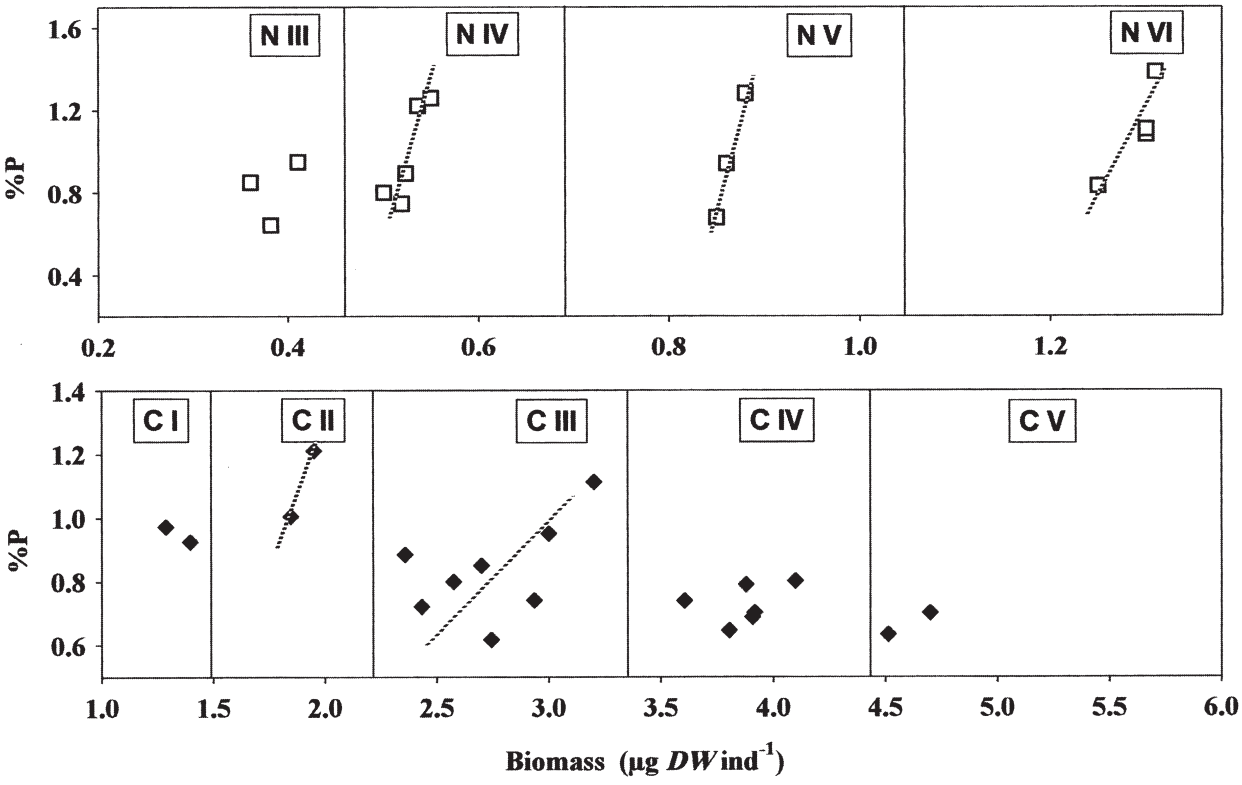

Fig. 3. Intra-stage variation in the %P as dry weight for *Mixodiaptomus laciniatus*. Nauplii (NIII–NVI) and copepodite stages (CI–CV). Lines denote intra-stage trends in the %P increase. Microscopic observations of developmental features for copepod stages allowed the grouping of individuals into stage categories.

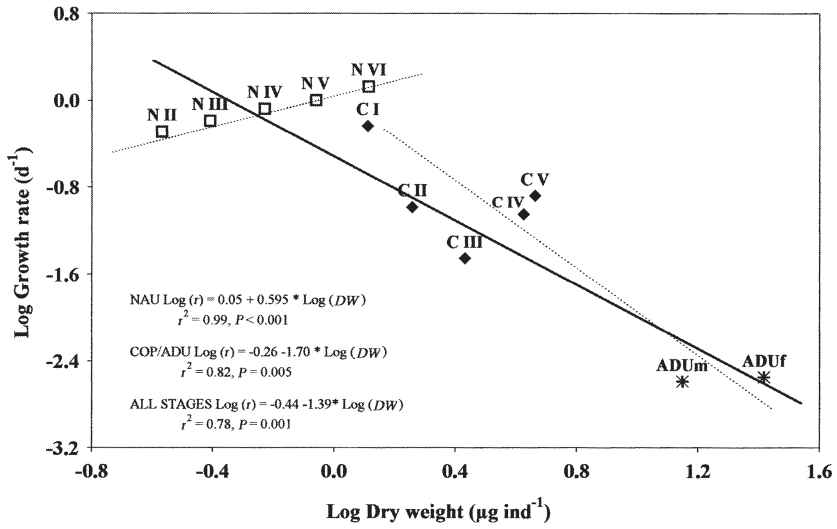

Fig. 4. Log-log scatter diagrams of growth rate and dry weight data for *Mixodiaptomus laciniatus* developmental stages. Dotted lines are linear regressions of log (growth rate) on log (dry weight) equations for nauplii and copepodites–adults. Solid line represents linear regression for all stages. Legend as in Figure 2.

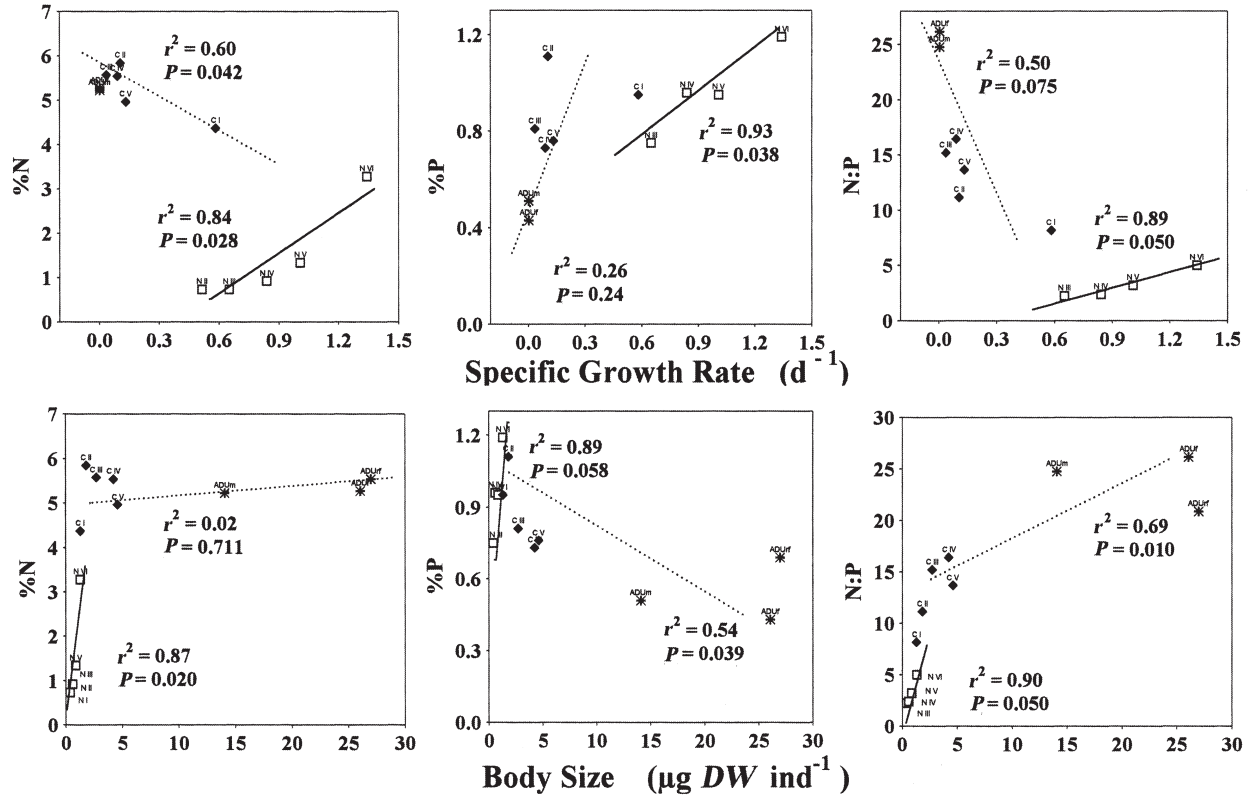

Fig. 5. Relationship between %N, %P and N:P ratio and growth rate (**upper panel**) and dry weight (**lower panel**) for *Mixodiaptomus laciniatus*. Solid lines represent the best fit from least-square regression for nauplii. Dotted lines represent the best fit for copepodites and adults. Key as in Figure 2.

Table II: Regression analysis between zooplankton stoichiometry (%N, %P and N:P ratio) and specific growth rate (r or r′, d^{-1}) and dry weight (DW, μg ind^{-1})

	Variables	*a* ± SE	*b* ± SE	*P*	r^2
%N	*r*	5.11 ± 0.65	–3.32 ± 1.05	0.010**	0.50
	r′ (nauplii mortality 2×)	4.89 ± 0.76	–3.99 ± 1.79	0.050*	0.33
	DW	3.07 ± 0.64	0.11 ± 0.06	0.080	0.25
%P	*r*	0.69 ± 0.08	0.33 ± 0.12	0.025*	0.44
	r′ (nauplii mortality 2×)	0.67 ± 0.07	0.51 ± 0.17	0.010**	0.50
	DW	0.94 ± 0.06	–0.016 ± 0.005	0.009**	0.51
N:P ratio	*r*	18.03 ± 2.20	–14.59 ± 3.51	0.002**	0.66
	r′ (nauplii mortality 2×)	18.17 ± 2.41	–20.79 ± 5.51	0.004**	0.61
	DW	7.42 ± 1.86	0.71 ± 0.16	0.001**	0.67

Regression model: %N, %P, N:P ratio = $a + b * (r, r', DW)$. *Significant at 5% level; ** significant at 1% level.

r′: *M. laciniatus* growth rate assuming double nauplii mortality.

metamorphosis processes (copepodites and adults) as well as independently of the growth rate value considered for nauplii, our results showed positive and significant relationships between the %N, %P and N:P ratio and specific growth rate for nauplii (all $P \leq 0.05$; Figure 5). Moreover, explained variance increased in comparison with regressions for data from all copepod stages considered together (Figure 5 and Table II). The %P and N:P ratio in copepodite and adult stages showed no relationship with growth rates ($P > 0.05$). However, when the body size was the ontogenetic parameter considered, the *DW* of copepodites and adults showed a positive and significant relationship with the N:P ratio ($r^2 = 0.69$; $P = 0.01$) and negative for P ($r^2 = 0.54$; $P = 0.04$), but no relationship between the %N and dry weight was found ($P > 0.05$). For nauplii, we found the same pattern as described above for growth rate, the strongest relationships being between the %N and N:P ratio and dry weight ($r^2 = 0.87$, $P = 0.02$ and $r^2 = 0.90$, $P = 0.05$, respectively).

DISCUSSION

Stoichiometric variability during life cycle

To the best of our knowledge, no data on naupliar N content are available in the literature for comparison. Nitrogen contents for copepodites and adults are slightly lower than those reported for other species (Andersen and Hessen, 1991; Hessen and Lyche, 1991; Gismervik, 1997). In contrast to these studies that find a nearly constant %N in zooplankton, in our study, the mean nitrogen content varied about sixfold throughout copepod ontogeny from nauplii to copepodites and adults.

Overall, the mean P content of 0.84% ± 0.34 as *DW* for all samples and stages of *M. laciniatus* agrees with previous reports for other freshwater (Hessen, 1990; Hessen and Lyche, 1991) and marine copepods (Båmstedt, 1986). Maximum P content occurred at nauplii stage NVI and a decrease in the %P coincided with the complex metamorphosis that copepods undergo between the last naupliar and the first copepodite stages. Recent studies have hypothesized that one of the events that occurs in several tissues at the time of metamorphosis is cell death (Clarke, 1976; Nicol *et al.*, 1992). These authors have documented mineral content losses of 9.2–30.6% and 34.5% respectively, for krill *Euphasia superba*. More recently, Vrede *et al.* (Vrede *et al.*, 1999) have documented Ca and P losses in the process of molting. It is likely that a decline in P content is, at least in part, related to these phenomena.

The N:P ratios showed strong intraspecific differences during the development of the copepod, and followed a trend similar to that predicted by Elser *et al.* (Elser *et al.*, 1996). Our data show that the N:P ratio in *M. laciniatus* nauplii is below the range of variation for the interspecific cladoceran–copepod N:P ratio differences yet reported (Andersen and Hessen, 1991). However, this result is consistent with findings by Båmstedt (Båmstedt, 1986), who indicated a lower protein:RNA ratio in copepod nauplii than in adults. Additionally, our results on copepodite and adult N:P ratios are in the lower limit of the range previously published for specimens of calanoids from fresh water (Andersen and Hessen, 1991; Hessen and Lyche, 1991) and marine systems [e.g., (Gismervik, 1997; Walve and Larsson, 1999)].

Consistent with the report by Hirst and Sheader (Hirst

and Sheader, 1997) of a significant relationship between growth rate and body size in calanoid copepods, the influence of the two variables in zooplankton stoichiometry was independently explored. Our values of growth rates across the ontogenetic development of *M. laciniatus*, assuming an identical stage mortality or a double naupliar mortality with respect to copepodites and adults (Table I), cover the range of variability found for different species of cladocerans by Main *et al.* (Main *et al.*, 1997). As previously observed by these authors for cladocerans, high growth rates in nauplii are consistent with high P contents (>1% P). Additionally, low P contents and growth rate values under 0.15 day^{-1} for copepodite stages CIII onwards reflect coupling of P and growth rate in copepods.

In contrast to the findings of Main *et al.* (Main *et al.*, 1997) for cladocerans, that there was a negative significant relationship between growth rate and N:P ratio in copepods due exclusively to P, we found that this relationship in copepods was due to both N and P. The steeper slopes in the N relationship with growth rate in comparison to P (Table II) suggests that copepods may be less sensitive to P constraints than cladocerans and demand more N. It is worth noting that the organisms in both studies, with very distinct life-history strategies and generation times, share growth rate as a common denominator to explain much of the variability in their composition and provide support for the hypothesis linking specific growth rates of organisms to their elemental composition, as proposed by Sterner (Sterner, 1995).

Our study reveals that life cycle is an important factor in determining population growth rates and presumably, is crucial to the evolutionary fitness of the organisms. Therefore, it is also critical to consider life history *in toto* in order to provide a complete picture of how growth rates are related to elemental composition (Sterner and Schulz, 1998). We should not expect all stages to show necessarily identical relationships between elemental content and ontogenetic parameters. Thus, these relationships may be explained as a consequence of distinct life-history strategies in nauplii versus copepodites and adults, or in other words, before and after metamorphosis. Firstly, the nauplii stages showed an increase in the %N during their development with a statistically significant positive relationship with both ontogenetic parameters (body size and growth rate). If high-P-content organisms do indeed have more RNA (Elser *et al.*, 1996), then increases in body P in nauplii would allow more rapid growth than in adults. This suggestion is in fact supported by the recently found relationship between growth rate and RNA content in copepods (Saiz *et al.*, 1998). High growth rates in nauplii would require a high ribosomal complement for an extensive protein synthesis before metamorphosis. As mentioned above, this may be the reason why N increased about sixfold from nauplii to copepodites. Secondly, copepodites and adults were characterized by a decrease in the specific P content as the organism's ontogenetic development progressed, reflecting the positive relationship between the body N:P ratio and dry weight.

A more complex life cycle in copepods may suggest that the high P content (in nauplii) is not only used in somatic growth, but also in preparing for metamorphosis (Sterner and Schulz, 1998). Furthermore, the P increase at given stages (Figure 3) suggests that this element plays an essential role in some ontogenetic processes such as molting (Vrede *et al.*, 1999). Therefore, it is reasonable to expect that the process of molting (ecdysis) in crustaceans might also result in peaks in cell activity and RNA content not associated with growth, as has been reported for herring larvae (Clemmensen, 1994).

To understand tissue growth during the molt cycle, we need further knowledge of the relationship between the cell cycle and the subphases of the molt cycle. As Freeman (Freeman, 1990, 1993) has noted, pre-molt is the dominant phase of the molt cycle and it is during this period, shortly before ecdysis, that mitosis in crustaceans takes place (Figure 6). This concept is supported by the observations of higher amounts of DNA in the gut cells of decapod crustaceans during pre-molt (Van Wormhoudt and Sellos, 1981). Therefore, it would not be surprising for P content to reach its maximum level shortly before ecdysis, when metabolic activity is also at its peak. Therefore, we suggest that molting processes could be strongly correlated with calanoid P content, and be responsible for the higher dispersion of this element, particularly in the naupliar stages (Table I).

In summary, body stoichiometry reflects the requirement for specific elements and provides a framework in which to interpret the growth rates and life history of copepods. Nauplii stages with the highest growth rates and lowest N:P tend to invest their resources in growth; copepodites with intermediate growth rates and N:P values split their available resources between growth and maintenance, whereas adults (slow growth rates) with a higher N:P ratio tend to invest their resources in survival and reproduction. Some evidence in cladoceran species suggests that a nutritional P constraint is more a factor for somatic growth than for the production of reproductive tissues (Sterner and Schulz, 1998). As previously suggested for cladocerans (Main *et al.*, 1997), demographic constraints on copepods may operate via juvenile growth under scarce P food.

The relationship found between ontogenetic parameters and elemental composition could be interpreted as an adaptive evolutionary mechanism to predictable and oligotrophic ecosystems. Nauplii grow fast when provided with a higher amount and quality of food after the thaw

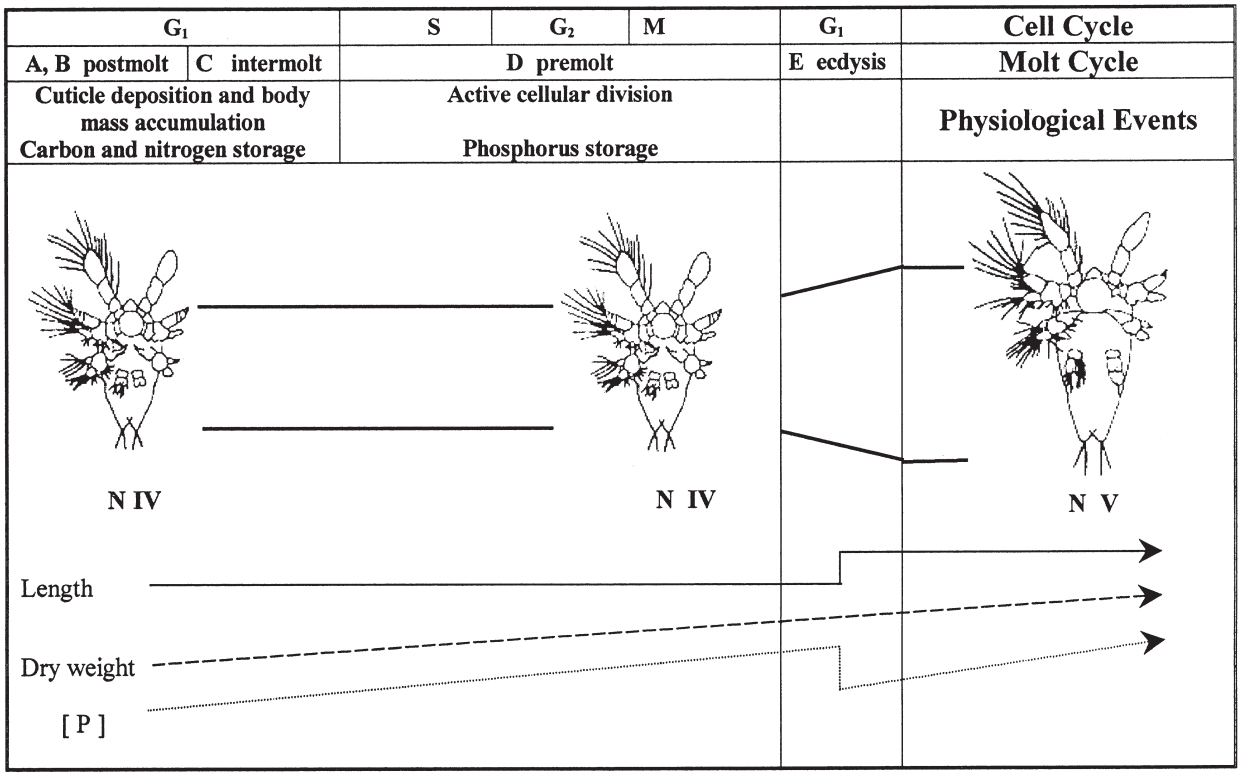

Fig. 6. Scheme of the relationship between the molt cycle of a crustacean copepod (nauplii stage IV and V), the cell cycle and some physiological events. The cell cycle is represented by a gap period (G_1), a DNA synthesis period (S) and a second gap period (G_2) before mitosis (M). Stages of the molt cycle included post-molt (A, B), inter-molt (C), pre-molt (D) and a brief period of ecdysis (E). Scheme modified from Freeman (Freeman, 1993). Arrows represent length (solid), dry weight (discontinuous) and phosphorus content (dotted) between two consecutive developmental stages of a copepod.

(Carrillo *et al.*, 1996a; Villar-Argaiz, 1999; Villar-Argaiz *et al.*, 2001). As food becomes depleted soon after the thaw, individuals, having reached copepodite stages with less somatic requirements, will grow slowly and will complete their development either the same year or, after a diapause period, during the following year. As argued by Sterner and Schulz (Sterner and Schulz, 1998), the trade-off for these organisms is between rapid growth and susceptibility to poor-quality food. Our results suggest that nauplii should be differently affected by food limitation (quality) than copepodites or adults, as a consequence of their different stoichiometric composition.

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