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Estuarine relationships between zooplankton community structure and trophic gradients

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Abstract. Zooplankton and water quality parameters were investigated at eight mesohaline stations in the lower Chesapeake Bay and Elizabeth River from January through December 1994 to identify the changes of zooplankton community structure with increased eutrophication. The total micro- and mesozooplankton biomass decreased with the increase of eutrophication. However, the relative proportion of microzooplankton increased with increased eutrophication. Within highly eutrophied waters, the small oligotrichs (<30 μm) and rotifers dominated the total zooplankton biomass (as carbon). However, tintinnids, copepod nauplii and mesozooplankton significantly decreased with the increase of eutrophication. These patterns were consistent throughout the seasons and had significant relationships statistically. These results suggest zooplankton community structures characterize an increasing eutrophication of an ecosystem.

Introduction

The Chesapeake Bay is a plankton based ecosystem in which the zooplankton act as trophic intermediates between the very productive phytoplankton and bacteria, and higher trophic levels, including many of the economically important fish and shellfish species. In the lower Chesapeake Bay and its tributaries, zooplankton consist primarily of protozoan ciliates, rotifers, and copepod nauplii as microzooplankton components, and copepods and metazoan nauplii as mesozooplankton (Birdsong *et al.*, 1987, 1988, 1989; Brownlee and Jacobs, 1987; Park and Marshall, 1993).

In view of the smaller body size of microzooplankton and higher specific rates of metabolism, it has been reported that the structure and composition of zooplankton assemblages are significantly altered with increasing eutrophication (Beaver and Crisman, 1982). Total zooplankton abundance increases and large species are replaced by smaller ones with increasing eutrophication (Gliwicz, 1969). However, the response of microzooplankton to eutrophication is not well documented in estuarine ecosystems. Some studies in lake systems have considered changes in the relative importance of microzooplankton and macrozooplankton with increasing eutrophication (Gannon and Stemberger, 1978; Ejsmont-Karabin *et al.*, 1980; Beaver and Crisman, 1982; Bays and Crisman, 1983, 1989; Pace, 1986). They state that zooplankton community structure shifts towards an increased relative biomass of microzooplankton with increased eutrophication. Bays and Crisman found an increase in the relative proportion of microzooplankton over macrozooplankton biomass with increased eutrophication in 35 Florida lakes (Bays and Crisman, 1983). Pace also found an increase of zooplankton biomass (Pace, 1986). However, no increase in relative importance of microzooplankton was observed with an increase of eutrophication. All the

above studies are limited to lake ecosystems and there are few studies in estuarine systems for comparison.

Accordingly, this study examines the changes in zooplankton community structure with an increase of eutrophication in the mesohaline stations in Chesapeake Bay and the Elizabeth River, a tidal tributary of the Chesapeake Bay.

Method

Monthly collections of water quality parameters and plankton samples were taken at eight stations in the southern Chesapeake Bay and Elizabeth River, Virginia, from January through December 1994 (Figure 1).

A whole water sampling and analysis protocol was used to overcome any under-estimations of smaller microzooplankters as reported in previous comparisons between whole water and net sampling methods (Beers and Stewart, 1967; Brownlee and Jacobs, 1987). On station, two sets of a vertical series of 3 l water samples were taken at approximately five equidistant depths between the pycnocline and surface. These were obtained using a battery powered bilge pump (PAR Model 34600–0000, ITT Jabsco Products) and a hose lowered to these depths with the water transferred to 15 l carboys. Both carboys were gently mixed and a 1 l sub-sample taken from each carboy and immediately preserved with 10 ml of acidic Lugol's solution. The pycnocline was determined using salinity measurements taken with a Hydro-Lab Surveyor II (H2O, Hydro-Lab Cooperation) at 1 m intervals with depth. To enhance enumeration and identification of the microzooplankton in these water samples that were very turbid, a specially designed methodology was followed. This produced a separation of the different size specimens into three Groups and reduced the amount of silt in the samples from interfering with the identifications and counts.

The paired 1 l water samples each had two series of settling and siphoning steps, at 72 h intervals, to obtain two 100 ml concentrates. These concentrates were then passed through a 73 μm mesh screen to remove larger specimens, such as rotifers and copepod larvae etc. Plankters trapped on the screen were washed into a Utermohl settling chamber, and represented the first subset (Group I). The two remaining 100 ml concentrates were then combined, gently mixed, and transferred to a graded glass cylinder. Three aliquots, totaling 5–10 ml (depending on high or low amounts of specimens/turbidity), were directly removed along the length of the cylinder and placed in a second settling chamber with buffered 5% formalin added to bring the total volume to 25 ml (Group II). Within 5 min, the upper 15 ml from this chamber was removed and transferred to a third settling chamber to represent Group III. The representative and typical size ranges within Group I, II and III were specimens >73, 30–73 and <30 μm, respectively. Each chamber was allowed to settle for 24 h before examination with an inverted microscope for specimen identification and counts at magnifications that ranged from ×100 to ×400.

The same carboys that provided water samples for microzooplankton analysis were also the sources of water used for phytoplankton and autotrophic picoplankton analysis, and for the determination of productivity rates and chlorophyll *a*

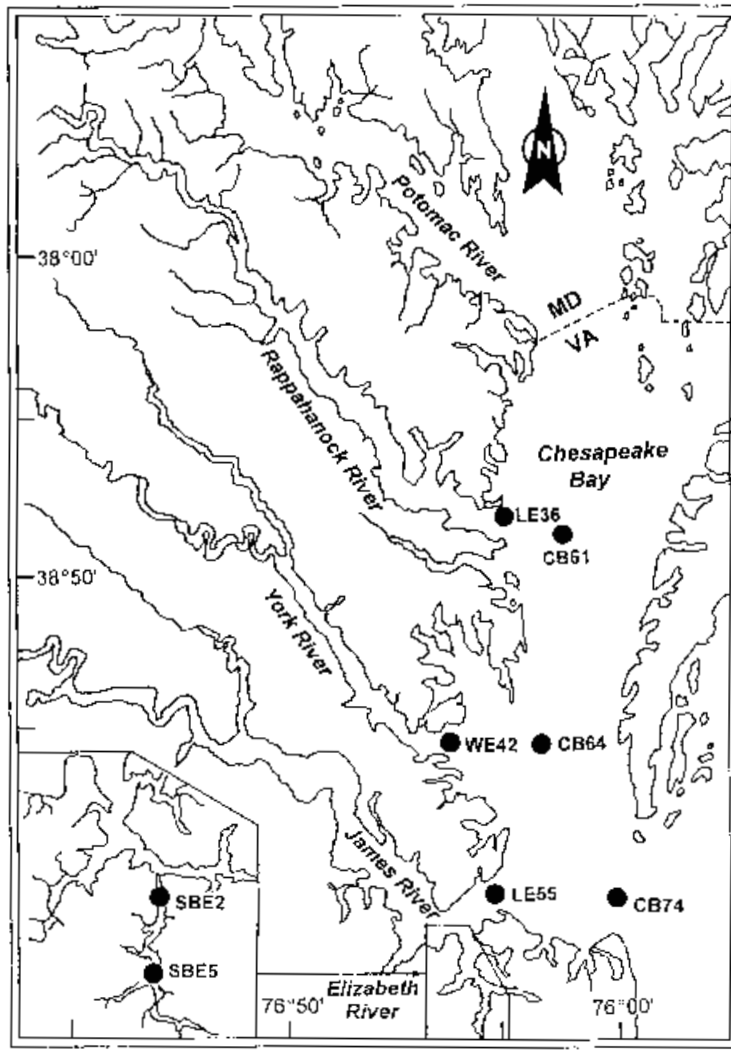

Fig. 1. Location of sampling stations in the lower Chesapeake Bay and Elizabeth River.

levels. The phytoplankton samples (500 ml) were preserved in Lugol's solution passed through a series of settling and siphoning steps, and then examined using a Zeiss inverted plankton microscope. Cell counts and identifications were made at ×315 and ×500 (Marshall and Alden, 1990). The autotrophic picoplankton samples (125 ml) were preserved with glutaraldehyde; 1–2 ml were filtered on a 0.2 μm Nuclepore filter, transferred to a glass slide and examined with a Zeiss Axioskop epifluorescence microscope, using green filter sets (G546, FT580, LP590), at ×1000 magnification for identification and counts (Marshall and Nesius, 1996). In the ^{14}C productivity measurements, four light and one dark 100 ml sample were taken from the 15 l carboy composites for analysis. These

were placed in 250 ml acid-washed milk dilution bottles, inoculated with 5 μmCi $NaH^{14}CO_3$, and incubated for 2–3 h upon return to the laboratory under saturated light conditions. Water temperature in the incubator was maintained at the same temperature as that recorded at the collection site. After incubation, 15 ml subsamples from each dilution bottle were filtered through a 0.45 μm Millipore filter, fumed over concentrated HCl, and placed in a scintillation vial containing 7 ml of scintillation fluid (6 g Omnifluor l^{-1} toluene). ^{14}C activity was determined using a Beckman LS1701 liquid scintillation counter. Alkalinity was determined from the samples taken at each station to calculate the amount of available organic carbon present. Carbon fixation rates were determined according to Strickland and Parsons (Strickland and Parsons, 1972), with daily production rates determined, using the 0.5 depth of the euphotic zone, and a 0.8 day length times the mean hourly carbon fixation rate.

Mesozooplankton were also collected at each station when the other collections mentioned above were taken. Replicate tows of 202 μm Nitex mesh Bongo nets were made obliquely in the water column for 5 min. The nets were equipped with flow meters to calculate the water volume sampled. A size fractionating procedure was followed to isolate groups for counts, with biomass estimates based on total dry weight values (Birdsong *et al.*, 1987).

Chlorophyll *a* measurements were collected above the pycnocline, transferred to a 1 l brown colored HPDE bottle and 1 ml of a 1% suspension of $MgCO_3$ added to each sample which was stored in a cooler in the field and returned to the laboratory for analysis. Samples were concentrated onto glass fiber filters by vacuum filtration, transferred to a grinding tube containing 34 ml of 90% aqueous acetone solution, and macerated using tissue homogenizers. Sample extracts were then centrifuged for 30 min and analyzed with a spectrophotometer at wavelengths of 750, 664, 647, 630, 480 and 510 nm before acidification, and at 750 and 665 nm after the addition of 1N HCl to the sample curvette. Chlorophyll *a* concentration was then corrected for phaeophytin.

Salinity, water temperature, dissolved oxygen (DO) and pH were measured on station at 1 m depth intervals using a Hydro-Lab Surveyor II. Secchi readings were also taken. Dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) values were provided by the Old Dominion University Applied Marine Research Laboratory.

Ciliate cell volumes were calculated using geometric formulae based on their size and shape, and tintinnid cell volumes were considered as half the lorica volume (Beers and Stewart, 1969). Biomass estimations employed conversion of cell volumes to dry weight (dry wt) using 0.279 pg dry wt μm^{-3} (Gates *et al.*, 1982), and to carbon content using a conversion factor of 0.19 pg C μm^{-3} (Putt and Stoecker, 1989). To estimate biomass (dry wt) of copepod nauplii, lengths were converted to dry weights using published length–dry wt regressions (McCauley, 1984), and then the dry wt was converted to carbon as 32.0% of the dry wt (Wiebe *et al.*, 1975). In the case of rotifers, biovolumes were calculated from the approximate geometric dimension, converted to dry wt (Ruttner-Kolisko, 1977; Pace, 1982) and finally to carbon as 50% of dry wt (Salonen *et al.*, 1976).

Using the nine environmental and biological data matrix at eight sampling

stations, principal component analysis (PCA) was performed to identify the similarity in environmental conditions between stations where the relative positions of the stations in hypospace reflect similarities. Pearson's correlation analysis and regression were employed to identify the relationship between nutrient concentrations and planktological parameters using SAS (SAS Institute, 1983). The data normality was checked before analysis if they were not normally distributed, and they were transformed into log $(x + 1)$ for data normality.

Results

Physical and chemical parameters

Descriptive statistics of the hydrological and biochemical parameters taken at the surface for each station are given in Table I. Mean salinity values ranged from 14.30‰ (LE36) to 26.16‰ (CB74). Water temperatures were similar at the bay stations, but were ~3–4°C higher at the Elizabeth River stations (SBE2, SBE5). This increase was mainly influenced by the cooling water discharge from the power plant located near the station SBE5. Dissolved oxygen and pH values for stations in the Elizabeth River were also significantly lower than those for the other stations, and DIN and DIP concentrations were significantly higher. Chlorophyll *a* and primary production in the Elizabeth River were among the highest compared with the other stations.

In terms of the DIN concentration, two stations in the Elizabeth River were in hypereutrophic water (>700 μg l^{-1}) and the others in mesotrophic (28–700 μg l^{-1}). All the other stations would be classified at mesotrophic levels (5–93 μg l^{-1}) based on the DIP concentrations (NFRDA, 1985).

Using the annual mean values of nine environmental and planktological variables in eight stations, an R-strategy PCA ordination was performed to summarize the environmental conditions at these stations (Figure 2). The relative positions of the stations reflect similarities in environmental variables among stations. Principal component I and II accounted for 80% of the variation (62% for PC I, 18% for PC II). Stations SBE2 and SBE5 were very similar; both were characterized by high water temperature, high chlorophyll *a* and increased nutrient (DIN, DIP) concentrations. However, Secchi depth, pH and dissolved oxygen were negatively correlated to these stations. The stations were clearly separated on PCA axis I based on their values within these environmental parameters. None of the parameters was significantly correlated with PC II. However, LE36 and CB61, with their low turbidity, were in the negative direction on PC II, whereas LE55, with high primary production, was in the positive direction on PC II. CB74 had the highest salinity and also showed a negative pattern on PC I. LE55 and SBE2 with high primary production were moved in the positive direction on PC I and PC II.

Zooplankton community structure

Pearson's correlation coefficients between nutrient concentration and chlorophyll *a*, primary productivity and phytoplankton density revealed no significant

Table I. Descriptive statistics of environmental and planktological variables. The values are annual means from January through December 1994 with one standard error of the means. Stations were ordered by DIN concentrations

Stations	Water temperature (°C)	Salinity (‰)	DO (mg l^{-1})	pH	DIN (μg l^{-1})	DIP (μg l^{-1})	Secchi depth (m)	Chlorophyll *a* (μg l^{-1})	Primary production (μg C l^{-1} h^{-1})
SBE5	18.76 ± 2.45	15.42 ± 1.82	7.12 ± 0.78	7.26 ± 0.08	1065.92 ± 88.42	45.75 ± 6.75	1.24 ± 0.14	7.32 ± 2.50	19.54 ± 5.59
SBE2	17.58 ± 2.39	15.69 ± 2.02	7.37 ± 0.84	7.38 ± 0.09	897.88 ± 43.76	43.33 ± 8.01	1.46 ± 0.28	9.59 ± 3.78	25.32 ± 8.44
LE36	14.85 ± 2.57	14.30 ± 0.91	9.83 ± 0.61	7.96 ± 0.07	557.63 ± 44.89	29.03 ± 3.43	2.02 ± 0.19	9.04 ± 1.64	13.96 ± 2.41
CB61	14.44 ± 2.61	15.43 ± 1.20	9.93 ± 0.71	7.99 ± 0.07	553.97 ± 53.29	27.41 ± 2.10	2.17 ± 0.27	7.69 ± 1.09	10.12 ± 1.46
WE42	15.74 ± 2.39	17.74 ± 0.89	10.24 ± 0.80	8.05 ± 0.08	448.71 ± 29.78	34.73 ± 2.47	1.69 ± 0.13	7.50 ± 1.19	14.90 ± 3.73
CB64	14.54 ± 2.45	18.54 ± 0.88	9.58 ± 0.69	8.11 ± 0.03	323.54 ± 40.27	9.67 ± 0.99	1.79 ± 0.15	7.11 ± 0.97	14.02 ± 2.46
LE55	14.91 ± 2.26	19.34 ± 1.19	9.01 ± 0.59	7.98 ± 0.03	304.63 ± 34.05	13.21 ± 1.94	1.19 ± 0.12	8.98 ± 1.26	27.89 ± 6.64
CB74	13.99 ± 2.17	26.16 ± 0.95	9.35 ± 0.53	7.99 ± 0.03	226.88 ± 22.41	11.13 ± 1.54	1.91 ± 0.16	5.83 ± 0.71	8.95 ± 1.92

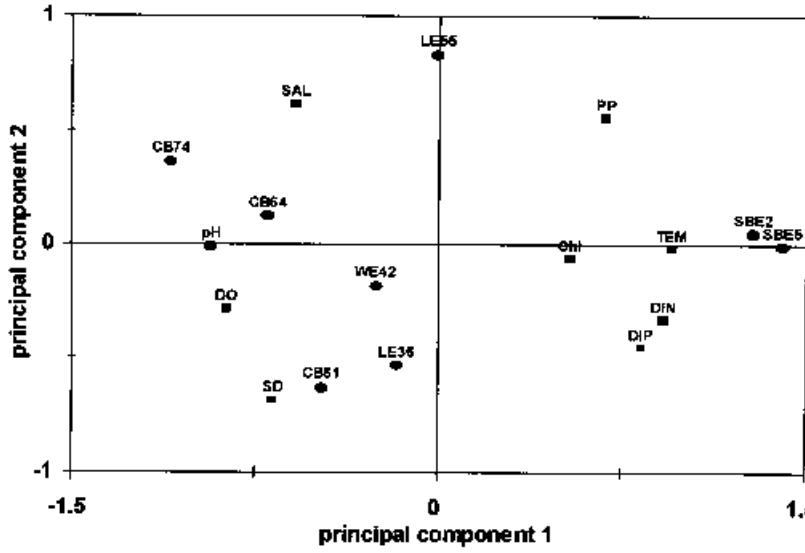

Fig. 2. Principal component ordination of eight stations, and environmental and planktological variables in the lower Chesapeake Bay and Elizabeth River. Abbreviations are TEM, water temperature; SAL, salinity; DO, dissolved oxygen; DIN; dissolved inorganic nitrogen; DIP, dissolved inorganic phosphorus; SD, Secchi depth; Chl, chlorophyll *a*; PP, primary production.

Table II. Pearson's correlation coefficients between dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) and phytoplankton density, primary productivity and chlorophyll *a* concentration. Top numbers are correlation coefficients and bottoms are calculated probabilities. Phytoplankton density is the density excluding picoplankton. Values are transformed into log $(x + 1)$ for data normality

Biological parameters/ nutrient	Chlorophyll *a*	Primary productivity	Picoplankton density	Phytoplankton density
DIN	0.36	0.35	–0.71	–0.20
	0.38	0.40	0.05	0.63
DIP	0.38	0.27	–0.59	–0.09
	0.35	0.51	0.12	0.82

relationship (Table II). Chlorophyll *a* and primary productivity were positively correlated with nutrients, but this was not statistically significant. However, there was a weak negative relationship between phytoplankton abundance and nutrients. The only significant correlation (negative) was between picoplankton density and dissolved inorganic nitrogen. Consequently, chlorophyll *a*, productivity and phytoplankton density varied independently with nutrient concentration at these sites in the lower Chesapeake Bay and Elizabeth River.

Microzooplankton were divided into four categories: loricate ciliates and aloricate ciliates, rotifers and copepod nauplii. Annual mean abundance and biomass (dry wt) for individual zooplankton components are given in Table III. Ciliates

Table III. Descriptive statistics of zooplankton abundance and biomass for the study sites in 1994. The values are annual means ± standard error of the mean. Density units are ind. l^{-1} for all the microzooplankton components and ind. m^{-3} for mesozooplankton. Biomass units are μg dry wt l^{-1} for all the plankton components. Stations were ordered by DIN concentrations

Stations	Aloricate ciliates		Loricate ciliates		Rotifers		Copepod nauplii		Mesozooplankton	
	Density	Biomass	Density	Biomass	Density	Biomass	Density	Biomass	Density	Biomass
SBE5	3559 ± 621	11.9 ± 2.5	807 ± 255	2.9 ± 0.9	149 ± 111	2.6 ± 1.8	77 ± 23	63.0 ± 18.2	3446 ± 1868	9.1 ± 3.3
SBE2	3269 ± 778	13.1 ± 4.0	1261 ± 570	3.8 ± 1.6	112 ± 43	2.4 ± 0.8	57 ± 13	44.2 ± 10.1	1930 ± 533	7.9 ± 1.7
LE36	2300 ± 392	9.8 ± 2.1	1531 ± 391	8.0 ± 3.9	61 ± 27	2.4 ± 1.1	77 ± 21	59.4 ± 16.3	7628 ± 3963	22.2 ± 10.4
CB61	1809 ± 396	9.6 ± 2.5	2065 ± 836	9.5 ± 5.0	75 ± 41	3.3 ± 1.9	103 ± 25	79.6 ± 19.6	7985 ± 3857	20.0 ± 7.0
WE42	2537 ± 607	12.3 ± 4.0	1873 ± 498	5.5 ± 1.1	111 ± 52	2.2 ± 1.1	100 ± 27	75.1 ± 21.1	6457 ± 2187	32.4 ± 10.1
LE55	2688 ± 555	14.7 ± 6.6	2424 ± 454	8.4 ± 1.6	80 ± 29	2.4 ± 0.8	98 ± 21	75.9 ± 16.7	7662 ± 3442	27.6 ± 10.4
CB74	2682 ± 998	8.8 ± 3.4	3077 ± 1484	10.2 ± 3.9	35 ± 19	1.2 ± 0.6	128 ± 32	99.5 ± 24.6	5835 ± 1455	28.3 ± 8.9

were numerically the major component of the zooplankton community and copepod nauplii comprised over 75% of the total zooplankton biomass (dry wt). Rotifers and mesozooplankton represented the remaining portion of the total zooplankton biomass and abundance.

To identify the relationship between eutrophication and zooplankton community structure, chlorophyll *a* and nutrient concentration were regressed against zooplankton biomass and percentage. All the values were transformed into log ($x + 1$) for data normality. The relationship between independent variable chlorophyll *a* and dependent variable zooplankton biomass and percentage are given in Table IV. Chlorophyll *a* was not significantly regressed against zooplankton biomass, and had only one significant correlation (negative) with copepod nauplii biomass. The relative contribution of copepod nauplii biomass to the total zooplankton biomass also decreased with increased chlorophyll *a* concentration. However, the percentage of rotifers and aloricate ciliates significantly increased directly with chlorophyll *a.* Nutrients were a better predictor for the changes of zooplankton community structure. DIN and DIP were highly correlated with zooplankton biomass and percentage. Loricate ciliates, rotifers, copepod nauplii, total microzooplankton, total mesozooplankton and total zooplankton biomass had a negative relationship with the DIN. The relative contributions of rotifers and total microzooplankton to the total zooplankton biomass were positively correlated with DIN (Table V). DIP had a significant correlation with loricate ciliates, total micro- and mesozooplankton biomass (Table VI). The percentage contribution of individual zooplankton biomass had a positive relationship with DIP. In general, regression of individual zooplankton biomass against DIN and DIP was highly significant. However, chlorophyll *a* was not significantly correlated with the above variables. Annual mean values of microzooplankton biomass as a percentage of total biomass for the eight stations in the lower Chesapeake Bay and Elizabeth River increased with eutrophication (Figure 3).

Table IV. Summary of regression models for zooplankton biomass and relative proportion using chlorophyll *a* as an independent variable. For each regression, number of observations is 8 and all the values are transformed into log ($x + 1$)

Dependent variables	Intercept	Slope	r^2	F	P
Biomass					
Aloricate ciliates	0.471	0.655	0.035	3.171	0.1253
Loricate ciliates	1.689	–0.848	0.093	0.615	0.4627
Rotifers	–0.109	0.685	0.189	1.397	0.2820
Copepod nauplii	3.162	–1.374	0.543	7.117	0.0371
Total microzooplankton	2.959	–1.041	0.467	5.251	0.0618
Total mesozooplankton	2.625	–1.385	0.154	1.090	0.3366
Total zooplankton	3.104	–1.104	0.385	3.753	0.1008
Percent					
Aloricate ciliates	–0.130	0.192	0.583	8.383	0.0275
Loricate ciliates	0.030	0.002	0.000	0.001	0.9823
Rotifers	–0.028	0.042	0.517	6.148	0.0445
Copepod nauplii	0.388	–0.148	0.746	17.641	0.0057
Total microzooplankton	0.233	0.028	0.021	0.126	0.7835

Table V. Summary of regression models for zooplankton biomass and relative proportion using dissolved inorganic nitrogen as an independent variable. For each regression, number of observations is 8 and all the values are transformed into log $(x + 1)$

Dependent variables	Intercept	Slope	r^2	F	P
Biomass					
Aloricate ciliates	0.901	0.070	0.055	0.351	0.5752
Loricate ciliates	2.569	–0.626	0.706	14.430	0.0090
Rotifers	–0.009	0.204	0.233	1.822	0.2257
Copepod nauplii	2.834	–0.361	0.522	6.548	0.0430
Total microzooplankton	2.817	–0.313	0.588	8.572	0.0264
Total mesozooplankton	3.602	–0.851	0.810	25.496	0.0023
Total zooplankton	3.148	–0.405	0.721	15.504	0.0076
Percent					
Aloricate ciliates	–0.067	0.044	0.425	4.441	0.0797
Loricate ciliates	0.097	–0.024	0.321	2.841	0.1483
Rotifers	–0.025	0.014	0.758	18.801	0.0049
Copepod nauplii	0.306	–0.022	0.220	1.692	0.2411
Total microzooplankton	0.148	0.042	0.627	10.088	0.0192

Table VI. Summary of regression models for zooplankton biomass and relative proportion using dissolved inorganic phosphorus as an independent variable. For each regression, number of observations is 8 and all the values are transformed into log $(x + 1)$

Dependent variables	Intercept	Slope	r^2	F	P
Biomass					
Aloricate ciliates	1.018	0.051	0.037	0.228	0.6500
Loricate ciliates	1.635	–0.541	0.649	11.108	0.0158
Rotifers	0.227	0.225	0.350	3.229	0.1225
Copepod nauplii	2.182	–0.229	0.259	2.100	0.1975
Total microzooplankton	2.259	–0.204	0.309	2.685	0.1542
Total mesozooplankton	2.244	–0.670	0.619	9.747	0.0205
Total zooplankton	2.459	–0.287	0.448	4.872	0.0694
Percent					
Aloricate ciliates	0.010	0.030	0.243	1.924	0.2148
Loricate ciliates	0.066	–0.025	0.429	4.502	0.0781
Rotifers	–0.005	0.012	0.721	15.498	0.0077
Copepod nauplii	0.264	–0.011	0.072	0.465	0.5206
Total microzooplankton	0.208	0.038	0.626	10.055	0.0193

Discussion

Microzooplankton represented over 85% of the total zooplankton biomass (as carbon) at the eight stations in the Bay and Elizabeth River. Copepod nauplii contributed the highest proportion of the total zooplankton biomass (47.5%). Ciliates and rotifers represented 33.5% and 4.5%, respectively. Mesozooplankton (mainly calanoid copepods and barnacle nauplii) constituted 14.5% of the total zooplankton biomass (annual mean). Buskey found a higher contribution by microzooplankton than mesozooplankton to the total zooplankton biomass in a subtropical estuary (Buskey, 1993). James and Hall also reported a high contribution by ciliates (33.4%) to the total zooplankton biomass (James and Hall, 1995), and Bays and Crisman found that microzooplankton biomass comprised

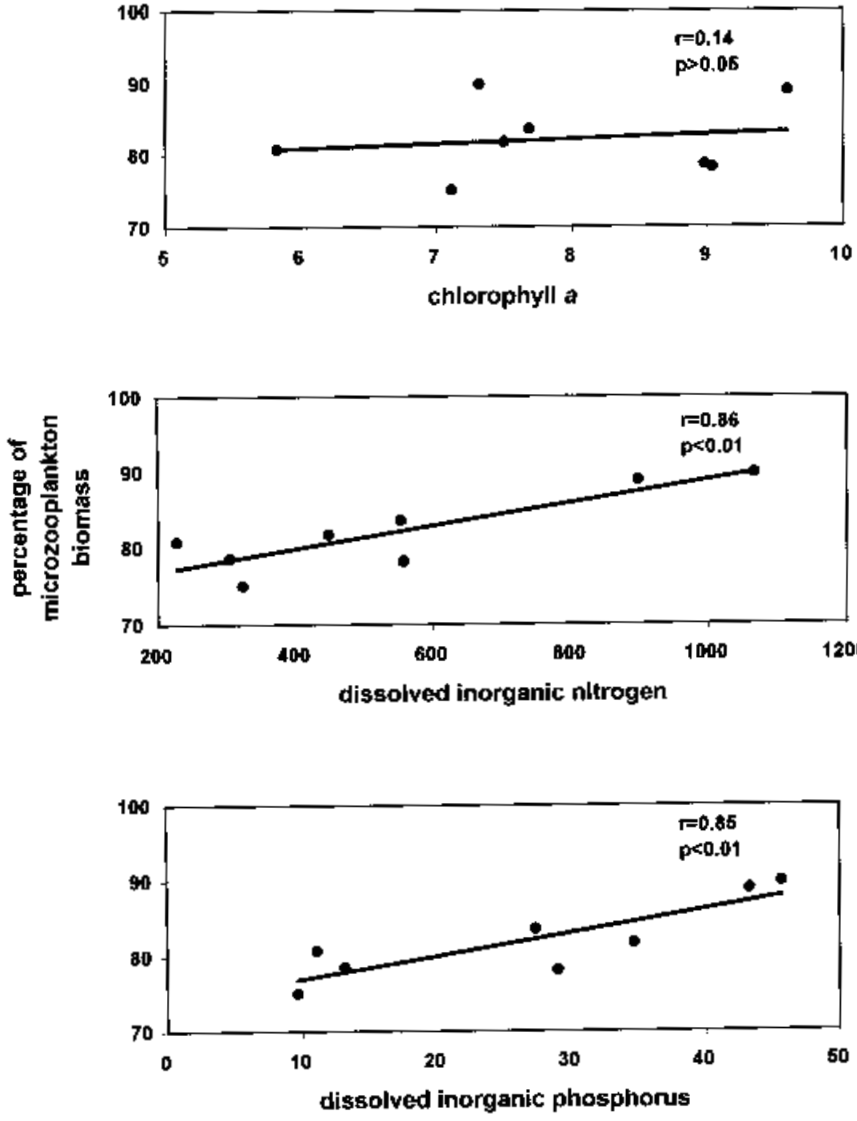

Fig. 3. Relationships of chlorophyll *a*, dissolved inorganic nitrogen, and dissolved inorganic phosphorus with microzooplankton biomass as a percentage of total biomass for the eight study sites. The values are annual means without transformation. Units are μg l^{-1} for the *x* axis.

50–90% of the total zooplankton biomass in Florida eutrophic lakes (Bays and Crisman, 1983). Pace reported zooplankton biomass (dry wt) composition, including micro- and mesozooplankton, from 12 lakes in Quebec (Pace, 1986). Recalculated from his paper, microzooplankton comprised ~40% of the total zooplankton biomass as dry wt. However, when considered as carbon content, the microzooplankton contribution increased to over 50%, since ciliate biomass

as carbon content assumes about 68% of the dry wt [estimated from (Gates *et al.*, 1982; Putt and Stoecker, 1989)]. This is in contrast to over 30% of mesozooplankton dry wt (Wiebe *et al.*, 1975). However, it is difficult to compare zooplankton biomass composition from study to study due to the different sampling methods and biomass estimation. Bays and Crisman (Bays and Crisman, 1983) and Pace (Pace, 1986) used a regression method (indirect method) to estimate mesozooplankton biomass, but in this study, direct estimation by weighing dry wt was used (Birdsong *et al*., 1987, 1988).

Copepod nauplii were a major component of the total zooplankton biomass throughout the seasons, even though their contribution to the total zooplankton density was low (<5%) during winter and spring. Mesozooplankton occasionally comprised a high proportion of the total biomass, reaching a maximum of 50%. However, the overall contribution by mesozooplankton to the total biomass was low. Rotifers made the smallest contribution to the total zooplankton biomass but during winter, they occupied over 20% of the total zooplankton biomass (mainly by *Synchaeta* spp.) at some stations (CB74, WE42, SBE5).

The correlation between percentage contribution by microzooplankton to the total zooplankton biomass and nutrients (DIN, DIP) was significantly positive. Ciliates were important to the spatial heterogeneity of zooplankton biomass structure. They comprised a high proportion of the zooplankton biomass in the highly eutrophied Elizabeth River, but their contributions in the meso- and eutrophic sites were relatively low. Few studies have tested a community shift toward microzooplankton with an increase in eutrophication. Bays and Crisman reported the dominance within zooplankton community shifts from macrozooplankton to microzooplankton with increasing trophic states at 39 Florida lakes (Bays and Crisman, 1983). However, Pace reported no relationship between lake trophy and zooplankton community shift from 12 lakes in Quebec (Pace, 1986). Both studies found an increase of zooplankton biomass with increased eutrophication. In this study, the meso- and microzooplankton biomass decreased with an increase of nutrient concentrations. The disparity between these results and those mentioned in the above lake studies regarding zooplankton biomass changes with eutrophication may be related to several factors. These would include the environmental gradients measured, the number of sampling sites, and the fundamental differences between freshwater lakes and estuarine ecosystems. Bays and Crisman incorporated 39 sampling sites in Florida and 12 lakes in Quebec, so their results may be considered more general (Bays and Crisman, 1983). The differences in their results may be due to the broad range of gradients for determining eutrophication. To divide lakes into different trophic levels, Bays and Crisman used annual mean chlorophyll *a* concentrations which ranged from 30 to 80 μg l^{-1} (Bays and Crisman, 1983). In this study, the annual mean values for chlorophyll *a* ranged from 5.8 to 9.6 μg l^{-1}. Even in the hypereutrophic (based on DIN, DIP) Elizabeth River stations, chlorophyll *a* concentrations were not significantly higher than those in the other eutrophic or mesotrophic sites.

Another great disparity was in microzooplankton biomass. Even with much higher chlorophyll *a* concentrations (about seven times) in Florida lakes, the total

microzooplankton biomass was slightly lower (32 μg C l^{-1} versus 38 μg C l^{-1} in this study), with the mesozooplankton biomass significantly higher in their study (30 μg C l^{-1} versus 7 μg C l^{-1}). These differences may be due to the different ecosystems between freshwater and estuarine water, sampling methods, and biomass estimations. They used 80 μm mesh to collect microzooplankton (except ciliates) and mesozooplankton, but in this study, mesozooplankton were collected using 200 μm mesh (Birdsong *et al.*, 1987, 1988) and microzooplankton using whole water samples. Pace also showed wide gradients in chlorophyll *a* concentrations (1.6–24.9 μg l^{-1}) from 12 lakes in Quebec (Pace, 1986). In general, the above two studies indicate increase of micro- and mesozooplankton biomass with the increase of eutrophication (based on both chlorophyll and total phosphorus), but in this study, there was a decrease of total zooplankton biomass with increase in eutrophication.

In addition to the above possibilities, high concentration of heavy metals in the hypereutrophic Elizabeth River may be another possible cause for zooplankton biomass disparity. Sunda *et al.* indicated that heavy metals were present at high concentration in Elizabeth River when compared with values in lower Chesapeake Bay (Sunda *et al.*, 1990). These metals included copper, mercury, zinc, cadmium, lead and nickel (Alden, 1988). Sunda *et al.* reported that the survival and reproduction of copepods were lower in Elizabeth River samples, which contained high levels of copper and zinc (Sunda *et al.*, 1987, 1990). In addition to growth inhibition to zooplankton, heavy metals can reduce nutrient uptake by phytoplankton (Singh and Yadava, 1984), the growth rates of various autotrophs (Heumann, 1987; Singh *et al.*, 1989; Lage *et al.*, 1994), and photosynthetic rates (Singh and Singh, 1987). Phytoplankton biomass (including autotrophic picoplankton) and primary production were not significantly higher in the Elizabeth River than in the other stations. When considering the amount of nutrients in the hypereutrophic Elizabeth River, phytoplankton biomass and primary production were considerably low, which indicates that nutrients may not be fully utilized by autotrophs in the river. This may suggest that the presence of heavy metals may inhibit autotroph growth and reproduction, and this low autotroph biomass may not be sufficient to support high zooplankton biomass, commonly present in eutrophic ecosystems.

However, the relative proportion of microzooplankton biomass increased with the increase of eutrophication. In the hypereutrophic system, small oligotrichs (<30 μm) and rotifers dominated total zooplankton biomass (carbon). Tintinnids, copepod nauplii and mesozooplankton significantly decreased with the increase of eutrophication. These patterns were consistent throughout the seasons and had significant relationships statistically. These results are an indication that zooplankton community structures characterize trophic states within an ecosystem.

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