

## Joint variation of zooplankton and seston stoichiometry in lakes and reservoirs

R. W. Sterner, J. H. Schampel, K. L. Schulz, A. E. Galford and J. J. Elser

### Introduction

With one exception, a predictive framework for the forces determining the structure of zooplankton communities has remained elusive. The single factor that is well understood and predictive is size-selective predation. Beyond this, we struggle for generalities and predictive power in spite of a wealth of detailed information. In recent years, several lines of evidence have indicated that perhaps the stoichiometry of carbon, nitrogen and phosphorus may be a major axis controlling zooplankton communities (STERNER & HESSEN 1994, HESSEN 1997). We know that lacustrine systems demonstrate a wide range in their C:N and C:P ratios (HECKY et al. 1993) and that they differ from marine systems (ELSER & HASSETT 1994). Further, we know that zooplankton demonstrate an interspecific variation in their C:P and C:N ratios (ANDERSEN & HESSEN 1991) and thus they presumably also differ in stoichiometric requirements for these elements. Individual zooplankton species have been characterized as "homeostatic", meaning that their C:N:P ratios remain relatively constant in spite of wide variation in these ratios in the food they eat (HESSEN & LYCHE 1991), though homeostasis is not perfect (DEMOTT et al. 1998). Reduced growth of high P (low C:P) zooplankton feeding on low P (high C:P) algae has been recorded in the laboratory (STERNER et al. 1993) and the field (MACKAY & ELSE 1998a).

Given these points, it is logical to deduce that there may be a consistent effect of stoichiometry on zooplankton communities, and there is evidence that this is so. Comparative data from Europe (GULATI et al. 1991, HESSEN 1992) and North America (HASSETT et al. 1997) indicate that the high-P herbivore *Daphnia* is generally not favored at high C:P ratios. The presumed mechanism is that zooplankton of low C:P such as *Daphnia* suffer growth penalties when the seston food base has a high C:P ratio, due to a direct (URABE et al. 1997) or indirect P limitation of animal growth. A general form of the question at hand is: is there a consistent pattern of species

replacements in zooplankton arranged along a seston C:P ratio gradient? If so, great inroads to another predictive axis for zooplankton communities would have been found. A test of this question is whether there are positive correlations between zooplankton C:N and C:P ratios and these same ratios in the seston food resources.

Stoichiometric feedbacks have also been examined. Due to the differential uptake and re-release by homeostatic consumers, nutrients that are in relative short supply from the perspective of the grazer should not be recycled, and instead should be efficiently funneled into production (STERNER et al. 1992, URABE 1993, MACKAY & ELSE 1998b). Nutrient recycling by homeostatic consumers is predicted to generate negative correlations between element ratios in consumers and their food because the animal recycles a greater proportion of the element not limiting its own growth (STERNER 1990).

There are thus three main hypotheses for possible joint dependence in chemical composition of zooplankton and their seston food base:

- Homeostasis – no correlation between zooplankton ratios and seston ratios.
- Food quality – positive correlation between zooplankton and seston.
- Recycling – negative correlation between zooplankton and algae.

It is noteworthy that these contrasting predictions are not all made for the same set of ratios. Homeostasis is thought to apply to all three ratios, C:N, C:P and N:P. Food quality is a function of the balance of N or P relative to C, and hence applies to C:P or C:N ratios. Trends in recycling should be most directly visible in the N:P ratio because zooplankton are not important in recycling C to the base of the food web.

As a first examination of the joint dependence of C:N:P ratios in zooplankton and seston, we constructed and analyzed a data set from several contrasting field sites that together span a wide range in

seston chemical qualities. A total of >600 observations of seston and zooplankton chemistry was included.

### Materials and methods

Zooplankton and seston stoichiometries were measured in five field sites. Three of these (Lakes 110, 240 and 227) are in the Experimental Lakes Area in Ontario (49° 40' × 93° 45'); they were sampled extensively in 1992, 1993 and 1994. The oligotrophic Lake 110 was subject to a piscivore manipulation (addition of northern pike, *Esox lucius*) during 1993 and 1994, and cyprinid minnow density declined during the 3-year study period. Zooplankton and algae showed little if any change during these years (ELSER et al. 1998). The zooplankton were generally dominated by non-daphnid cladocerans. Lake 240 (unmanipulated) is a naturally oligotrophic lake with a zooplankton community dominated by non-daphnid cladocerans (especially *Holopedium*) and a fish community that includes *E. lucius* and *Coregonus artedii* (ELSER et al. 1998). Lake 227 is an artificially fertilized eutrophic headwater lake (FINDLAY et al. 1994, HECKY et al. 1994) which also was manipulated via addition of *E. lucius* in 1993 and 1994 and thus also had declining minnows. There were no dramatic changes to the zooplankton community of L227 during 1992–1994, though the mid-summer algae showed some shifts from dominance by N-fixing nostocalean taxa to non-fixing *Microcystis*. The zooplankton comprised mainly calanoid and cyclopoid copepods. ELA zooplankton were sampled both day and night, but for consistency with the other lakes where only daytime observations are available, only the daytime samplings are used here. The fourth lake was Lake Superior. Here, several sites along a transect extending from Superior entry of Duluth harbor (46° 20' × 92° 0') to a site in 250 m depth in the North Shore trench in the western arm (47° 03' × 91° 25') were occupied for a number of cruises in 1996. The zooplankton were generally dominated by calanoid copepods. A total of three sites in the fifth basin, Joe Pool Lake – a reservoir in North Texas (97° 0' × 32° 35') – was sampled during portions of 1991 and 1992. Zooplankton were diverse and comprised mainly non-daphnid cladocerans and copepods. For background and location of sample sites, see STERNER (1994).

For the ELA lakes, samples were taken during the ice-free season between May and September. Once per week, a single site at the deepest part of each of these lakes was occupied. Seston samples were taken from three depths equally spaced in the mixed layer. Zooplankton were taken from Schindler trap (80 µm) samples of the same depths. Both seston

and zooplankton samples were performed in triplicate (i.e. three replicates of combined samplings of three depths). For Lake Superior, seston was sampled from three to eight depths depending on water column depth, and zooplankton was taken from vertical hauls of a large diameter, calibrated 80-µm net. In Joe Pool Lake, seston came from duplicate samples of a single depth in the mixed layer, and zooplankton came from duplicate vertical hauls of an 80-µm zooplankton net (net efficiency assumed to be 100%). In all cases, seston samples were pre-screened with 80-µm netting. Data presented here are means for a given sample date, and hence are generally made up of either triplicate or duplicate chemical analyses.

For chemical analysis, seston and zooplankton C and N were determined by CHN analysis (Perkin Elmer 2400) on pre-combusted GF/F filters. Measurements of P came from persulfate-oxidized samples that were subsequently analyzed for SRP using the molybdate reaction. All concentrations were converted to moles/liter and all ratios are reported as molar. Statistical analyses were performed with Statistica V. 5.1.

### Results

The lakes spanned a wide range in trophic status from the oligotrophic offshore Lake Superior to the highly eutrophic L227. Measurements of C, N and P in seston hence spanned a wide range (Table 1). Likewise, seston chemistry (C:P and N:P ratios) was highly variable. Average C, N and P in zooplankton expressed in µM were 0.02, 0.03, and 0.10 of average C, N and P in seston (expressed as fraction of total, i.e. 1.0 = 100%). The increase from C to N to P in these figures reflects the generally higher N and especially P content (as measured by N:C or P:C ratios for instance) in zooplankton than algae. Similar trends are visible in a plot of the fraction of total particulate matter (seston plus zooplankton) made up by zooplankton (Fig. 1A). Note the increase in median from C to N to P in this figure. It is apparent that, typically, zooplankton element pools are less than 10% of the total particulate matter in these sites, but there are occasional observations where zooplankton made up 30–50% of the elements. The contribution of zooplankton to the particulate matter varied systematically with lake trophic status (Fig. 1B), with the greatest contribution at

Table 1. Summary statistics of individual parameters. Individual observations are means of each sampling day.

	Valid N	Mean	Median	Lower Quartile	Upper Quartile	Std. Dev.
Seston C $\mu\text{M}$	193	118	80.0	49.1	119	118
Seston N $\mu\text{M}$	190	12.7	6.84	3.85	12.2	15.8
Seston P $\mu\text{M}$	193	390	160	103	668	379
Seston C:P	186	399	366	200	585	218
Seston N:P	186	35.6	37.0	20.5	48.2	17.3
Seston C:N	186	11.5	10.8	8.46	12.9	2.89
Zooplankton C $\mu\text{M}$	202	2.39	2.02	.907	3.59	1.90
Zooplankton N $\mu\text{M}$	202	.416	.330	.142	.645	.338
Zooplankton P $\mu\text{M}$	206	.0190	.0159	.0070	.0281	.0143
Zooplankton C:P	200	138	127	108	151	55.4
Zooplankton N:P	199	23.0	22.4	18.8	26.0	7.22
Zooplankton C:N	199	6.10	5.75	5.18	6.35	1.88

lower seston concentration as measured by seston P.

Plots of zooplankton chemistry versus seston chemistry emphasize the strong homeostatic nature of the bulk zooplankton community compared to seston, their potential food source (Fig. 2). Zooplankton C:P, N:P, and C:N are confined to narrow bands compared to the wide range in seston chemical parameters. Joint dependence of zooplankton and seston chemistry was examined by linear regression analysis. Zooplankton C:P was not significantly related to seston C:P ( $r^2 < 0.001$ ,  $P = 0.99$ ). In contrast, zooplankton N:P (Z) was weakly, but negatively, related to seston N:P (S) by the equation

$$Z = 26.7 - 0.998 S$$

( $F_{1,176} = 10.7$ ,  $r^2 = 0.05$ ,  $P = 0.001$ ; Fig. 2B). Though a linear model is probably inadequate, in that it implies negative zooplankton N:P ratios at very high seston N:P ratios, residual analysis of the linear model did not indicate that a different model was a statistical necessity; hence the linear model was retained. Also, zooplankton C:N was weakly and positively related to seston C:N by the equation

$$Z = 4.82 + 0.121 S$$

( $F_{1,176} = 5.75$ ,  $r_2 = 0.03$ ,  $P = 0.02$ ; Fig. 2C).

A somewhat more detailed analysis of joint dependence of C:P ratios was performed via distance-weighted least squares regression (McLAIN 1974), a procedure similar to the perhaps more familiar LOWESS regression (CLEVELAND 1979, TREXLER & TRAVIS 1993). The curve fit with this procedure suggests a slight increase in zooplankton C:P with increasing seston C:P in certain portions of the range of seston C:P (Fig. 3). Linear regression on the samples with seston C:P ratios  $< 200$  indicated a significant positive trend ( $F_{1,43} = 6.1$ ,  $P = 0.02$ ), as did a regression for C:P ratios  $< 300$  ( $F_{1,68} = 4.1$ ,  $P = 0.04$ ), though these trends are weak ( $r^2 = 0.10$  and  $0.04$ , respectively), and the P values must be regarded cautiously given that the trend was suggested by the data. Perhaps the most direct prediction of the food quality hypothesis would be that there should be an exclusion of high P zooplankton taxa from sites possessing high seston C:P ratios. Such a potential region of exclusion is hinted at by visual examination of the plot in Fig. 3, and it is indicated with a diagonal solid line. This line suggests an exclusion of a bulk zooplankton community with a C:P of  $\sim 80$  (similar to reports of *Daphnia*) from sites with seston C:P 700 or greater.

## Discussion

This analysis of a large collection of bulk zoop-

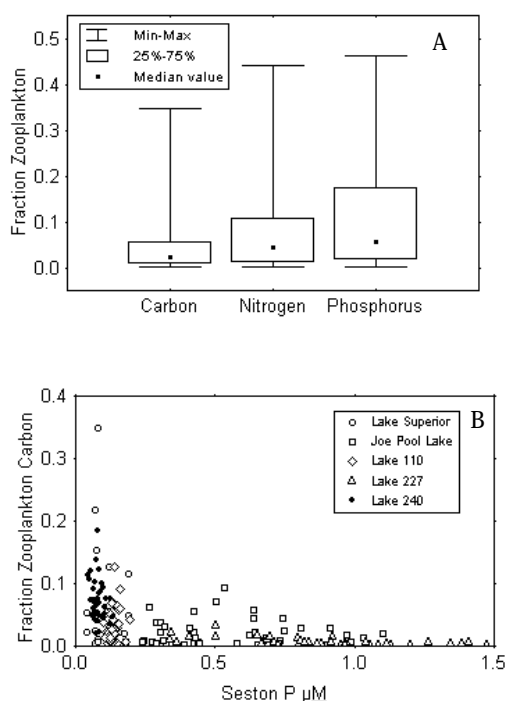


Fig. 1. Contribution of zooplankton ( $>80 \mu\text{m}$ ) to the total particulate matter. A. Fraction of the elements C, N and P in zooplankton (median, quartiles and range). Zooplankton makes up an increasing fraction of C, N and P. B. Contribution of zooplankton to total particulate carbon as a function of seston P. Zooplankton make up a smaller fraction of total particulate carbon with increasing lake trophy.

lankton nutrient measurements, and their joint dependence with seston chemistry, provides very strong support for overall zooplankton nutrient homeostasis in the presence of chemically variable food resources. Homeostasis of individual taxa has been studied by a number of investigators. Though a perfect homeostasis is not held (DEMOTT et al. 1998), variation in individual zooplankton species is less than in algae (HESSEN 1990). Our data indicate that, even at the community level, zooplankton biomass maintains a narrow range of C:N:P.

It is worthwhile also to compare the range of zooplankton bulk chemistry to reported values of individual taxa. Most of this data apply to species relatively easy to collect in sufficient

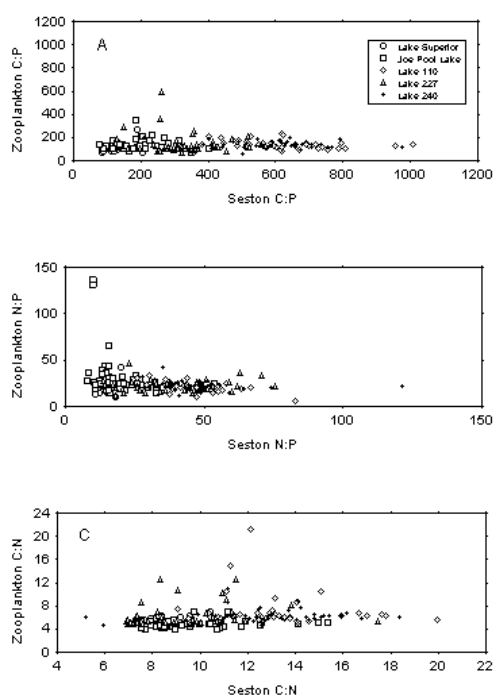


Fig. 2. Zooplankton stoichiometry (C:P, N:P, and C:N ratios) plotted against seston stoichiometry. A strong signal of homeostasis is apparent for all three ratios, with much less variation in the zooplankton than in the seston.

quantity for accurate measurements. Nevertheless, when we compare our measures of bulk zooplankton to values for a high C:P taxon (*Acanthodiptomus*) (ANDERSEN & HESSEN 1991) and a low C:P taxon (*Daphnia*), we see good agreement. The range in bulk measurements almost perfectly matches the values for the high and low species (Fig. 3). It appears that we have a fairly good idea of the potential variability in zooplankton chemical contents.

The food quality hypothesis predicted a positive correlation between zooplankton and seston chemical content. This might be either a true linear association, or a positive association determined by a progressively larger region of exclusion with higher and higher seston C:P or C:N ratios. Our data provide some support for a predictable pattern of species replacement along a seston C:P axis. There were some linear trends in certain ranges of the C:P data, though

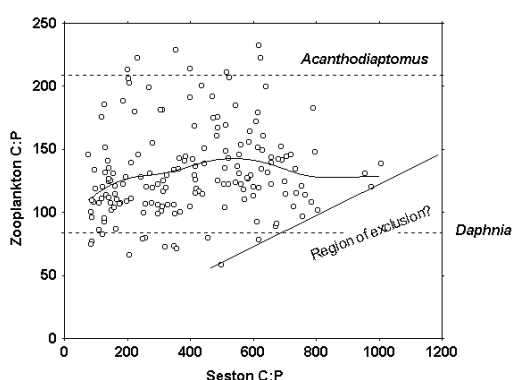


Fig. 3. Expansion of the vertical scale of Fig. 2A. Horizontal dotted lines indicate reported values of C:P for a high and a low C:P zooplankton taxon (from STERNER et al. 1992). The wavy line is a distance-weighted least squares regression. The solid inclined line represents a hypothetical region of exclusion (high seston C:P precludes dominance by low C:P zooplankton).

not in others. A potential region of exclusion is perhaps visible in the data, but the limited number of samples at very high C:P limits our confidence in the reality of this region of exclusion. There was a highly significant linear relation in the C:N data, but it explained only a small amount of the variation. Trends are in the right direction, but they are very weak. We must conclude that these data do not support a strong link between seston chemical content and zooplankton chemical content. In this regard, though, it is unfortunate that the sampling of lakes in our data did not include any strongly *Daphnia*-dominated systems. It is possible that these lakes, thought to be restricted to low seston C:P systems (ELSER et al. 1998), would generate a much more noticeable trend.

The nutrient recycling hypothesis made the opposite prediction, namely that the chemical content of zooplankton and the seston would be negatively correlated. There was a highly significant, though again a very weak, negative correlation in the N:P data. It seems intriguing that these correlations in C:P, C:N and N:P, though slight, occur in the directions they do: positive for C:P and C:N, negative for N:P. Perhaps this is an indication that both food quality

and nutrient recycling processes produce visible signals.

To close, we can list several reasons why the joint dependence in the element ratios in zooplankton and seston are so low. 1) Homeostasis itself is clearly a highly constraining force, limiting the variation in the zooplankton chemical ratios. 2) The highest C:P zooplankton taxa are calanoid copepods; however these taxa have high C:P as adults. Data for naupliar stages are sparse, but nauplii appear to have very low C:P ratio (SCHULZ 1996). Perhaps calanoid copepods are limited from high C:P lakes due to a life history bottleneck in the juvenile stages. 3) Finally there are several important methodological issues. Bulk seston might not be a good indication of the true particle spectrum used by most zooplankton species; selective feeding is well known for many taxa. Our measure of bulk zooplankton includes an unknown contribution of omnivores and carnivores. Finally, the 80- $\mu\text{m}$  cutoff might not be a reliable separation of consumer and resource. The present results of course are not definitive, but they represent perhaps the largest sampling of direct chemical measurements of zooplankton communities to date.

## Summary

We tested several stoichiometric hypotheses for the joint dependence in element ratios in zooplankton and their resources using direct chemical measurements of zooplankton (>80  $\mu\text{m}$ ) and seston. Our five field sites included three small boreal lakes, a warm water reservoir and Lake Superior. Zooplankton element pools were generally not large compared to the total seston. We found a tight homeostasis in bulk zooplankton element ratios compared to their resources. Weak correlations consistent with stoichiometric hypotheses were seen. Zooplankton C:P and C:N were very slightly positively correlated with seston ratios, but the zooplankton N:P ratio was negatively correlated with the same ratio in the seston.

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## Authors' addresses:

R. W. STERNER\*, J. H. SCHAMPEL, K. L. SCHULZ, A. E. GALFORD, Department of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, MN, USA.

J. J. ELSE, Department of Zoology, Arizona State University, Tempe, AZ, USA.

\*Corresponding author: stern007@tc.umn.edu