Limnol. Oceanogr., 50(6), 2005, 2043–2056

Reproduction as one of the main causes of temporal variability in the elemental composition of zooplankton

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Abstract

With the aim to determine the contribution of development and reproduction to the variability in the elemental composition of zooplankton, we measured the carbon (C), hydrogen (H), nitrogen (N), and phosphorus (P) elemental composition of the planktonic crustacean assemblage in an alpine lake for a whole seasonal cycle. The species included three distinct living modes: a cladoceran, *Daphnia pulicaria*; a cyclopoid copepod, *Cyclops abyssorum*; and a calanoid copepod, *Diaptomus cyaneus.* For the three species, reproduction was the main cause of elemental variability. Adult females of the three species lost from 32% to 48% of their initial absolute C and H content during reproduction, which corresponded to a similar decrease in their lipid and carbohydrate content. The N content did not change in any of the three species, nor did the protein and chitin content. *Daphnia* and *Diaptomus* lost 35% and 56%, respectively, of their initial absolute P content during reproduction, whereas the P content of *Cyclops* did not change. The three species stored energy compounds under unfavorable conditions for later use in offspring production, but only *Diaptomus* and *Daphnia* mobilized stored P. Corresponding stoichiometric changes with reproduction included a decrease in C : N ratio for the three species; an increase in N : P ratio for *Daphnia* adult females and adults of *Diaptomus*; and a C : P ratio increase in *Diaptomus* females and decline in *Cyclops* females. Differences in C : P ratio changes corresponded with differences in allocation to their respective reproductive tissues. *Diaptomus* males and *Daphnia* females did not change their C : P ratio with reproduction.

One of the fundamental challenges of modern ecology is to understand the influence of the ecological context on the evolution of species traits, and vice versa, to elucidate how certain species traits affect ecological dynamics (Partridge and Harvey 1988). A difficulty that arises when comparing the life histories of species is how to select comparable species traits. Ecological stoichiometry overcomes such difficulty by comparing the elemental main body constituents (Sterner and Elser 2002), usually focusing on carbon (C), nitrogen (N), and phosphorus (P). This approach has been used for identifying living constraints in organisms from freshwater, marine, and terrestrial environments (Elser et al. 2000*b*). Crustacean zooplankton is the animal group most frequently studied, and the results obtained have shown the relevance of using stoichiometry within the ecological context. Pioneering experimental (Hessen 1990) and field studies (Andersen and Hessen 1991; Hessen and Lyche 1991) showed low variability in the elemental composition of zooplankton. Other studies have shown that the homeostatic nature of the stoichiometric composition of crustacean zooplankton (e.g., N : P ratio) has an influence on the recycling ratios between distinct elements, and in consequence, on the entire dynamics of plankton (Sterner and Hessen 1994). More recently, some studies have found that certain zooplankton species exhibit a relevant temporal elemental variation (Main et al. 1997; DeMott et al. 1998; Villar-Argaiz et al. 2002; Boersma and Kreutzer 2002; DeMott et al. 2004). For these species, the direct application of nutrient recycling models (Olsen et al. 1986) based on the assumption of strict elemental composition is inaccurate. Therefore, there is a need for elucidating why the stoichiometric composition is more (or less) strict for distinct species. It has been found that differences in ribonucleic acid (RNA) content are related to differences in growth rates (Main et al. 1997) and thus are responsible for part of the N : P variation. Moreover, changes in food quality (C : P ratio) have been shown to significantly affect the P balance of *Daphnia* (DeMott et al. 1998, 2001; Boersma and Kreutzer 2002; DeMott et al. 2004). Surprisingly, a potential cause of variability in stoichiometry, reproduction, has not yet been explored. Two main reproduction modes have been identified within zooplankton organisms (Gilbert and Williamson 1983). Some species store the energy required for reproduction during their development and use it at a certain point of their life cycle with little complement of feeding resources during reproduction time. Having reached the mature stage, these species are able to delay egg production, and they therefore decouple reproduction from immediate food supply or quality. This strategy is called the food-independent reproduction mode. Other species are not able to stop egg production once they are mature. The egg production is totally dependent on simultaneous food supply, a reproductive

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Acknowledgments

We are grateful to Ll. Camarero, B. Claramunt, A. Miró, P. Renom, T. Buchaca, and G. Cots for assistance in the field, R. Franco for help in the laboratory, and Ll. Camarero for advice on the P analysis. The total amino acid analysis and the elemental C, H, N, and S analyses were performed in the Serveis Científico Tècnics of the University of Barcelona. We thank T. Buchaca, S. Pla, and E. Martí and two anonymous referees for helpful comments on the manuscript.

This research was supported by the European Commission projects MOLAR (ENV4-CT95-0007) and EUROLIMPACS (GOCE-CT-2003-505540). M. V. was partially supported by a Marie Curie post-doctoral grant (MEIF-CT-2005-010554).

strategy that is known as the food-dependent reproduction mode. It has been suggested that these reproduction modes are adaptations to habitats with different levels of food availability (Hirche and Kosobokova 2003), the food-dependent reproduction mode being more suitable for productive systems (Tessier et al. 1983).

The physiological mechanism used by species with the food-independent reproduction mode is storage of high quantities of lipid substances that are later used for reproduction (Vanderploeg et al. 1992; Hagen and Schnack-Schiel 1996). Therefore, as reserve substances are enriched in a few elemental compounds, we expect organisms with food-independent reproduction mode to undergo relevant changes in elemental composition throughout their life cycle. Although some studies have indicated changes in the C : N ratio during overwintering (Tande 1982; Andersen 1997), detailed studies of elemental composition variability caused by reproduction modes are lacking.

In this study we compared the elemental and biochemical changes of three crustacean species of zooplankton in an alpine lake throughout their life cycles by examining the influence of development and reproduction on the elemental composition of each species. The species are representative of the main freshwater crustacean groups with contrasting reproductive modes: the cladoceran *Daphnia pulicaria* Forbes, the cyclopoid copepod *Cyclops abyssorum* Sars, and the calanoid copepod *Diaptomus cyaneus* Gurney. *Cyclops* takes an entire year to complete its life cycle, whereas that of *Diaptomus* lasts a short period during the ice-free season (Ventura et al. 2000). *Daphnia* is also present throughout the year and has a single cohort, a feature also observed in other European alpine lakes (Gliwicz et al. 2001) and unlike lowland lakes, where *Daphnia* usually has several cohorts per year. The three contrasting living and reproductive modes provide an excellent opportunity for undertaking field studies of the sources of stoichiometric variability. To elucidate further the mechanisms behind the possible elemental changes, the seasonal variation in the major biochemical compounds (proteins, lipids, carbohydrates, and chitin) was also assessed in adult individuals of the three species.

Materials and methods

Study site—Lake Redon (formerly Redó) is an oligotrophic glacial cirque lake located at 2,240 m above sea level in the central Pyrenees (42°38′N, 0°46′E). It is relatively large (0.24 km^2) and deep (73 m) for an alpine lake. It is dimictic, covered by ice for one-half of the year (usually from mid-December until June). Details of its physical structure and general water chemistry can be found in Ventura et al. (2000), of its productivity aspects in Camarero et al. (1999), and of its plankton composition and abundance in Felip et al. (1999).

Sampling—The lake was surveyed on 14 occasions from December 1998, just after the lake became ice-covered, until December 1999, when it was ice-covered again. Samples were collected at the deepest part of the lake, either by drilling the ice cover or from a platform anchored at the same location throughout the summer. The temperature in the water column was measured at 1-m-depth intervals using a WTW TA-197 temperature and oxygen meter. Seston was sampled for particulate carbon (PC), particulate nitrogen (PN), and particulate phosphorus (PP) analysis using a Ruttner bottle at 2 m, 10 m, 20 m, 25 m, and 60 m. The samples were stored in polypropylene bottles and transported cold and dark to the laboratory. Zooplankton samples were collected by vertical net hauls from 65 m to the surface with a 200-μm net. Two replicate samples were collected: one sample that was immediately preserved in formaldehyde for individual counts and one sample for elemental and biochemical analyses. Individuals from the latter sample were kept alive and transported cold (4°C) to the laboratory where they were frozen (−20°C) a few hours after collection.

Population evaluation—Individuals were counted using an Olympus inverted microscope. At least 100 individuals of *Daphnia* and 50 adults of the two copepod species were measured per sample for body length (*Daphnia*: from the upper edge of the head to the base of tail spine; copepods: from the anterior end of the cephalothorax to the posterior end of the furca). Copepods were classified at each stage. *Daphnia* females were divided into juveniles and adults using the criterion proposed by Edmondson and Litt (1982), which is based on the relative length of the abdominal process.

Elemental analysis—Seston samples were analyzed by collecting the particles on precombusted Whatman GF/F filters (the samples were prefiltered through a Nytal mesh to remove particles >200 μm). Frozen zooplankton samples were thawed, and from 10 to several hundred individuals of each species or developmental phase were quickly sorted under a dissecting microscope and placed in a preweighed tin (for C, N, H [hydrogen], and S [sulfur] analyses) or Teflon capsules (for P, protein, chitin, carbohydrate, and lipid analyses). For all sampling events, at least three composed sample replicates (composed of several individuals) were analyzed for each species and for each developmental phase, except for a few cases when the material available did not suffice. Individuals were kept cold (<4°C) during the sorting process, which was undertaken within a few hours after thawing. Eggs from egg-bearing females were carefully removed before analysis. Dry weight (dry wt) was determined for all samples after drying at 60°C for 24 h using a microbalance (Ohaus Analytical Plus, AP250D-0). Between 300 μg and 500 μg of dry wt was required for C, N, H, and P analyses, 600 μg and 800 μg dry wt for the biochemical analysis, and 3 mg and 5 mg dry wt for the S and ash content analyses. For all the analyses, blank capsules were used during the whole sorting and drying process. The ash content was determined after combustion at 450°C for 5 h. Elemental C, N, H, and S were analyzed on EA 1108 CHNS-O Carlo Erba Instruments after drying and packing of the samples into tin capsules with vanadium pentaoxide as a catalyzer to ensure complete combustion. Elemental P was determined by acid-persulphate digestion (Grasshoff 1983), followed by phosphate analysis using the malachite green method (Camarero 1994). All elemental ratios reported in the manuscript were expressed in atoms.

Biochemical analyses—Protein content was measured as the total amino acid composition. This method was chosen in preference over more traditional total protein methods (Zamer et al. 1989) because *N*-acetylglucosamine, the molecular constituent of chitin, is an amino-sugar (Strayer 1988), appearing in the aminogram as glucosamine after hydrolysis. Therefore, total amino acids and chitin could be analyzed simultaneously. Samples for total amino acid analysis were vacuum-sealed and hydrolyzed with 6 mol L^{-1} HCL at 116°C for 24 h. An internal norleucine standard was included in every sample before hydrolysis to maximize reproducibility. The analysis was performed on a Biochrom20 (Amersham-Pharmacia) ion-exchange amino acid auto analyzer, following the ninhydrin method of Spackman et al. (1958). A standard solution of 20 amino acids and glucosamine was run for every 10 samples. Tryptophan was not considered in this study because it degrades under acid hydrolysis. Total carbohydrates were analyzed following the phenol-sulfuric acid method, as described by Meyer and Walther (1988). Total lipids were quantified gravimetrically. Weighed dry samples were placed in a dichlormethane : methanol (2 : 1, v/v) solution (Folch et al. 1957) and sonicated for 30 min in an ultrasonic bath. Then the nonlipid constituents were collected in preweighed GF/F Whatman filters, which were dried and reweighed.

Results

Seston concentration—The seston concentration was low throughout the whole sampling period, which is typical of ultra-oligotrophic conditions (Fig. 1). The average water column concentration was relatively high in December 1998 when the ice cover was only composed of black ice, allowing the formation of a chlorophyll maximum below the ice, with PC reaching 172 μg C L^{-1}. This was comparable with the values measured during the following ice-free period. Soon after sampling in December 1998, snow covered the black ice, light penetration declined to very low levels, and phytoplankton growth below the ice stopped. Then PC concentration decreased progressively until March 1999, remaining at low values during the rest of the ice-covered period. After the melting of the ice cover, the PC concentration increased again, reaching the highest values at the end of September 1999. From October to December 1999, PC declined to winter concentrations again (Fig. 1). The quality of seston in terms of C : P and N : P ratios was higher during the ice-covered than during the ice-free period (C : P of 221 ± 57 and N : P of 27 ± 9 for the ice-covered period and C : P of 301 ± 69 and N : P of 35 ± 6 for the ice-free period; Fig. 1). The highest C : P and N : P ratios were measured when the lake was stratified, which was from July to October 1999.

Life cycles—*Cyclops abyssorum*: At the beginning of the study in December, adults of *Cyclops* were found in the lake (Fig. 2). Initially, adult males and adult females were present in similar proportions, but adult males decreased suddenly in March and were not found from mid-April until October, when they reappeared. Females started to reproduce below the ice cover when the males started to disappear in March. However, the greatest number of reproductive females was found when the ice cover melted. Although the proportion of females with eggs was low, almost all adult females already had ripened ovaries on the sampling date previous to the ice cover melting. Nauplii first appeared after the ice cover melted and after the overwintering females started laying their eggs. Copepodites of the different instars peaked in almost each consecutive month: the first two stages were dominant at the end of July; the third stage in August; the fourth in September; and the fifth was dominant in October and November. Adult males appeared again in October, but it was not until December that they, together with fifth-instar female copepodites, again dominated the cyclopoid abundance.

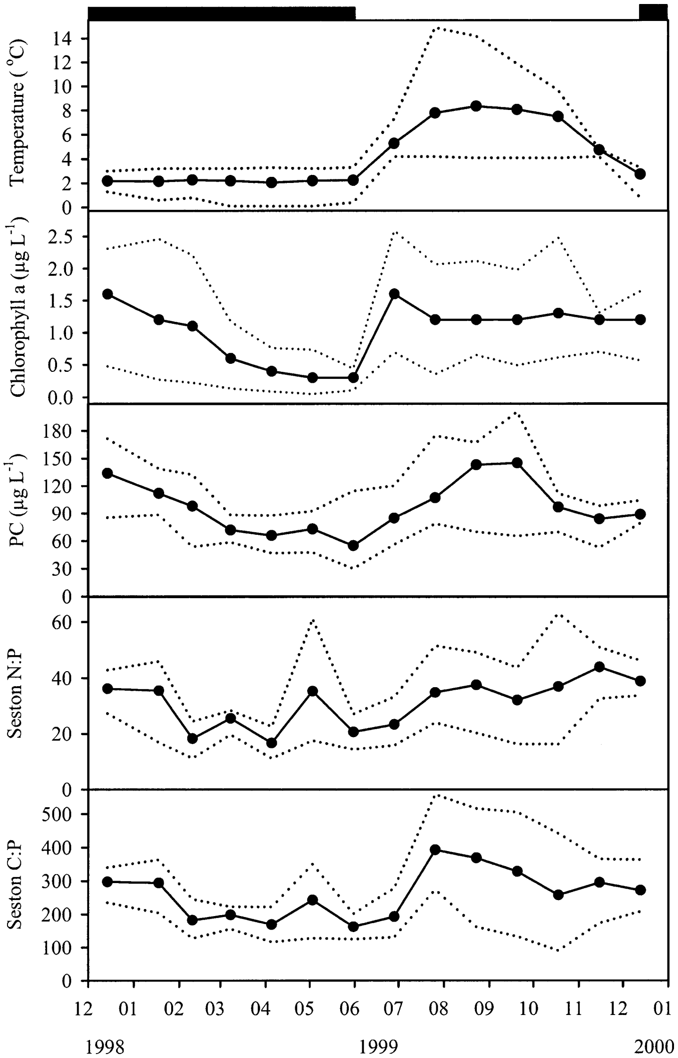

Fig. 1. Seasonal changes in water temperature, particulate carbon, seston nitrogen to phosphorus ratio (N : P), and seston carbon to phosphorus ratio (C : P) in Lake Redon. Both ratios are expressed in atoms. Filled circles are the water column average values; dotted lines follow maximum and minimum values recorded on each sampling occasion.

Diaptomus cyaneus: Nauplii of this copepod appeared immediately after the ice melted at the end of June. No stages

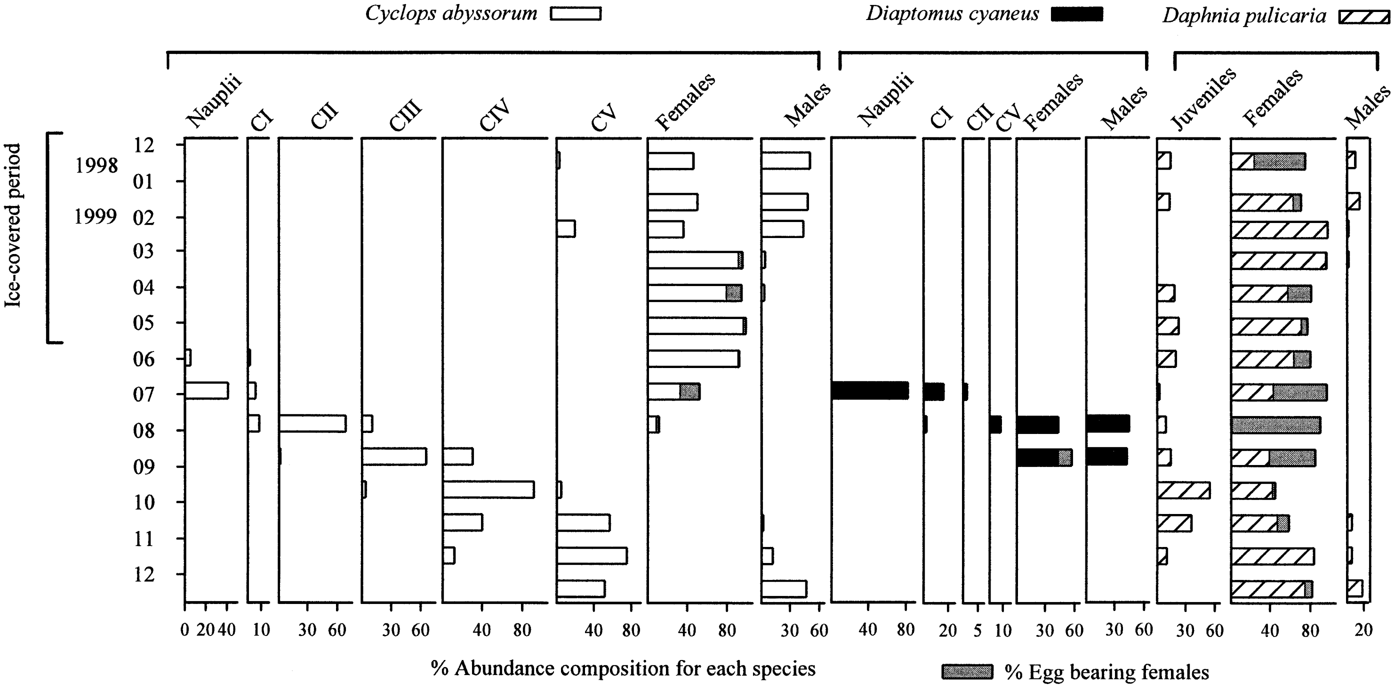

Fig. 2. Seasonal changes in the three crustacean zooplankton species of Lake Redon.

were found during the ice-covered period, indicating that nauplii emerged from overwintering eggs. The development time of copepodites was very short: whereas at the end of June the population was mainly composed of nauplii and a few first-stage copepodites, by the end of July, most of the diaptomids were adults and copepodites of the fifth instar (Fig. 2). Adults were divided evenly between males and females during their 2-month presence. Females had ripened ovaries already in the July sample, although no egg-bearing females were found until the end of August. Then, almost all adult females were carrying eggs or had many spermatophores attached, which is indicative of intense reproduction. From September onward, no more diaptomids were found in the surveys; thus, this species completed its life cycle in 3 months.

Daphnia pulicaria: Males appeared sporadically from December 1998 until March 1999 below the ice cover, and again from October 1999 until the end of the study in December 1999, always in very low numbers (Fig. 2). Ephippial females were occasionally found in male presence. Adult females produced offspring during most of the year, but reproducing females formed a majority of the population only in December 1998 and from June to August 1999, matching the highest seston concentrations. The population size distribution (Fig. 3) showed that the reproductive pulse of December 1998 was less relevant than the one during the ice-free period. It also revealed that many adult females survived the ice-covered period and laid eggs during the following summer. After reproducing, most of the old adult females died; in fact, almost none of them survived after August 1999 (Fig. 3). During the ice-free period, adult females from the overwintering cohort were easily distinguished from the 1999 newborn females; the former were initially larger, had a transparent carapace, and had few or no lipid droplets, whereas the young females had melanin in the dorsal part of the carapace and lipid droplets accumulating in their body. The newborn females of summer 1999 rarely bore eggs during autumn, but waited until the following summer to lay their eggs.

Elemental and biochemical composition—The three species had similar C and H contents, as expressed in percentage of dry wt (Table 1), with no significant differences between the three species (Table 2). The two copepods had a significantly higher N content ($p < 0.001$) than the cladoceran, whereas within the copepods, the calanoid was significantly richer than the cyclopoid ($p < 0.001$). The P content was similar in the two copepods (Table 1), but they both had almost one-half the P of the cladoceran in terms of percentage of dry wt (Table 2; $p < 0.001$). We had to use a great many individuals per S analysis because of its low concentration, and, in consequence, the number of samples analyzed was lower. *Diaptomus* had a higher S percentage than the other two species, but the differences among the three species were not statistically significant (Table 2). In summary, the two copepods had greater similarity in their atomic ratios C : N : P : S to each other than to the cladoceran (*Cyclops* 390 : 48 : 1 : 1; *Diaptomus* 346 : 53 : 1 : 0.7, *Daphnia* 238 : 23 : 1 : 0.4).

The ash content of the three species was very low (Table 1). *Diaptomus* had the highest value, whereas *Cyclops* and *Daphnia* had a lower and similar content. The lipid fraction and proteins accounted for most of the biomass of the three species, whereas chitin and total carbohydrates were <5.5%. The calanoid copepod had the highest protein and chitin content, followed by *Cyclops* and *Daphnia*; all differences between them were statistically significant (Table 2). However,

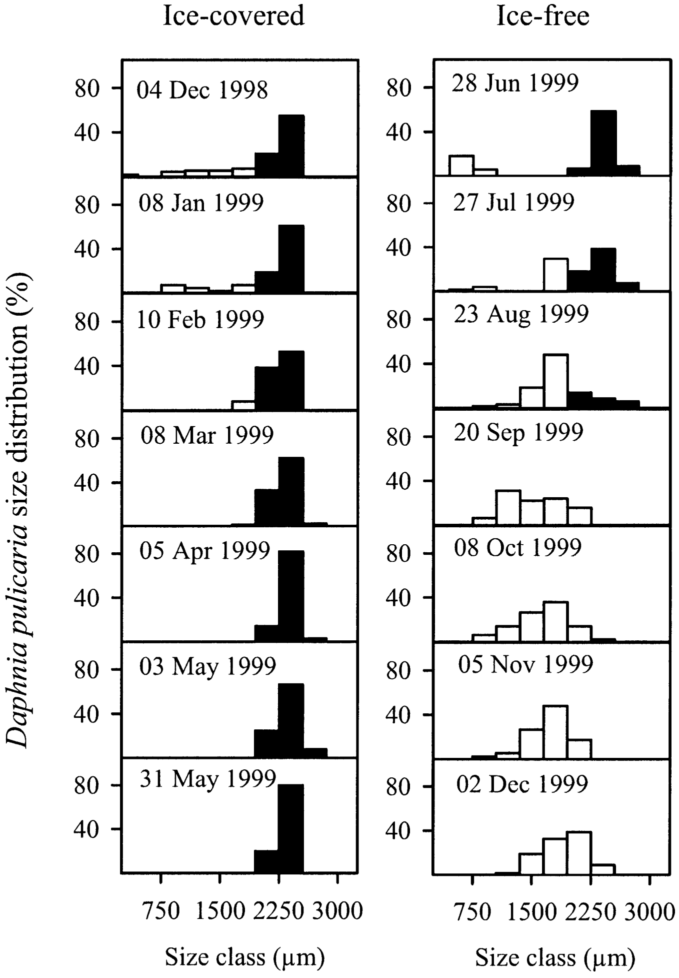

Fig. 3. Body size distribution in the *Daphnia pulicaria* population of Lake Redon from 14 Dec 98 to 12 Dec 99. Black bars correspond to overwintering adult females and white bars to juveniles and females born during summer 1999.

Diaptomus had significantly lower lipid content than the other two species, lipid substances accounting for, on average, >50% of dry wt (Table 1). Total carbohydrates followed an inverse pattern to proteins and chitin, *Daphnia* having the highest content followed by *Cyclops* and *Diaptomus.*

Intraspecific variability in the elemental composition—Variation in the elemental composition was low during ontogenetic development and mainly restricted to the last developmental phase (i.e., adults) of the three species (Fig. 4). There was a significant linear relationship between absolute content of each of the considered elements and the individual's dry wt for all the species, although the proportion of variance explained by the relationship was different for each element. For C and H, the variance explained was high, between 97% and 99% (Fig. 4), whereas for P and N, the variance explained by the regression was lower, between 63% and 89%, mainly because of greater variability in the adult females of *Cyclops* and *Daphnia.* When excluding adult females in the linear regression of *Cyclops* and *Daphnia* (Fig. 4, dashed lines), the variance explained increased from 2.6% to 21.4% depending on species and element, P still being the element with the highest variation. Despite the lower degrees of freedom for the case of *Diaptomus,* the results for this species are consistent with those of the other two species, the adult stage showing the greatest variation in elemental composition.

Elemental and biochemical composition of reproducing versus nonreproducing individuals—The changes in dry wt of overwintering adult females of both *Cyclops* and *Daphnia* followed a similar pattern: during most of the winter period, the weight was constant. In March for *Cyclops* and in April for *Daphnia,* the weight began to decrease, a tendency that lasted until the overwintering adult females disappeared (Fig. 5). During this period, *Cyclops* and *Daphnia* lost 34% and 52%, respectively, of their dry wt. This decrease in dry

Table 1. Average elemental and biochemical composition of the three species of zooplankton from Lake Redon. Data are expressed as mean values ± SE of the percentages; in brackets, the number of composite samples analyzed. SE for elemental ratios indicates seasonal and development phase variability, and elemental and biochemical SE includes replicate variability. Percentages are calculated in relation to dry weight, elemental ratios are in atoms. nc, not calculated because of availability of only one replicate.

	Diaptomus cyaneus		*Cyclops abyssorum*		*Daphnia pulicaria*	
C (%)	56.9±1.2	(16)	58.8±1.0	(42)	58.7±0.9	(54)
N (%)	9.9±0.5	(16)	8.3±0.2	(42)	6.4±0.2	(55)
H (%)	7.8±0.1	(16)	7.9±0.1	(42)	8.1±0.1	(45)
P (%)	0.47±0.04	(19)	0.46±0.03	(48)	0.71±0.02	(52)
S (%)	0.47±0.06	(4)	0.30±0.05	(8)	0.26±0.04	(14)
Ash (%)	3.8±nc	(1)	2.4±0.1	(4)	2.5±0.2	(4)
C:N	7.0±0.6	(7)	8.4±0.5	(18)	11.2±0.7	(19)
C:P	345.6±34.1	(7)	389.8±43.3	(18)	238.4±15.8	(18)
N:P	52.7±8.4	(7)	47.8±4.9	(18)	23.1±2.2	(19)
C:S	421.0±95.9	(4)	536.2±105.1	(3)	639.2±77.3	(8)
N:S	53.4±10	(4)	56.7±6.2	(3)	51.3±3.6	(8)
P:S	1.5±0.6	(4)	1.0±0.2	(3)	2.7±0.2	(8)
Protein (%)	58.7±4.8	(8)	43.6±1.6	(22)	27.0±1.1	(17)
Chitin (%)	5.4±0.4	(8)	3.9±0.2	(22)	2.2±0.1	(17)
Lipid (%)	35.5±5.5	(9)	51.6±2.9	(26)	53.5±2.6	(26)
Carbohydrate (%)	2.5±0.1	(12)	3.4±0.2	(36)	4.4±0.1	(23)

Table 2. One-way ANOVAs examining interspecies differences of *D. cyaneus* (*D.c.*), *C. abyssorum* (*C.a.*), and *D. pulicara* (*D.p.*) in terms of their elemental (C, H, N, P, S) and biochemical (lipids, protein, carbohydrate, and chitin) composition. Data were, when required, previously standarized with either logarithmic or square root transformation, and a test of homogeneity of variances was performed before ANOVA. df stands for degrees of freedom; MS, for mean squares; *F* for the result of the *F*-test; and *p* for the probability of significance. Tukey HSD test was used to test the difference between the three species. Post hoc comparison: $<$ or $>$ significantly higher or lower at $p < 0.01$ respectively; $\ll$ or $\gg$, significantly higher or lower at $p < 0.001$; $=$, not significantly different.

	Source	df	MS	*F*	*p*	Post hoc comparison
C (%)	Interspecies	2	25.9	0.7	0.511	
	Error	100	38.3			
	Total	102				
H (%)	Interspecies	2	1.1	2.5	0.086	
	Error	91	0.4			
	Total	93				
N (%)	Interspecies	2	0.3	34.0	0.000	*D.c.* $\gg$ *C.a.* $\gg$ *D.p*
	Error	101	0.0			
	Total	103				
P (%)	Interspecies	2	0.5	33.1	0.000	*D.c.* $=$ *C.a.* $\ll$ *D.p.*
	Error	116	0.0			
	Total	118				
S (%)	Interspecies	2	0.1	3.7	0.039	
	Error	26	0.0			
	Total	28				
Lipid (%)	Interspecies	2	8.6	7.0	0.002	*D.c.* $\ll$ *C.a.* $=$ *D.p.*
	Error	59	1.2			
	Total	61				
Carbohydrate (%)	Interspecies	2	0.3	21.3	0.000	*D.c.* $<$ *C.a.* $\ll$ *D.p.*
	Error	64	0.0			
	Total	66				
Protein (%)	Interspecies	2	0.4	60.5	0.000	*D.c.* $\gg$ *C.a.* $\gg$ *D.p.*
	Error	44	0.0			
	Total	46				
Chitin (%)	Interspecies	2	0.5	68.5	0.000	*D.c.* $\gg$ *C.a.* $\gg$ *D.p.*
	Error	43	0.0			
	Total	45				

wt was mostly caused by a decline in H and C, and it coincided with the reproductive periods of the two species. There were significant differences in the individual C and H content between adult females in the reproducing period and those in the rest of the winter for the two species (*U*-test, $p \leq 0.001$). The absolute content of N was rather constant during the complete seasonal cycle for the two species (there were no differences between the reproductive period and the nonreproductive period, $p = 0.934$ and $p = 0.102$ for *Cyclops* and *Daphnia*, respectively). However, the two species varied in relation to P content. Whereas the P content of *Cyclops* was constant throughout its life cycle (no differences between the reproductive period and the nonreproductive period, $p = 0.664$), for *Daphnia*, the P content increased until March and then steadily decreased until the end of August, when up to 58% of the March content was lost. *Daphnia* had a significantly lower P content during the reproduction period than before reproduction ($p = 0.003$). Of the *Daphnia* females born during the summer, the absolute content of all the main elements changed between August and December 1999. During the last 2 months, however, there was a steeper increase in C and H (Fig. 5) because of a relatively higher accumulation of lipids (data not shown). The stoichiometric composition of these females when they reached maturity (December 1999) was very similar to those of the females of the previous year (December 1998; 262:17:1 and 240:17.5:1 for C:N:P of the females in December 1999 and December 1998, respectively).

For *Diaptomus*, there were also substantial differences in the composition of both adult females and adult males before and after reproduction (Table 3). The dry wt of adult females and adult males decreased by 35% because of a reduction of C, H, and P (Fig. 6), but N content and total length did not change significantly from 1 month to the other.

The changes in the absolute elemental content resulted in differences in the relative proportions of elements between the nonreproductive and reproductive individuals (Fig. 6). For the adult females of the three species and the adult males of *Diaptomus*, the percentage of N increased significantly (*U*-test, $p \leq 0.05$), whereas the percentage of C and H significantly decreased ($p \leq 0.05$). In contrast, both *Daphnia* and *Cyclops* reproductive females had a higher relative P content ($p \leq 0.05$), whereas *Diaptomus* reproductive females had a lower proportion of P ($p \leq 0.05$).The relative P content of *Diaptomus* adult males changed only insignificantly ($p = 0.275$). As a consequence, the stoichiometric changes were not the same for the distinct ratios and species (Fig. 6). The C:N ratio of the three species significantly decreased ($p \leq 0.05$). The N:P ratio of adult females of *Daphnia* and adult males and females of *Diaptomus* in-

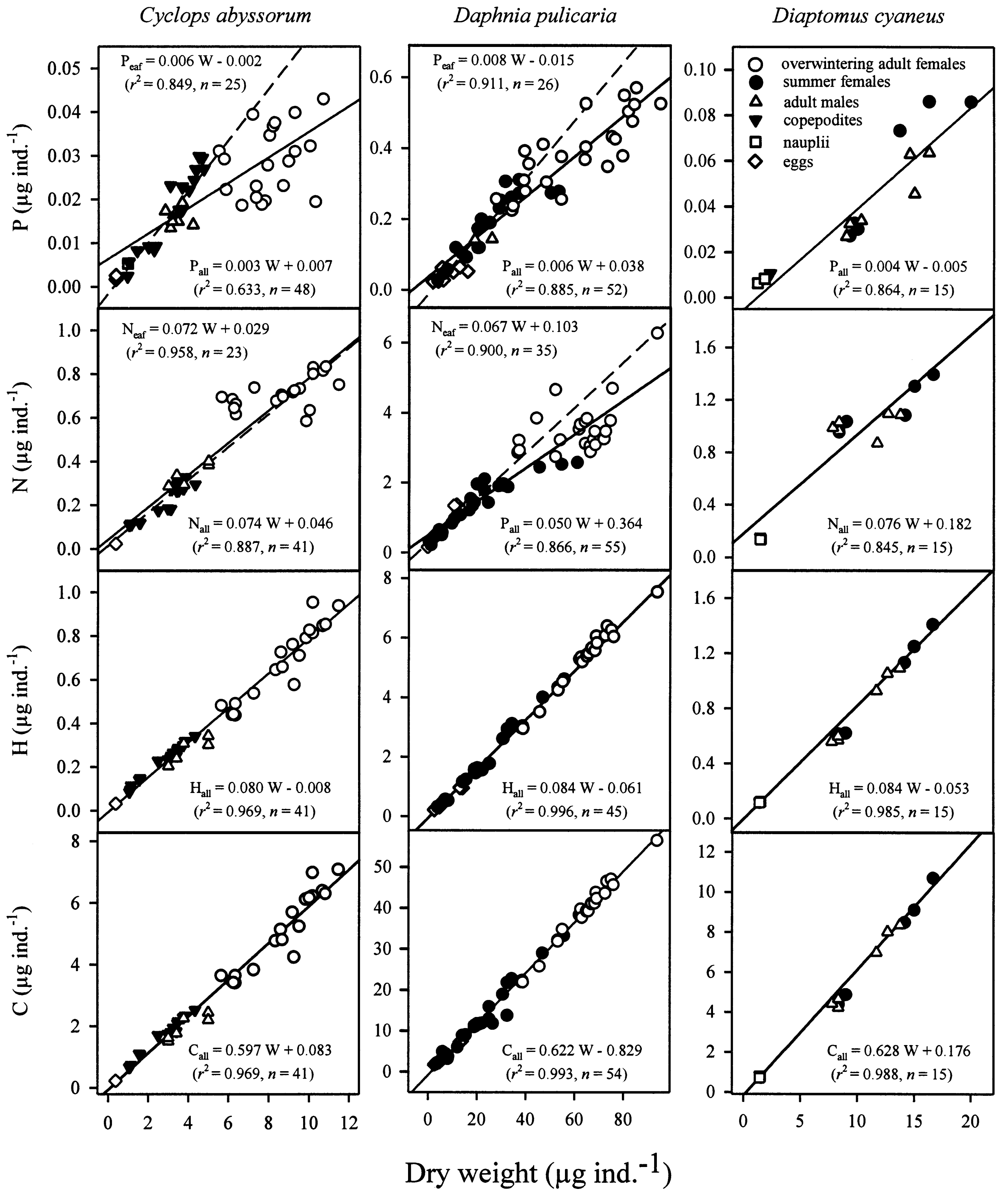

Fig. 4. Relationship between dry weight (W) and elemental composition for the three crustacean zooplankton species of Lake Redon. Solid lines are regression fits including all stages (all) and dashed lines are the fits excluding adult females (eaf).

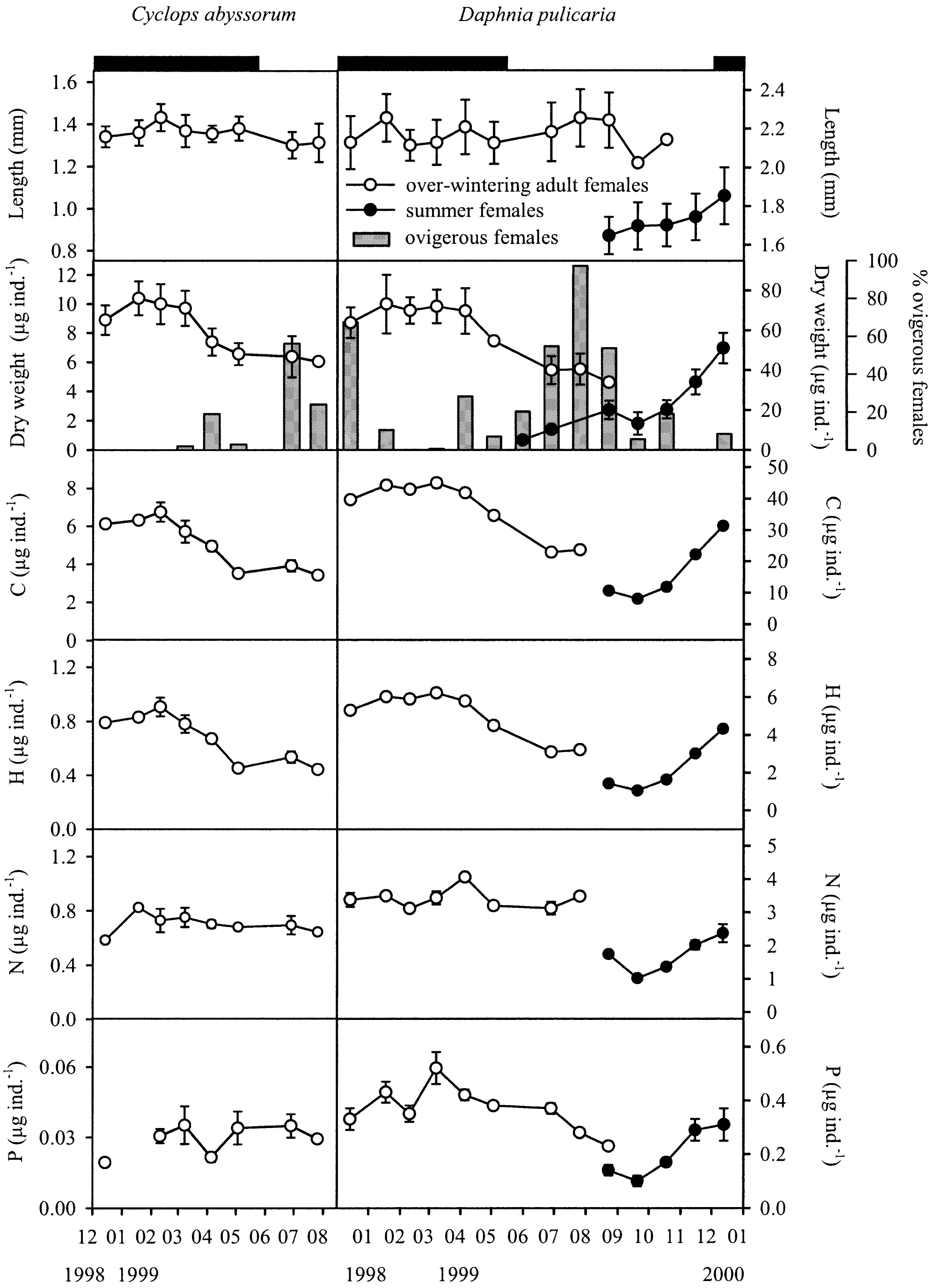

Fig. 5. Seasonal changes in *Cyclops abyssorum* and *Daphnia pulicaria* females as to size, dry weight, ovigerous percentage, and elemental composition. The black filled bar shows the ice-covered period. Circles are averages and error bars show SD.

creased ($p \leq 0.05$), whereas that of *Cyclops* did not change significantly ($p = 0.149$). Finally, *Diaptomus* and *Cyclops* reproductive females had, respectively, a higher and lower C : P ratio ($p \leq 0.05$), whereas that of *Diaptomus* adult males and *Daphnia* adult females remained unchanged.

Because the major elemental changes of the adults of the three species coincided with the onset of reproduction, we compared the main biochemical compounds before and after reproduction for *Cyclops* and *Daphnia* adult females and *Diaptomus* adults (Fig. 7). As expected, differences in adult elemental composition occurred simultaneously with changes in biochemical composition. The absolute lipid and carbohydrate contents of adult females of the three species and adult *Diaptomus* males declined significantly (*U*-tests, *Cyclops* $p = 0.013$, *Daphnia* $p = 0.003$, and *Diaptomus* $p = 0.014$, respectively, for lipids, and $p = 0.009$, $p = 0.003$,

Table 3. Changes in male and female *D. cyaneus* during their 2-month presence in Lake Redon. Values represent the average of composite sample replicates ± SE; in brackets, the number of composite samples analyzed.

	Diaptomus cyaneus adult females		*Diaptomus cyaneus* adult males	
	26 Jul. 1999	23 Aug. 1999	26 Jul. 1999	23 Aug. 1999
% reproductive females	0	23		
Maximum length (mm)	1.837±0.015 (28)	1.834±0.015 (32)	1.689±0.010 (27)	1.665±0.008 (24)
Dry weight	15.7±1.8 (13)	10.2±1.4 (14)	13.3±1.8 (13)	8.7±0.8 (14)
C (μg ind.$^{-1}$)	9.68±0.36 (3)	5.45±0.09 (3)	8.14±0.25 (3)	4.74±0.29 (3)
H (μg ind.$^{-1}$)	1.30±0.04 (3)	0.73±0.03 (3)	1.07±0.03 (3)	0.62±0.02 (3)
N (μg ind.$^{-1}$)	1.30±0.08 (3)	1.18±0.02 (3)	1.07±0.08 (3)	1.07±0.05 (3)
P (μg ind.$^{-1}$)	0.08±0.009 (3)	0.03±0.003 (3)	0.05±0.009 (3)	0.03±0.002 (3)

and $p = 0.025$, respectively, for carbohydrates). This decrease was commensurate with a reduction of 58.0%, 62.7%, and 74.5%, respectively, for lipids, whereas the total carbohydrate loss was 55.6%, 55.0%, and 36.8%. Unlike these two major compounds involved in energy storage, the two main structural compounds, protein and chitin, did not significantly change per individual for any of the three species.

Discussion

Life cycles—The three species studied had contrasting reproduction strategies, although they were all iteroparous annual species (Hairston and Bohonak 1998). The production of a single cohort was a common feature, but they differed in their diapausing strategy. *Daphnia* showed both diapausing and nondiapausing strategies. Coinciding with the end of the autumn overturn and the start of the ice cover, adult males appeared, and ephippia were subsequently produced. However, most adult females survived below the ice cover, waiting to reproduce until the next ice-free period. A similar behavior has been described for the same species in an alpine lake in the Tatra mountains (Gliwicz et al. 2001), with the difference that males were always absent in the Tatras lake. *Cyclops* adults also survived below the ice cover, probably synchronizing its life cycle with the *Daphnia,* as the former prey on the latter (Gliwicz et al. 2001). However, unlike in other northern European lakes (Nilssen and Elgmork 1977), the *Cyclops* population of Lake Redon has up to now not been observed to overwinter as copepodites in the sediments. *Diaptomus* was the only strict diapausing species, completing its life cycle within a short period, which contrasts with other calanoid copepods with a longer life cycle (Hairston and Bohonak 1998). In Lake Redon, *Diaptomus* emergence coincides with the spring production maxima (Ventura et al. 2000), and during their presence in the plankton of the lake, they completely dominate the zooplankton biomass.

Interspecific differences—The elemental and biochemical composition of the three crustacean species described in this study are generally consistent with the compositions found by other authors researching crustacean species (Båmstedt 1986; Elser et al. 2000*b*). C values were among the highest reported and were similar to those characteristic of marine copepods living in cold-oligotrophic areas (Mayzaud and Martin 1975). The high C content can be explained by the high lipid content of the three species, because lipids are C-rich compounds (e.g., 77% of triacylglycerol weight is C). H was within the range described for marine copepods (Omori 1969) and freshwater cladocerans (Badouin and Ravera 1972). The P content found in *Daphnia* was consistent with the value reported by DeMott (1998), 0.81% in weight, which confirms that, within the genus *Daphnia, D. pulicaria* is the species with the lowest P content. This feature may facilitate the survival of *Daphnia* under highly oligotrophic conditions (DeMott 1998), which are typical of large alpine lakes such as Lake Redon. A low P content is also consistent with the slow growth of *Daphnia* observed in Lake Redon, which would require less RNA and therefore lower P content according to the growth rate hypothesis (Main et al. 1997). In contrast, in other extreme habitats such as Arctic lakes, *Daphnia* of the same group have been found to have a higher P content associated with a higher growth rate, which has been attributed to an adaptation of the short growing season (Elser et al. 2000*a*).

The differences in elemental composition between the three species can mainly be ascribed to their N and P content. The two copepods had, on average, one-half the P of the cladoceran, N being 2% and 4% higher. To check the generality of these differences, we compared our results with those for 21 cladoceran species and 11 copepod species reported by Elser et al. (2000*b*). We found that copepods have a significantly higher N and a lower P content than cladocerans. N is 9.9% ± 0.4% and 8.6% ± 0.2% for copepods and cladocerans, respectively, and 0.8% ± 0.1% and 1.2% ± 0.1% for P (*U*-test, $p = 0.006$, for the two elements). Therefore, the small difference in N between copepods and cladocerans (1.3%) seems to be constitutional, as is the case of P (Hessen and Lyche 1991; Elser et al. 2000*b*).

Almost all elements of organisms consist of molecules that can be grouped into a few broad types (Strayer 1988). Among them, the four biochemical groups measured in this study are known to be the most abundant in zooplankton (Elser et al. 1996). These can be roughly separated into structural (protein and chitin) and energy compounds (lipids and carbohydrates) (Sterner and Elser 2002). Proteins are mainly found in the muscular tissue; chitin is found in the exoskeleton; lipids (mainly triacylglycerol) are found in energy reserves, although some lipids such as phospholipids are mainly structural; and carbohydrates are involved in energy resources for immediate use rather than storage. The elemental composition of these four biochemical compounds

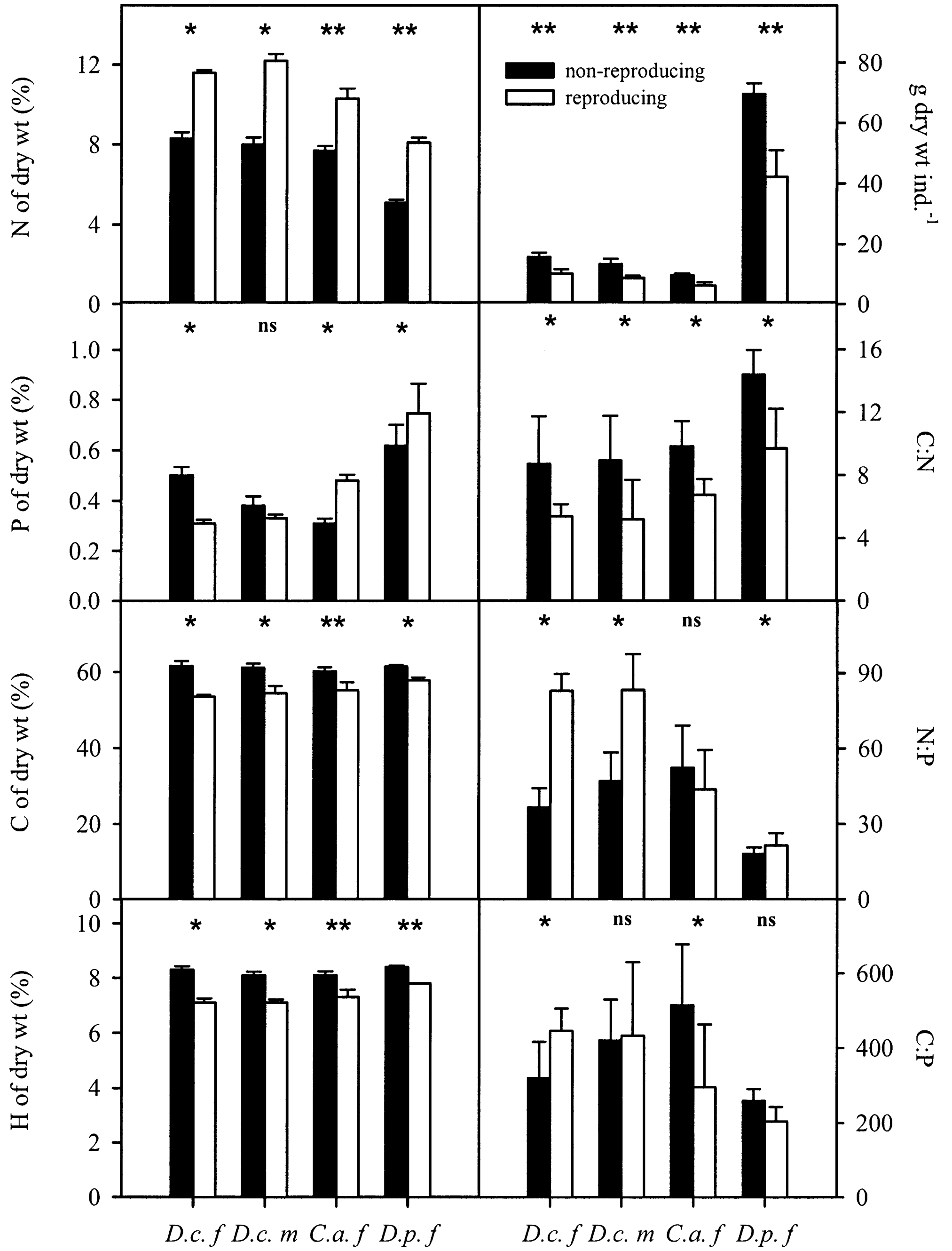

Fig. 6. Dry weight, elemental content, and atomic ratios (by atoms) before and after reproduction for adult females of *Diaptomus cyaneus* (*D.c.f.*), *Cyclops abyssorum (C.a.f.)*, and *Daphnia pulicaria* (*D.p.f.*) and adult males of *Diaptomus cyaneus* (*D.c.m.*) from Lake Redon.* and **Significant differences between before and during/after reproduction at $p < 0.05$ and $p < 0.005$, according to a *U*-test (Legendre and Legendre 1998). ns, not significant.

consist mainly of C, H, O, and N (Sterner and Elser 2002) and are very constant in zooplankton (Ventura 2005). The elemental composition measured, and that calculated from the measured biochemical composition according to Ventura (2005), are compared in Fig. 8. The good fit shows the expected consistency between the biochemical and elemental composition. Given that the slope of the relationship is close to unity, it can reasonably be assumed that almost all the variability of C, H, N, and S is explained by the variability of the four main biochemical groups. In particular, in our case, we showed that the higher N content of copepods was primarily caused by higher protein content. Copepods have higher maximum swimming speeds than *Daphnia* (escape velocities) (Fryer 1957), which probably is related to a higher proportion of muscular body tissue, and with it, more proteins.

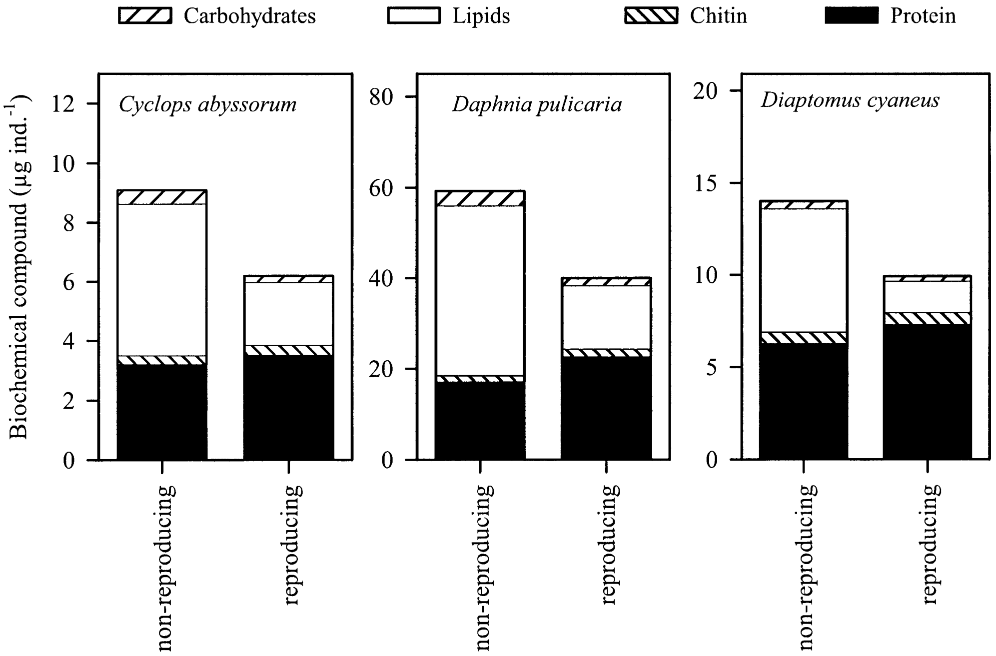

Fig. 7. Biochemical content before and after reproduction for adult females of the three crustacean zooplankton species in Lake Redon.

Reproduction as a cause of intraspecific elemental variability—The consequence of allocating resources to reproduction was a marked decrease in energy-related biochemical compounds. This decrease was comparable for the three species, and a similar pattern has been documented in other studies. Various authors (e.g., Ohman 1987; Smith 1990) found reductions in lipids of marine copepods with reproduction, and increased reproduction success with increasing amounts of lipids stored during previous productive periods. Vanderploeg et al. (1992) described a decrease in lipid content of a freshwater calanoid from 33–40% for overwintering copepods to 9% during the reproduction period, which is similar to the measurements for the three species included in this study. In cladocerans, Tessier et al. (1983) documented a close relationship between lipid accumulation during the intermolt cycle and egg production in an experimental study of two cladoceran species. They also found lipid content to be the main source of dry wt changes. In the *Daphnia* of Lake Redon, this intermolt accumulation was replaced by seasonal accumulation followed by a progressive decrease in lipid content caused by offspring production.

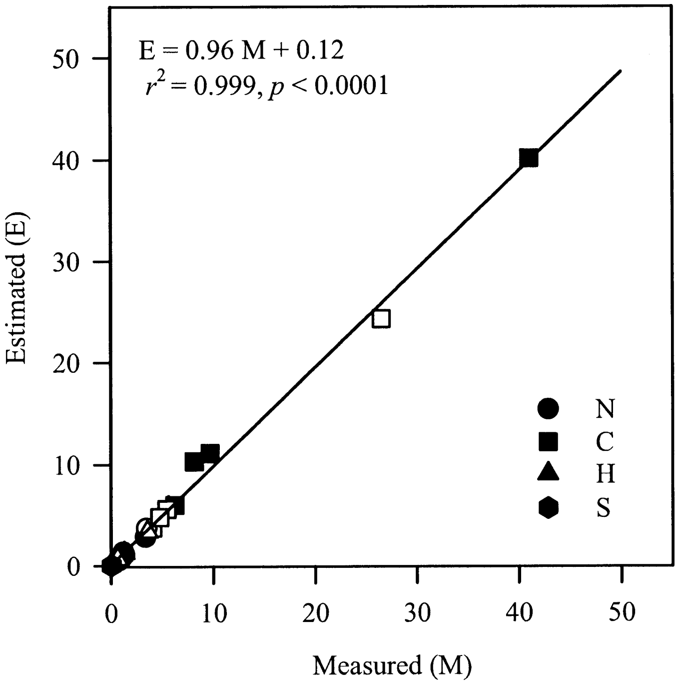

Fig. 8. Measured (M) versus biochemically calculated elemental composition (estimated; E) (Ventura 2005) for reproducing (open symbols) and non-reproducing (filled symbols) individuals of the means of the three crustacean zooplankton species in Lake Redon (*Cyclops abyssorum, Daphnia pulicaria,* and *Diaptomus cyaneus*).

The elemental consequences of the biochemical changes observed were related to a decrease in C and H. Therefore, C and H are less homeostatic compounds, whose changes are closely associated with dry wt. Unlike these two highly variable compounds, N was the only element that remained constant when expressed in absolute value because of its presence in the two structural components, protein and chitin. These two components are very stable, even under starvation (Elendt 1989). Differences among the three species existed in their P content. *Cyclops* did not change its absolute P content during reproduction (Fig. 6), whereas that of the other two species decreased. Because the P content of *Daphnia* is almost twice as high as that of *Cyclops,* even occasional predation by the latter on the former (Gliwicz 1994) will ensure an excellent P source without any need for storage over the winter. A certain synchronization of the

Table 4. Average stoichiometric composition (C:N:P by atoms) of adult females and their eggs for the three crustacean zooplankton species inhabiting Lake Redon.

	Adult Females	Eggs
Cyclops abyssorum	400:50:1	273:28:1
Diaptomus cyaneus	368:54:1	213:26:1
Daphnia pulicaria	229:19:1	259:38:1

life cycles of *Cyclops* and *Daphnia* has been established (Gliwicz et al. 2001). Whatever the dependence of *Cyclops* on *Daphnia* for P, our results provide field evidence that some species store P for later offspring production in addition to storage of energy compounds. These findings corroborate the experimental study of Urabe et al. (1997), who showed that certain *Daphnia* stored inorganic P directly.

As a result of the elemental changes with reproduction, the stoichiometric composition of the three species also changed. The C : N ratio fell because of the decline in carbon. In contrast, there were differences between the three species in the changes of the N : P and C : P ratios. Both *Daphnia* and *Diaptomus* increased their N : P ratios because of the reduction of P content, whereas the ratio remained constant for *Cyclop*s. Also for *Cyclops* females, the C : P ratio declined because of the decrease in C. The C : P content of *Daphnia* females and *Diaptomus* males did not change, whereas that of *Diaptomus* females increased. To investigate if there were differences in the somatic tissues and reproductive tissues, we compared a summary of the stoichiometric composition of adult females with the mean values of some egg measurements carried out during the study (Table 4). The copepod females of the two species had almost twice as high C : P and N : P ratios than those of their eggs because of a lower P content in somatic tissues, whereas the C : N ratio was slightly smaller. In contrast, the C : P ratio of *Daphnia* females was only slightly smaller than that of their eggs; being their N : P much smaller and the C : N much higher because of a higher N content in the eggs. A higher C : P ratio in *Daphnia* eggs than juveniles was found by Færøvig and Hessen (2003); however, the C : P ratios were substantially lower for both *Daphnia magna* and *Astracus* (120 for both species) than in our study. The C : P ratios measured in this study are closer to those found by Sterner and Schulz (1998), who reported egg C : P ratios for *Daphnia magna* and *Daphnia obtusa* of 192 and 310, respectively, *D. magna* eggs having almost twice the C : P ratio of adult females (83). Previous studies have reported changes in zooplankton elemental composition (mainly related to changes in the relative P content) because of feeding on highly P-deficient algae (DeMott et al. 1998, 2001; Boersma and Kreutzer 2002; DeMott et al. 2004). The growth of *Daphnia* consuming algae with C : P ratios $>$300–400 has been shown to be reduced (revised by Brett et al. 2000), even under very oligotrophic conditions, as in this study (Boersma and Kreutzer 2002). Lake Redon seston C : P ratios remained low during most of the study period (e.g., $<$300), except at the end of July and August, when the average ratio was almost 400. Therefore, it is very unlikely that any of the observed elemental changes in the three species can be attributed to changes in the quality of food.

Under oligotrophic conditions, small reductions in food availability may lead to food concentrations so low that grazers cannot meet their metabolic requirements. For *D. magna,* this concentration has been calculated to be 100 μg C L^{-1} (Boersma and Kreutzer 2002). In Lake Redon, the seston concentration decreased progressively from December 1998, it was almost always $<$100 μg C L^{-1} below the ice cover. In contrast, *Daphnia* dry wt was constant until April, when the first egg-bearing females appeared in the lake, suggesting that this reproduction investment was the main cause of dry wt loss. In addition, two experimental studies of the effects of starvation on the biochemical composition of cladocerans (Lemcke and Lampert 1975; Elendt 1989) reported a preferential reduction of lipids and carbohydrates after several days of starvation; this was associated with a reproduction investment rather than metabolic consumption.

Despite their three distinct life cycles, the three species exhibited a similar pattern of elemental changes, showing the highest variation during the last developmental phase, whereas there was less elemental variability during the first developmental phases. The only previous field study examining the ontogenetic changes in elemental composition of a calanoid copepod (*Mixodiaptomus laciniatus*) (Villar-Argaiz et al. 2002) showed substantial variations during ontogeny. However, because of the overlapping cohorts in the population, they also recorded high seasonal variability in elemental composition at each stage, to which most of the ontogenetic variability may be ascribed, at least for copepodites. Intrastage seasonal variability was, however, not the explanation in the case of Lake Redon because during the entire study period, *Cyclops* copepodites were present in the lake only during a short period of time at each stage, with an almost complete monthly replacement of each previous stage.

The ability of some marine species to store compounds for later use in reproduction (the food-independent reproduction mode) has been interpreted as an adaptation to long periods of starvation, either because of occasional overcrowded patchiness (Dagg 1977) or to seasonality (Hirche and Kosobokova 2003). The two longest-living species in this study (*Cyclops* and *Daphnia)* had a reproduction strategy similar to that of such marine species. In both habitats, female embryo development started well in advance of the production maxima, which allowed the young stages to grow during the productive periods. Because they are smaller and have high energy demands for development, the young stages are usually less resistant to starvation (Gliwicz and Guisande 1992). Therefore, this strategy is an adaptation enabling survival in these low food environments. Unlike the two long-lived species of this study, the life cycle of *Diaptomus* lasted only a short period, and it lost a considerable proportion of its body elements and biochemical components during reproduction. The reproduction strategy of *Diaptomus* is comparable with that of the other two species, with the difference that it does not need to spend resources on survival during winter. Our observations of food-independent reproduction species complement those made for species with food-dependent reproduction modes (Gilbert and Williamson

1983). It seems that the elemental composition of species with food-independent reproduction varies considerably more than that of food-dependent reproduction species because the latter have a shorter time to acquire all the resources for every clutch. This has probably been the case in several experimental studies reporting *D. magna*, *D. galeata*, and *Eudiaptomus gracilis* to be elementally homeostatic (Hessen 1990; Main et al. 1997; Vrede et al. 1999), although Main et al. (1997) found another species (*D. lumbolzy*) exhibiting significant elemental changes during its ontogeny. Finally, studies conducted under extremely low food quality conditions (e.g., C : P ratio $>$ 900) have shown that significant changes in the P content may occur independently of the reproduction mode (e.g., DeMott 2003).

The elemental composition of adult males differed between the three species. The dry wt of adult males of *Cyclops* did not change significantly from December 1998 to January 1999 and from November to December 1999, and as for *Daphnia* males, their composition remained the same as that of preadult instars (Fig. 4). In contrast, the dry wt of *Diaptomus* adult males decreased and their elemental composition changed similarly to that of adult females during reproduction (Table 3); thus, they yielded a reproduction effort comparable with that of adult females. Adult males of *Cyclops* survived less time than adult females, whereas *Diaptomus* adult males survived for the same period of time as adult females. The reason for these differences is probably related to a difference in mating requirements of the two copepod groups. Cyclopoid females need to be impregnated only once during their lifetimes, and fertile subitaneous eggs can be produced for extended periods of time in the absence of males (Whitehouse and Lewis 1973). Most calanoid females, however, must mate repeatedly to maintain their fertility (Watras and Haney 1980). In this context, our results suggest that calanoid copepod males may have a reproduction effort resembling more closely that of the females than is the case for cyclopoid males. This has already been documented for some marine calanoid species (Mauchline 1998).

In conclusion, the results reported in this study show that the changes in elemental and stoichiometric composition of the three species of zooplankton present in Lake Redon, being representative of each of the main freshwater zooplankton groups, followed a similar pattern, which is mainly attributable to reproduction. Previous studies have shown that highly P-deficient food (DeMott et al. 1998, 2001; Boersma and Kreutzer 2002; DeMott et al. 2004) or interstage variability (Villar-Argaiz et al. 2002) can be a relevant cause of elemental variability in some species. Our results indicate that reproduction may also be a cause of significant elemental variation in zooplankton, the extent probably depending on the reproductive mode of the species.

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Received: 15 December 2004
Accepted: 10 July 2005
Amended: 6 August 2005