Helgol Mar Res (2001) 55:150–159
DOI 10.1007/s101520000067

ORIGINAL ARTICLE

N. Bojanić · M. Šolić · N. Krstulović · I. Marasović
Ž. Ninčević · O. Vidjak

Seasonal and vertical distribution of the ciliated protozoa and micrometazoa in Kaštela Bay (central Adriatic)

Received: 15 February 2000 / Received in revised form: 8 November 2000 / Accepted: 12 November 2000 / Published online: 7 March 2001
© Springer-Verlag and AWI 2001

Abstract Seasonal and vertical distribution of tintinnids, non-loricate ciliates and micrometazoa were studied in Kaštela Bay (central Adriatic Sea) throughout 1995. The species composition of tintinnids and copepods were studied as well. This is the first estimation of non-loricate ciliate biomass in the coastal area of the central Adriatic. Non-loricate ciliates were quantitatively the best represented ciliated protozoa, whereas nauplii were the most numerous micrometazoan organisms. Temperature affected the distribution of most micrometazoan components of the zooplankton and that of non-loricate ciliates. The temperature-dependent presence of individual size categories of non-loricate ciliates was also established. Apart from the interaction between microzooplankton groups, the influence of biotic factors, such as phytoplankton, bacteria, non-pigmented nanoflagellates (NNF) and mesozooplankton, was also discussed. The abundance of ciliates was controlled by both food supply (phytoplankton and NNF) and micrometazoan grazing. The results point to very complex trophic relationships within the planktonic community, suggesting that microzooplankton could be an important link between the microbial food web and higher trophic levels.

Keywords Ciliated protozoa · Micrometazoa · Seasonal distribution · Vertical distribution · Adriatic Sea

Introduction

A small-dimension fraction of zooplankton forms an important part of the food web of any marine ecosystem, relating bacteria and phytoplankton to large-dimension zooplankton fractions, benthic invertebrates and fish larvae (Margaleff 1963; Rassoulzadegan et al. 1988; Pelegrí et al. 1998). Some earlier studies (Beers and Stewart 1971) show that microzooplankton (organisms from 20 to 200 µm) may consume up to 70% of the daily phytoplankton production. Herbivore activity of microzooplankton may exceed copepod grazing by an order of magnitude (Burkill et al. 1993). In addition, ciliates along with bacteria and non-pigmented nanoflagellates (NNF) form a "microbial loop", which may channel an important fraction of photosynthetically fixed carbon (Azam et al. 1983; Hagström et al. 1988; Stone and Berman 1993). Their short generation time makes them easily adaptable to ecosystem changes (Heinbokel 1978), stabilising their marine populations and preventing en-ergy losses (Capriulo and Carpenter 1980). Therefore, studies of bacterioplankton, protozooplankton and small metazooplankton contribute to a better understanding of trophic relations within a plankton community.

Microzooplankton has been studied rather intensively along the eastern Adriatic coast for the past two decades (Kršinić 1980a, 1982, 1987, 1995; Revelante and Gilmartin 1983; Revelante et al. 1985). Species composition, density and vertical distribution of organisms were observed. Those data showed that the microzooplankton density was higher in enclosed areas, particularly in bays and estuaries.

Whereas earlier studies in Kaštela Bay were carried out over short periods of time only, we aimed at recording the annual changes in densities of ciliated protozoa and micrometazoa, their vertical distribution and the species composition of tintinnids and copepods. This is the first attempt to estimate the biomass of non-loricate ciliates in this area. Effects of individual abiotic and biotic factors on the seasonal and vertical distribution of ciliated protozoa and micrometazoa in Kaštela Bay were also studied.

Communicated by H.-D. Franke

N. Bojanić (✉) · M. Šolić · N. Krstulović · I. Marasović
Ž. Ninčević · O. Vidjak
Institute of Oceanography and Fisheries, PO Box 500,
21000 Split, Croatia
e-mail: bojanic@jadran.izor.hr

Materials and methods

Sampling methods and measurement techniques

Sampling was done on a monthly basis in 1995 at a station located in the middle of Kaštela Bay (43°31′N and 16°22′E) (Fig. 1). Samples were collected at 5 m depth intervals, between the surface and bottom (35 m), using 5-l Niskin bottles. The material was preserved in formaldehyde at a final concentration of 2.5%, previously neutralised with $CaCO_3$.

Samples were sedimented in the laboratory for 24 h, and thereafter decanted down to the volume of approximately 2 l. After another 24 h sedimentation, their volume was decanted to approximately 0.2 l. For microscopic analysis the material needed to be sedimented and the volume had to be reduced again (Kršinić 1980b). The material was analysed using an inverted microscope, "Olympus" IMT-2, at 100× magnification.

Non-loricate ciliates were sampled using a 1.7-l Nansen bottle. An aliquot volume of 100 ml was used for the analysis. Counting was done at 200× magnification. The size of individuals was measured using an ocular micrometer at 400× magnification. Non-loricate ciliates were also fixed with formaldehyde in the same way as the tintinnids and metazoa. The biovolume of non-loricate ciliates was calculated to estimate their biomass. The shape of the plasmatic body of each individual organism was compared to one or more geometrical bodies. After measurement of biovolume, non-loricate ciliates were divided in four size categories: (I) biovolume $<10^3$ µm³; (II) biovolume from 10^3 to 10^4 µm³; (III) biovolume from 10^4 to 10^5 µm³; (IV) biovolume $>10^5$ µm³. The biovolume of non-loricate ciliates was converted into units of carbon based on a C volume ratio of 0.14 pgC μm^{-3} (Putt and Stoecker 1989).

Sea-water temperature was measured using a reversible thermometer attached to the Nansen bottle. Salinity was determined in the laboratory with an inductive salinometer (model RS10).

The estimate of phytoplankton biomass was performed by measuring the chlorophyll *a* concentration by fluorimetric methods (Yentsch and Menzel 1963; Holm-Hansen et al. 1965; Strickland and Parsons 1972).

Enumeration of bacteria and NNF was made by epifluorescence microscopy using the standard AODC technique for bacteria (Hobbie et al. 1977) and the proflavine staining technique for NNF (Haas 1982), respectively.

Mesozooplankton was collected using a Nansen net (net mesh diameter 125 µm and 0.255 m^2 surface area) vertical hauls from bottom to surface. Samples were analysed with a WILD stereomicroscope at 80× magnification.

Principal component analysis

Principal component analysis (PCA) was used to extract the main patterns of seasonal changes in abundance. The data input to each analysis consisted of a set of variables representing seasonal fluctuations in abundance. The analyses were all based on correlation matrices involving the standardisation of each variable to zero mean and unit variance. The purpose of this is to eliminate differences in abundance between the groups studied, leaving only the relative month-to-month changes in abundance.

Hydrography

The entire winter period was characterised by homothermy with a gradual temperature increase from 11.2°C to 13.2°C (January to April). A vertical temperature gradient appeared in spring (May), attaining its maximum in July. The thermocline disappeared in September and temperature inversion was followed by homothermy recorded again in November (Fig. 2A).

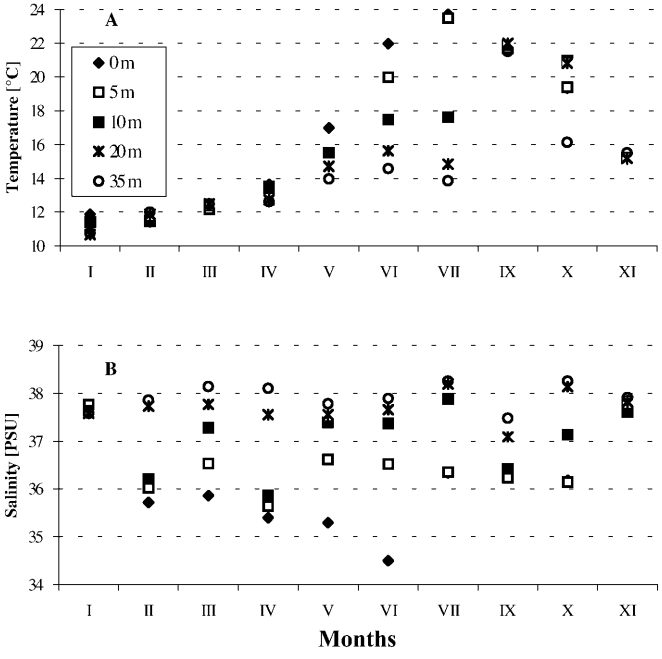


Fig. 2 Seasonal changes of temperature (**A**) and salinity (**B**) at different depths in Kaštela Bay, 1995

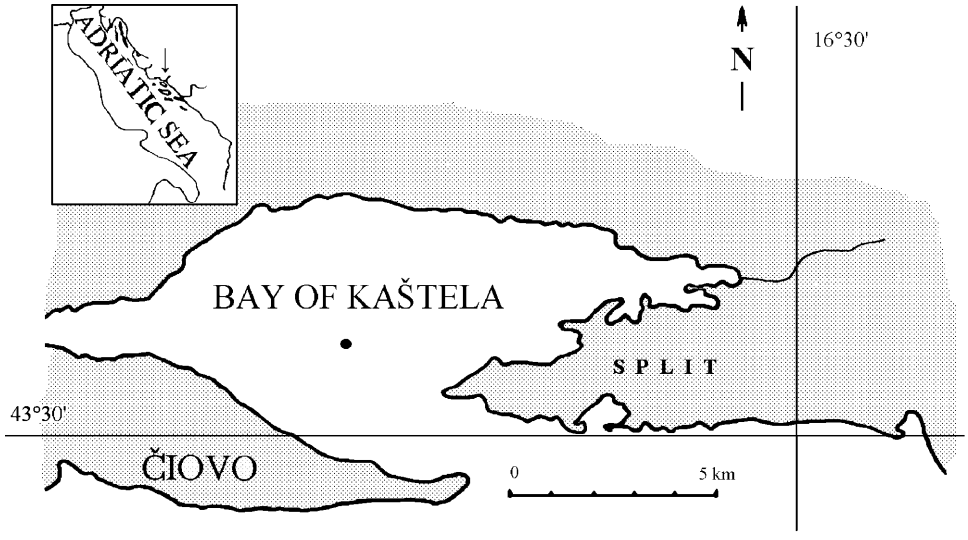


Fig. 1 Area studied (Kaštela Bay) with sampling locations

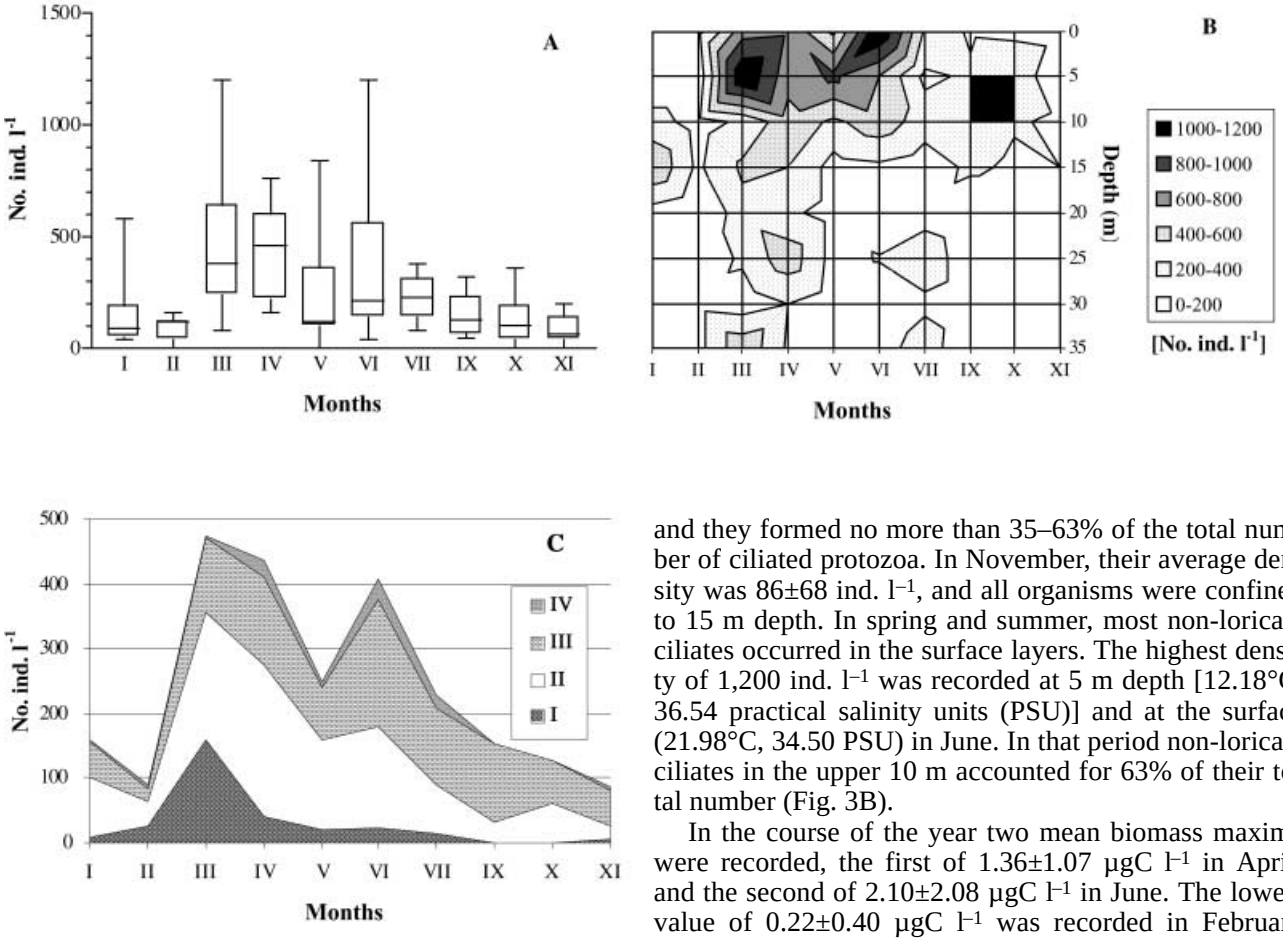


Fig. 3 Population density of non-loricate ciliates illustrated by **A** box and whiskers column graph of the seasonal distribution; **B** vertical depth distribution; and **C** distribution of density of four size categories of non-loricate ciliates (*I* $<10^3$ µm³; *II* 10^3–10^4 µm³; *III* 10^4–10^5 µm³; *IV* $>10^5$ µm³)

Throughout the year, salinity ranged from 34.50 to 38.25. Salinity increased with depth, while fluctuation of salinity decreased in the same direction. From February onwards, salinity of the surface layers dropped (Fig. 2B). The greatest vertical salinity gradient was recorded in June. During the colder period of the year all the layers showed almost the same salinity value of 37.7.

Salinity of this area is affected by fresh water sources such as the Jadro River runoff in the eastern part of the bay, along with the small Pantan Brook and submarine freshwater springs in the western part of the bay. Due to nearby industrial plant, the bay receives a defined quantity of urban and industrial effluents, which stimulate phytoplankton production (Pucher-Petković and Marasović 1988).

Results

Non-loricate ciliates

The highest average density of non-loricate ciliates exceeding 408 individuals ind. l^{-1}, was recorded in March, April and June, when they represented 76–93% of the total counts of ciliated protozoa (Fig. 3A). During the colder period of the year, their average density was low, and they formed no more than 35–63% of the total number of ciliated protozoa. In November, their average density was 86±68 ind. l^{-1}, and all organisms were confined to 15 m depth. In spring and summer, most non-loricate ciliates occurred in the surface layers. The highest density of 1,200 ind. l^{-1} was recorded at 5 m depth [12.18°C, 36.54 practical salinity units (PSU)] and at the surface (21.98°C, 34.50 PSU) in June. In that period non-loricate ciliates in the upper 10 m accounted for 63% of their total number (Fig. 3B).

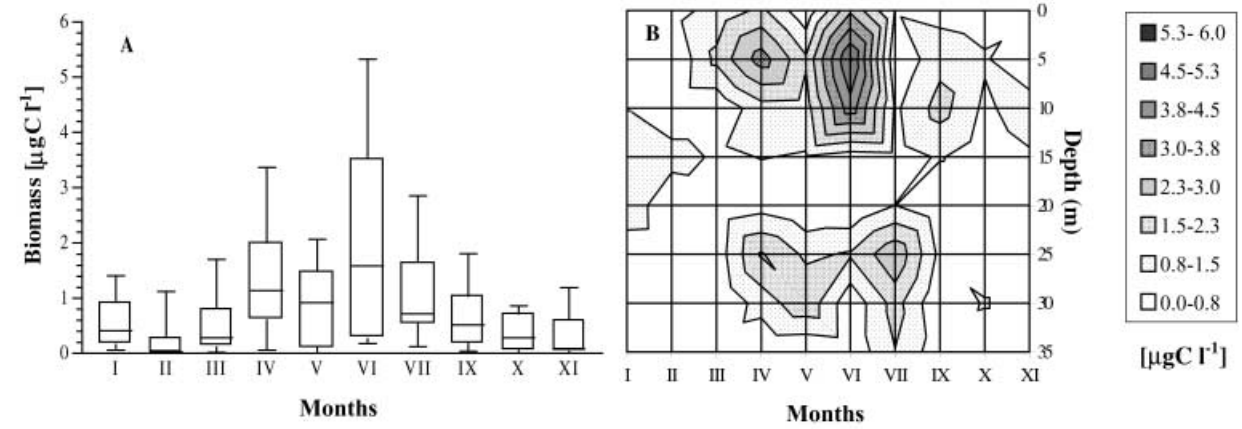
In the course of the year two mean biomass maxima were recorded, the first of 1.36±1.07 µgC l^{-1} in April, and the second of 2.10±2.08 µgC l^{-1} in June. The lowest value of 0.22±0.40 µgC l^{-1} was recorded in February (Fig. 4A). The highest biomass value of 5.32 µgC l^{-1} was established at 5 m depth (19.98°C, 36.53 PSU) in June. Seasonal differences in the biomass were most pronounced at 5 m depth, while the variations were smallest at 15 m depth (Fig. 4B). With the sea-water heating by April, the biomass maximum occurred in the surface layer. Temperature stratification caused the redistribution of the biomass, with the higher values in deeper layers, particularly in July.

As shown in Fig. 3C, the spring increase in population density was due to organisms of the first two size categories, which contribute 75% of the non-loricate population. During the summer stratification, organisms of these two size categories decreased in number, while non-loricate ciliates from the third size category became dominant.

Contribution in the total biomass of non-loricate ciliates of the first two size categories did not exceed 20%, whereas the share of the third size category ranged from 34% in February to 96% in September (Fig. 4C).

Tintinnids

A total of 38 species of tintinnids was identified from the study area. The highest number of species was recorded in October (24 species) and the lowest in June (11 species). The quantitatively most important tintinnid species



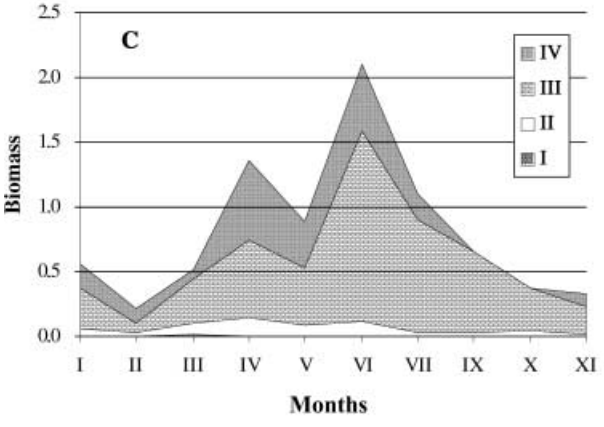


Fig. 4 Biomass of non-loricate ciliates (µgC l^{-1}) illustrated by **A** box and whiskers column graph of the seasonal distribution; **B** vertical depth distribution in Kaštela Bay; and **C** distribution of biomass of four size categories of non-loricate ciliates (*I* <10^3 µm³; *II* 10^3–10^4 µm³; *III* 10^4–10^5 µm³; *IV* >10^5 µm³)

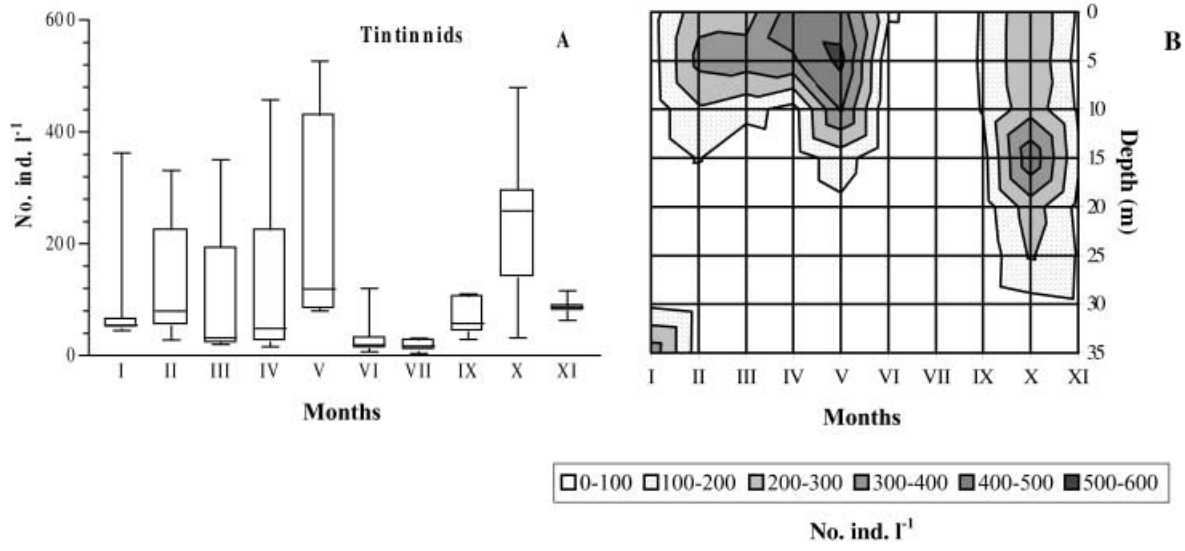
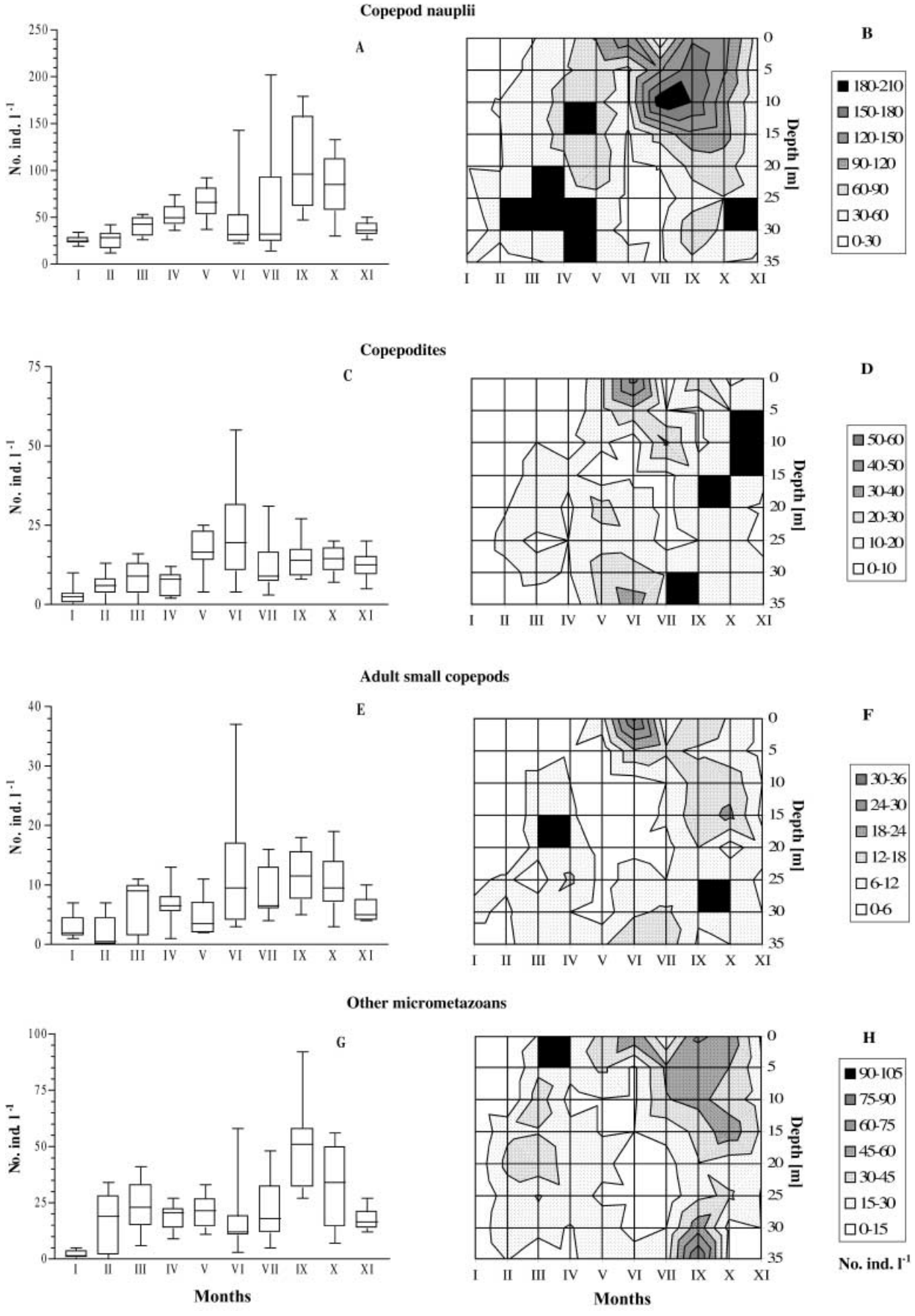


Fig. 5 Distribution of tintinnids illustrated by **A** box and whiskers column graph of the seasonal distribution and **B** vertical depth distribution

of the Kaštela Bay was *Helicostomella subulata*, which made up 33% of the total number of tintinnids. This species was particularly abundant between March and May. The species *Stenosemella nivalis*, *Stenosemella ventricosa* and *Tintinnopsis campanula* were dominant in January and February, while *Salpingella rotundata* and *Codonellopsis schabi* dominated in autumn.

The seasonal distribution of tintinnid abundance showed two maxima, the first in May with 234±192 ind. l^{-1}, and the second in October with 237±141 ind. l^{-1} (Fig. 5A). They constituted 65% of the total ciliated protozoan counts in October. Summer was characterised by very low numbers of tintinnids, with densities not exceeding 18 ind. l^{-1}.


Copepod nauplii
A
No. ind. l-1
250
200
150
100
50
0
I II III IV V VI VII IX X XI
B
Depth [m]
0 5 10 15 20 25 30 35
180-210
150-180
120-150
90-120
60-90
30-60
0-30
Copepodites
C
No. ind. l-1
75
50
25
0
D
50-60
40-50
30-40
20-30
10-20
0-10
Adult small copepods
E
No. ind. l-1
40
30
20
10
0
F
30-36
24-30
18-24
12-18
6-12
0-6
Other micrometazoans
G
No. ind. l-1
100
75
50
25
0
Months
H
90-105
75-90
60-75
45-60
30-45
15-30
0-15
No. ind. l-1

The vertical distribution of tintinnids showed that in January they mostly occurred in the bottom layer (Fig. 5B). The sea-water heating encouraged them to get close to the surface. During the spring period, approximately 64% of the population occurred in the upper 5 m depth. With the disappearance of the thermocline from September onwards, the vertical distribution of tintinnids became more uniform.

Copepod nauplii

Nauplii were the most numerous micrometazoan organisms, contributing an average of 57% to the total micrometazoan counts. Their annual distribution showed average density values to be lowest in winter with 25±5 ind. l^{-1} (Fig. 6A). With the sea-water heating towards the end of summer, the number of nauplii increased, resulting in a maximum average value of 107±52 ind. l^{-1}, when they formed 58% of the total micrometazoan counts. Their highest density of 202 ind. l^{-1} was found at 10 m depth in July (17.63°C, 37.88 PSU).

The vertical distribution of nauplii was uniform from January to May (Fig. 6B). In summer, with a well pronounced thermocline, 61% of the nauplii population occurred in the layer from 5 to 10 m depth.

Copepodites

The lowest average density values of copepodites were recorded in winter, and the highest during the warmer part of the year (Fig. 6C). Average values varied within the range of 3±3 ind. l^{-1} in January and 23±17 ind. l^{-1} in June, when their contribution to the total micrometazoa was maximal. During the colder part of the year, copepodites were restricted to the deeper layers. With sea-water heating they moved to the surface layer down to 10 m depth. In June, 45% of the copepodite population occurred in the 0–5 m depth layer, with the highest density of 55 ind. l^{-1} at the surface (21.98°C, 34.50 PSU). With the cooling of the surface layers and the disappearance of the thermocline, their vertical distribution became more uniform (Fig. 6D).

Calanoid copepodites made up an average of 43% of the total copepodite counts. Copepodites of the genera *Oithona* and *Oncaea* contributed to the total copepodite population with 34% and 23%, respectively.

Adult small copepods

The species *Euterpina acutifrons* and *Oithona nana* were the best represented adult small copepods in Kaštela Bay. They were most numerous in June in the surface layer with densities of 25 and 8 ind. l^{-1}, respectively.

The numbers of small copepods increased with sea-water temperature, from 2±3 ind. l^{-1} in February to 13±11 ind. l^{-1} in June, when they constituted 13% of the total micrometazoan population (Fig. 6E, F). In the winter–spring period they were confined to deeper layers. In May they were uniformly distributed, with an equal percentage of the population in the surface and bottom layers. In June, however, 37% of the organisms could be found at the surface, with a maximum density of 37 ind. l^{-1}. In November, 62% of the adult small copepods inhabited the layers below 20 m depth.

Other micrometazoans

Other micrometazoans comprised the following groups: Cladocera, Pteropoda, Appendicularia, Chaetognatha, as well as the larvae of Echinodermata, Polychaeta and Bivalvia. Their annual distribution was characterised by a very wide range of density values, from several individuals per litre in January to 22 ind. l^{-1} in July. The annual maximum of 50±21 ind. l^{-1} was recorded in September, with a dominance of Bivalvia larvae and the cladoceran species *Penilia avirostris* (Fig. 6G, H).

The analysis of the relationship between ciliated protozoa and micrometazoa groups

The correlation matrix between tintinnids, non-loricate ciliates and micrometazoan groups is given in Table 1. High coefficients of correlation indicate similar annual cycles of the groups studied. This particularly applies to copepods and their developmental stages.

The highest correlation coefficient of 0.68 was recorded between the number of nauplii and other micrometazoans, and between copepodites and adult small copepods (r=0.67). Positive and statistically significant correlations were also found between nauplii and adult copepods (r=0.48), between nauplii and copepodites (r=0.42) and between adult small copepods and other micrometazoans (r=0.47).

Table 1 Correlation coefficients between principal groups of ciliated protozoa and micrometazoa (*NLC* non-loricate ciliates; *TIN* tintinnids; *NAUP* copepod nauplii; *COP* copepodites; *ACOP* adult small copepods; *OM* other micrometazoans)

	Zooplankton groups					
	NLC	TIN	NAUP	COP	ACOP	OM
NLC	1.00	0.34*	0.18	0.10	0.19	0.14
TIN	0.34*	1.00	0.17	–0.09	–0.11	0.15
NAUP	0.18	0.17	1.00	0.42*	0.48*	0.68*
COP	0.10	–0.09	0.42*	1.00	0.67*	0.44*
ACOP	0.19	–0.11	0.48*	0.67*	1.00	0.47*
OM	0.14	0.15	0.68*	0.44*	0.47*	1.00

* Statistically significant at P<0.05; n=79

◀ **Fig. 6** Distribution of micrometazoans illustrated by box and whiskers column graphs of the seasonal distribution of **A** copepod nauplii, **C** copepodites, **E** adult small copepods and **G** other micrometazoans; vertical distribution of **B** copepod nauplii, **D** copepodites, **F** adult small copepods and **H** other micrometazoans

A PCA was carried out on the data sets for non-loricate ciliates, tintinnids, and micrometazoan groups. All time-series were previously detrended and standardised (zero mean, unit variance). The PCA extracted three clusters enclosing zooplankton groups with similar patterns of seasonal distribution. The first cluster comprised all micrometazoan groups: nauplii, copepodites, adult small copepods and other micrometazoans; the second cluster consisted of tintinnids; and the third contained non-loricate ciliates (Fig. 7).

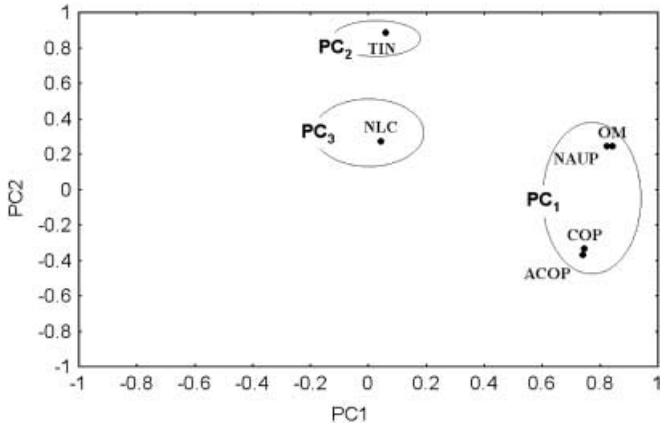


Fig. 7 Clustering of ciliated protozoan and micrometazoan groups obtained by principal component analysis (*PCA*)

Spearman rank correlation showed a relationship of ciliated protozoans and micrometazoans to several abiotic and biotic parameters (Table 2). Fluctuations in the abundance of bacteria, NNF and total mesozooplankton, as well as in the concentration of chlorophyll *a* are shown in Fig. 8.

High and statistically significant rank correlations were recorded between temperature and the micrometazoan zooplankton component (copepod nauplii, copepodites, adult small copepods, other micrometazoans). In addition, a statistically significant correlation was recorded for bacteria and adult small copepods. The coefficients of Spearman rank correlation between individual groups of microzooplankton are not given in Table 2, since they agree with the results of the Pearson correlation given in Table 1.

Table 2 Spearman rank correlations between average monthly densities of ciliated protozoa and micrometazoa, and individual abiotic and biotic factors, n=7 for bacteria and non-pigmented nanoflagellates (NNF), n=10 for other parameters (*NLC* non-loricate ciliates; TIN tintinnids; NAUP copepod nauplii; COP copepodites; ACOP adult small copepods; OM other micrometazoans)

Factor	NLC	TIN	NAUP	COP	ACOP	OM
Temperature	–0.12	–0.24	0.89**	0.79*	0.81*	0.81*
Salinity	–0.35	–0.16	–0.35	–0.14	–0.25	–0.09
Phytoplankton	0.36	0.56	–0.40	–0.59	–0.52	–0.30
Bacteria	–0.61	–0.11	0.32	0.29	0.89*	0.32
NNF	0.39	–0.61	–0.71	–0.21	0.11	–0.43
Mesozooplankton	–0.26	–0.47	0.16	0.41	0.65*	0.36

*P<0.05, **P<0.01

Fig. 8 Seasonal fluctuations in the abundance of bacteria (**A**), non-pigmented nanoflagellates (*NNF*) (**B**) and mesozooplankton (**C**). Seasonal cycle of chlorophyll *a* concentration (**D**)

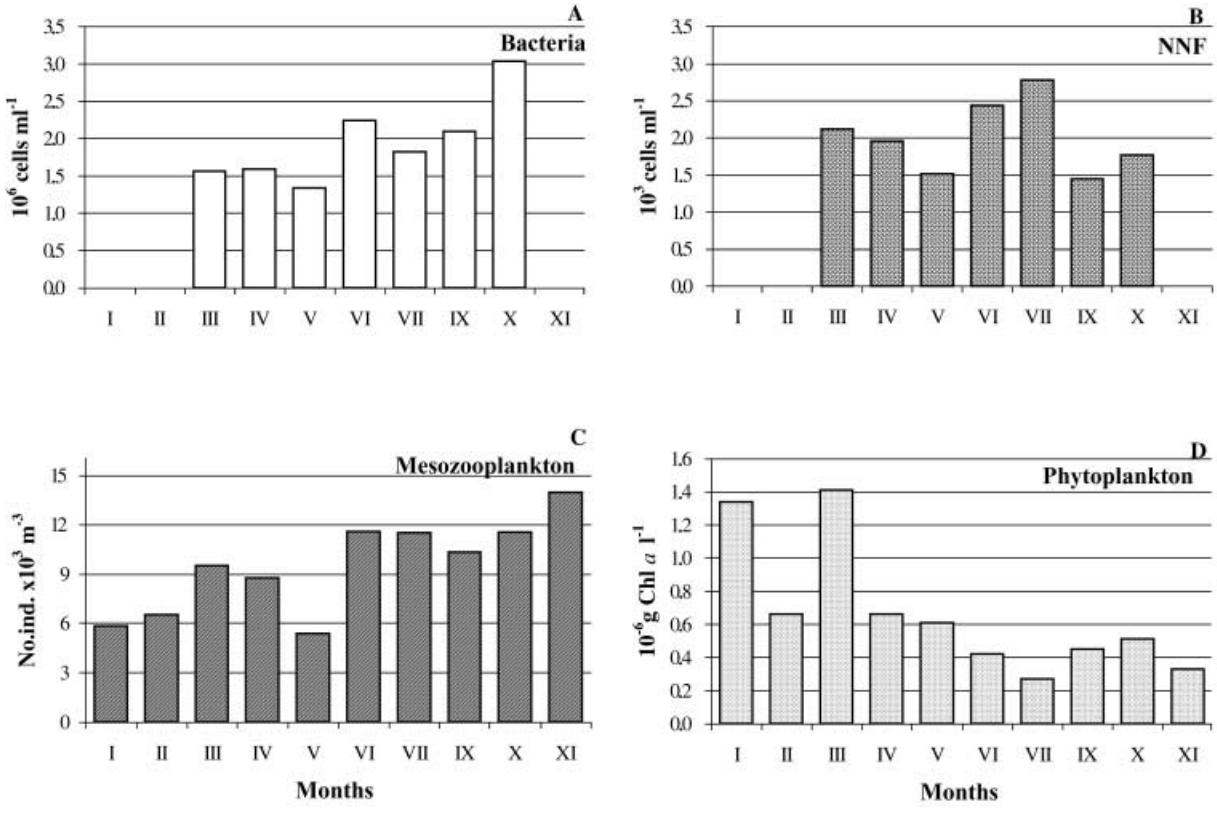


Table 3 Correlation coefficients between four size categories of non-loricate ciliates and individual abiotic and biotic factors (*NLC* non-loricate ciliates; *I* <10^3 μm³; *II* 10^3–10^4 μm³; *III* 10^4–10^5 μm³; *IV* >10^5 μm³)

Factor/NLC	I	II	III	IV
Temperature	–0.57*	–0.26	0.01	–0.14
Salinity	–0.25	–0.62*	–0.31	–0.21
Phytoplankton	0.73*	0.42*	0.31	0.02
Bacteria	–0.24	–0.18	0.11	0.18
Non-pigmented nanoflagellates	0.23	0.60*	0.16	0.04
Tintinnids	0.21	0.50*	0.11	–0.08
Copepod nauplii	–0.45*	–0.18	0.10	–0.32
Copepodites	–0.17	0.24	0.10	–0.06
Adult small copepods	–0.25	0.10	0.18	–0.03
Other micrometazoans	–0.26	0.03	0.04	–0.46*

* Statistically significant at $P<0.05$, $n=27$

On the basis of the relationship between some abiotic and biotic factors, and individual size categories of non-loricate ciliates, a significant correlation was recorded between phytoplankton and the smallest size category of non-loricate ciliates ($r=0.73$). The second size category of non-loricates was significantly correlated with NNF, tintinnids and phytoplankton. Furthermore, the smallest size category of non-loricate ciliates was negatively correlated with temperature and copepod nauplii, while the second size category of non-loricates was negatively correlated with salinity (Table 3).

Discussion

Non-loricate ciliates constitute the majority of ciliated protozoans. The highest density of these organisms in the Adriatic Sea (approximately 39,000 ind. l^{-1}) was recorded by Revelante and Gilmartin (1983) in the vicinity of the Po River Estuary during vertical water column stratification. According to Smetacek (1981), the protozooplankton biomass is highest in spring and autumn and lowest in winter. The total number of non-loricate ciliates in the Maine Estuary was highest in May with 5.4×10^5 ind. l^{-1} (Sanders 1987). These data agree with those for the Kaštela Bay, with the highest values recorded in spring and the lowest in winter.

The majority of non-loricates are found in the surface layers down to 10 m depth, as described by many earlier studies (Revelante et al. 1985; Edwards and Burkill 1995). At the same time, most of the phytoplankton biomass remains in the layer above the thermocline in the Kaštela Bay in summer. The bacterioplankton shows a similar vertical distribution. Such a vertical distribution of non-loricate ciliates is also affected by the temperature gradient and pycnocline depth (James and Hall 1995). The present study shows that low sea-water temperature and homothermy reduce the differences in the vertical distribution of non-loricates, and that the confinement of organisms to the surface layers in summer is probably due to the occurrence of the thermocline.

Of the known Adriatic tintinnid species, 38% were recorded from the Kaštela Bay. Such species diversity is due to the influence of both the mainland and the open sea. As in earlier studies, the species *Helicostomella subulata* was dominant. This species plays an important role in the coastal area food web. It occurs in high numbers in other, particularly eutrophicated, areas such as the northern Adriatic (Kršinić et al. 1988), Narragansett Bay (Hargraves 1981), and Bedford Basin (Paranjape 1980).

In contrast to protozoa, the average density of micrometazoa shows a gradual increase by the end of summer, which coincides in time with the reproduction of the summer copepods. Long-term studies of copepods in the Kaštela Bay show their high numbers for the whole period from spring to autumn (Regner 1985).

According to a number of authors, nauplii are the most numerous micrometazoa in the coastal area of the Adriatic Sea (Kršinić 1982; Revelante and Gilmartin 1983). The maximum density of nauplii is recorded from the Kaštela Bay in the summer–autumn period. An increase in their numbers in autumn is probably due to the reproduction of many copepod species and to a reduced importance of their predators. A decrease of nauplii density in June is accompanied by an increase in the number of copepodites. Positive correlation coefficients between nauplii, copepodites and adult small copepods are expected because these groups present the different developmental stages of copepods.

A year's study of microzooplankton in the Kaštela Bay in 1982 (F. Kršinić, unpublished data), found that nauplii population density at the surface in July was almost four times higher than that recorded in the present study. So far, the highest nauplii concentration in the Adriatic (1,139 ind. l^{-1}) was recorded from the subsurface layer of Šibenik Harbour in July 1995, with a dense population of the dinoflagellate species, *Prorocentrum minimum* (Kršinić and Njire 1996). However, it should be emphasised that vertical distribution of nauplii could not be observed without knowledge of ontogenetic migrations for each individual species, since the youngest developmental stages of many species inhabit the surface area during copepod reproduction, and descend to deeper layers with age.

With respect to their qualitative composition, the youngest developmental stages of calanoid copepods constitute the majority of copepod plankton of the Adriatic Sea. Their percentage presence in the Kaštela Bay was estimated to 27.5% (Gamulin 1938). Data gathered from the literature indicate that the highest recorded copepodite density of 72 ind. l^{-1} was measured at 5 m depth in Trieste Bay in June 1990 (Milani et al. 1991/1994). The vertical distribution of copepodites which we recorded was similar to that observed slightly further south in the Bay of Mali Ston (Rudenjak-Lukenda 1985) and at Gruz Harbour, Dubrovnik (Mikuš 1990). Some authors suggest that such a vertical distribution is probably due to the density gradient of phytoplankton, which concentrates in the layer above the pycnocline (Tiselius et al. 1994). However, our data indi-

cate that the correlation between the number of copepodites and phytoplankton biomass is not statistically significant for the observed yearly period.

Temperature is the most important abiotic factor affecting the distribution of micrometazoans. A positive correlation coefficient with temperature may account for the high density values of these organisms in the warmer part of the year. It was observed that the sea-water temperature affects the presence of individual size categories of non-loricate ciliates in the plankton. During the colder part of the year smaller organisms prevail in the plankton, whereas bigger ones prevail during the warmer part. The time of reproduction is characterised by a greater variety in the size of the organisms. Similar studies in the temperate seas show the dominance of small ciliates almost everywhere, particularly in winter, whereas bigger ciliates dominate in summer months (Leakey et al. 1992). High values for non-loricate biovolume in Kaštela Bay (Bojanić 1998) are probably due to the eutrophication of the area caused by high organic and inorganic matter discharges, which favour the development of phytoplankton and bacteria. The results of Beers and Stewart (1967) also show that waters closer to the coast contain higher densities and biovolume of microzooplankton.

Annual variations of abundance and biomass of microzooplankton and mesozooplankton in subtropical estuaries point to the fact that their abundances are partly dependent on food concentration (Buskey 1993). The present study shows that the phytoplankton bloom is accompanied by an increase in the number of ciliated protozoans and that this correlation is statistically significant (r=0.58; n=27; P<0.05). Thus the increase in density of the first two size categories of non-loricate ciliates after the phytoplankton bloom leads to the conclusion that there is a strong trophic relationship between these organisms. In addition, the metabolic products of non-loricates may affect an increase in primary production after remineralisation (Bishop et al. 1977; Revelante and Gilmartin 1983). The summer maximum of non-loricate ciliates may be due to high primary production caused by the eutrophication of the area. Studies of Revelante et al. (1985) showed that the eutrophication process leads to proportionate increases in the biomass of all ciliate categories.

NNF may be another food source for ciliates (Šolić and Krstulović 1994), as well as bacteria and other picoplankton (Sherr et al. 1986). The spring minimum of the bacterial biomass coincides in time with the maximum number of tintinnids. However, since the number of tintinnids varied between 80 and 526 ind. l^{-1}, with a mean value of 234±192 ind. l^{-1}, it is unlikely that their grazing considerably affects the size of the bacterial population. Adult small copepods and nauplii also significantly correlated with bacteria, which was probably the result of marked maxima in abundance during the summer for both groups.

Low numbers of herbivore microzooplankton in winter means a decrease in primary consumption, which probably accounts for the high phytoplankton biomass in that period. The second increase of phytoplankton biomass in spring was accompanied by an increase in the density of non-loricate ciliates and copepodites, and thereafter tintinnids and copepod nauplii. Since ciliates may utilise 20–70% of phytoplankton autotrophic organisms, they may have the same or even greater importance in grazing than small crustaceans (Raymont 1963; Leakey et al. 1992). The high density of ciliates in Kaštela Bay persisted until June and apart from being controlled by the quantity of available food, population size was also affected by metazoan grazing. In the summer the number of ciliates was low, and thus no considerable effect of these organisms on phytoplankton, bacteria and NNF densities could be expected. This is in accordance with the results, which showed that, during the warmer part of the year, the NNF population size was controlled by high bacterial production and not by ciliate grazing (Šolić and Krstulović 1995; Šolić et al. 1998). The second density maximum of tintinnids in October coincided in time with phytoplankton, bacteria and NNF biomass increase. High densities of copepodites of the genera *Oncaea*, juvenile Appendicularia, Bivalvia and Polychaeta larvae were also recorded, and these groups may also affect the number of protozoa.

This study pointed at the microzooplankton as an important zooplankton fraction characterised by high biomass and intensive population dynamics. There are very complex trophic relationships between microzooplankton groups, but also between them and a number of other groups of picoplankton and nanoplankton, both autotrophic and heterotrophic. Some micrometazoan groups could be an important link between the microbial food web and higher trophic levels. This study gives some new information for the coastal Adriatic Sea. However, this area warrants further investigation towards a better understanding of processes and relationships in the planktonic communities.

Acknowledgements Many thanks are due to Prof. Dr. F. Kršinić for his advice and assistance during the preparation of this manuscript. We are also grateful to the anonymous reviewers for their most valuable comments and helpful criticism.

References

Azam F, Fenchel T, Field JG, Gray JS, Mayer-Reil LA, Thingstad F (1983) The ecological role of water – column microbes in the sea. Mar Ecol Prog Ser 10:257–263

Beers JR, Stewart GL (1967) Micro-zooplankton in the euphotic zone at five locations across the California Current. J Fish Res Bd Can 24:2053–2068

Beers JR, Stewart GL (1971) Micro-zooplankton in the plankton communities of the upper waters of the eastern tropical Pacific. Deep-Sea Res 18:861–883

Bishop JKB, Edmond JM, Ketten DR, Bacon MP, Silker WB (1977) The chemistry, biology and vertical flux of particulate matter from the upper 400 m of the equatorial Atlantic Ocean. Deep-Sea Res 24:511–548

Bojanić N (1998) Annual variability of the population density and biovolume of non-loricate ciliates in the Kaštela Bay. Rapp Comm Int Mer Medit 35:376–377

Burkill PH, Edwards ES, John AWG, Sleigh MA (1993) Microzooplankton and their herbivorous activity in the northeastern Atlantic Ocean. Deep-Sea Res II 40:479–493

Buskey EJ (1993) Annual pattern of micro- and mesozooplankton abundance and biomass in a subtropical estuary. J Plankton Res 15:907–924

Capriulo GM, Carpenter EJ (1980) Grazing by 35 to 202 µm micro-zooplankton in the Long Sound. Mar Biol 56:319–326

Edwards ES, Burkill PH (1995) Abundance, biomass and distribution of microzooplankton in the Irish Sea. J Plankton Res 17:771–782

Gamulin T (1938) Report on research on the appearance of plankton copepods in the surroundings of Split between 1936–1937 (in Croatian). Godišnjak Ocean Inst 1:51–61

Haas LW (1982) Improved epifluorescence microscopy for observing planktonic micro-organisms. Ann Inst Oceanogr 58: 261–266

Hagström A, Azam F, Andersson A, Wikner J, Rassoulzadegan F (1988) Microbial loop in an oligotrophic pelagic marine ecosystem possible roles of cyanobacteria and nanoflagellates in the organic fluxes. Mar Ecol Prog Ser 49:171–178

Hargraves PE (1981) Seasonal variations of tintinnids (Ciliophora Oligotrichida) in Narragansett Bay, Rhode Island, USA. J Plankton Res 3:81–91

Heinbokel JF (1978) Studies on the functional role of tintinnids in the Southern California Bight. I. Grazing rates in laboratory cultures. Mar Biol 47:177–189

Hobbie JE, Daley RJ, Jasper S (1977) Use of nucleopore filters for counting bacteria by fluorescence microscopy. App Environ Microbiol 33:1225–1228

Holm-Hansen O, Lorenzen CJ, Holmes RW, Strickland JDH (1965) Fluorometric determination of chlorophyll. J Cons Perm Int Explor Mer 30:3–15

James MR, Hall JA (1995) Planktonic ciliated protozoa their distribution and relationship to environmental variables in a marine coastal ecosystem. J Plankton Res 17:659–683

Kršinić F (1980a) Qualitative and quantitative investigations of the tintinnids along the eastern coast of the Adriatic (in Croatian). Acta Adriat 21:19–104

Kršinić F (1980b) Comparison of methods used in micro-zooplankton research in neritic waters of the Eastern Adriatic. Nova Thalassia 4:91–106

Kršinić F (1982) Microzooplankton in the Bay of Kaštela and the adjacent area (in Croatian). Acta Adriat 23: 89–96

Kršinić F (1987) Tintinnines (Ciliophora, Oligotrichida, Tintinnina) in Eastern Adriatic Bays. Estuar Coast Shelf Sci 24:527–538

Kršinić F (1995) Changes in the microzooplankton assemblages in the Northern Adriatic Sea during 1989 to 1992. J Plankton Res 17:935–953

Kršinić F, Njire J (1996) Controlling the quality of the coastal sea (Project Vir–Konavle, Split, 1995) (in Croatian). In: Study and elaboration by IOR, Split, pp 55–62

Kršinić F, Mušin D, Rudenjak-Lukenda M (1988) Microzooplankton and eutrophication of the northern Adriatic (in Croatian). Pomorski Zbornik 26:601–612

Leakey RJG, Burkill PH, Sleigh MA (1992) Planktonic ciliates in Southampton Water their abundance, biomass, production and role in pelagic carbon flow. Mar Biol 114:67–83

Margaleff R (1963) Rôle des ciliés dans le cycle de la vie pélagique en Méditerranée. Rapp Comm Int Mer Medit 17:11–512

Mikuš J (1990) Microzooplankton of Gruz Harbour (in Croatian). Degree thesis, University of Zagreb, Zagreb

Milani L, Cabrini M, Martecchini E, Braun E, Fonda Umani S (1991/1994) Il microzooplancton in una stazione del Golfo di Trieste da ottobre 1988 a dicembre 1990. Nova Thalassia 12:311–369

Paranjape MA (1980) Occurrence and significance of resting cysts in a hyaline tintinnid *Helicostomella subulata* (Ehre.) Jorgensen. J Exp Mar Biol Ecol 48:23–33

Pelegrí SP, Christaki U, Dolan J, Rassoulzadegan F (1998) The use of *Echerichia coli* as food source for protozoa in excretion experiments: preliminary results. Rapp Comm Int Mer Medit 35:358–359

Pucher-Petković T, Marasović I (1988) Change of productivity conditions in the open middle Adriatic – eutrophication indicators (in Croatian). Pomorski Zbornik 26:585–593

Putt M, Stoecker DK (1989) An experimentally determined carbon:volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. Limnol Oceanogr 34:1097–1103

Rassoulzadegan F, Laval-Peuto M, Sheldon RW (1988) Partitioning of the food ration of marine ciliates between pico- and nanoplankton. Hydrobiologia 159:75–88

Raymont JEG (ed) (1963) Plankton and productivity in the oceans, vol 2. Pergamon, Oxford

Regner D (1985) Seasonal and multiannual dynamics of copepods in the middle Adriatic. Acta Adriat 26:11–99

Revelante N, Gilmartin M (1983) Microzooplankton distribution in the Northern Adriatic Sea with emphasis on the relative abundance of ciliated protozoans. Oceanol Acta 6:407–415

Revelante N, Gilmartin M, Smodlaka N (1985) The effects of Po River induced eutrophication on the distribution and community structure of ciliated protozoan and micrometazoan populations in the Northern Adriatic Sea. J Plankton Res 7:461–471

Rudenjak-Lukenda M (1985) The vertical and seasonal distribution of microzooplankton in the Bay of Mali Ston (in Croatian). MSc thesis, University of Zagreb, Zagreb

Sanders RW (1987) Tintinnids and other microzooplankton – seasonal distributions and relationships to resources and hydrography in a Maine estuary. J Plankton Res 9:65–77

Sherr EB, Sherr BF, Fallon RD, Newell SY (1986) Small, aloricate ciliates as a major component of the marine heterotrophic nanoplankton. Limnol Oceanogr 31:177–183

Smetacek V (1981) The annual cycle of protozooplankton in the Kiel Bight. Mar Biol 63:1–11

Stone L, Berman T (1993) Positive feedback in aquatic ecosystems: the case of the microbial loop. Bull Math Biol 55: 919–936

Strickland JDH, Parsons TR (1972) A practical handbook of seawater analysis. Bull Fish Res Bd Can 167:1–310

Šolić M, Krstulović N (1994) Role of predation in controlling bacterial and heterotrophic nanoflagellate standing stocks in the coastal Adriatic Sea seasonal patterns. Mar Ecol Prog Ser 114:219–235

Šolić M, Krstulović N (1995) Bacterial carbon flux through the microbial loop in the Kaštela Bay (Adriatic Sea). Ophelia 41:345–360

Šolić M, Krstulović N, Bojanić N, Marasović I, Ninčević Ž (1998) Seasonal switching between relative importance of bottom-up and top-down control of bacterial and heterotrophic nanoflagellate abundance. J Mar Biol Ass UK 78:755–766

Tiselius P, Nielsen G, Nielsen TG (1994) Microscale patchiness of plankton within a sharp pycnocline. J Plankton Res 16:543–554

Yentsch CS, Menzel DW (1963) A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Deep-Sea Res 10:221–231