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# Spatial and temporal variability in abundance and biomass of oligotrich ciliates in Kaštela Bay (middle Adriatic Sea)

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The nonloricate ciliate (NLC) community was investigated in Kaštela Bay from January 1998 until November 1999. Samples were collected at four sites characterized by different trophic states. Cell abundance, carbon content, and size categories of the oligotrich ciliates were investigated. NLC abundance was highest in spring with average values of 400-608 ind. $l^{-1}$ . Abundance during the second highest period, autumn-winter, was less pronounced. In the eutrophicated part of the bay, conditions were most favorable for NLC proliferation in summer, with the highest average density (1430 $\pm$ 1348 ind. $l^{-1}$ ) in August 1998. The size structure of the NLC populations changed with the trophic gradient. Organisms of <10 $^4$  µm $^3$  were useful in distinguishing trophic conditions in the marine ecosystem. Eutrophication favored growth of organisms of <10 $^4$  µm $^3$  while the succession of NLC categories changed without a clear seasonal pattern. Eutrophication also increased NLC biomass and affected seasonal fluctuations of biomass values. Average monthly biomass values ranged from 0.012 $\pm$ 0.014 to 3.925 $\pm$ 5.094 µgC  $l^{-1}$ . The high abundance and biomass in Kaštela Bay suggest that NLC play a critical role in the secondary production of the area.

Key words: Oligotrich ciliates, abundance, biomass, size categories, Adriatic Sea

#### INTRODUCTION

Ciliated protozoa are an important component of the plankton community (AZAM et al., 1983; SHERR et al., 1986). Because of their grazing activity, they are important as a carbon link in the microbial food web (CAPRIULO et al., 1991; GONZALEZ, 1999). Ciliates are consumed by larger zooplankton, benthos invertebrates, and fish larvae, forming a vital pathway for energy transfer to higher trophic levels in marine eco-

systems (STOECKER & CAPUZZO, 1990; PAFFEN-HOFER, 1998; VIDJAK *et al.*, 2006). Environmental variability plays a major role in determining spatial and temporal patterns of zooplankton distribution in shallow-water ecosystems. In general, the abundance of ciliates is associated with changes in standing stocks of phytoplankton, bacterioplankton, and heterotrophic nanoflagellates as well as with local hydrographic variables (KRSTULOVIĆ *et al.*, 1997; PARK & MAR-

SHALL, 2000; KAMIYAMA et al., 2003; STELFOX-WIDDICOMBE et al., 2004).

The structure of the zooplankton community reflects trophic states within an ecosystem (PARK & MARSHALL, 2000). The structure and composition of zooplankton assemblages are significantly altered by eutrophication, resulting in a smaller body size of ciliates and higher specific rate of metabolism (KIVI *et al.*, 1996; SCHIEWER, 1998; GISMERVIK *et al.*, 2002).

There are few studies on seasonal variability of abundance and biomass of ciliates in the Adriatic Sea. In earlier studies (KRŠINIĆ, 1982; BOJANIĆ et al., 2001, 2005), Kaštela Bay was chosen as an ideal location for analyzing the impact of eutrophication on the community structure of microzooplankton. In this paper, the abundance and carbon contents of oligotrich ciliate populations (NLC) in Kaštela Bay were determined. Emphasis was placed on determining whether the density and biomass of NLC size categories varied seasonally and spatially and, if so, whether such variations were related to seasonal and spatial trends in salinity and temperature. This is the first research of this kind in Kaštela Bay and one of very few in the Adriatic Sea.

#### MATERIAL AND METHODS

Kaštela Bay is a semi-enclosed coastal bay in the Middle Adriatic with a total area of 61 km<sup>2</sup>, volume of 1.4 km<sup>3</sup>, and average depth of 23 m (Fig. 1). The most important

fresh water source is the Jadro River, with an average annual inflow of ~10 m<sup>3</sup>s<sup>-1</sup>. The discharge of several submarine springs and Pantan Brook are of lower intensity. Kaštela Bay receives large quantities of untreated municipal and industrial effluents. Precipitation waters carry considerable quantities of nutrients. The geographic characteristics of the bay, the vicinity of the land, and anthropogenic impacts greatly influence the bay's hydrographic parameters. Water circulation is generated mostly by local winds related to the passage of mid-latitude cyclones over the area (GAČIĆ et al., 1987). These are more frequent during the winter; therefore water circulation and exchange with the open sea are more intense during winter. The eastern part of the bay is weakly coupled with the rest of the basin during calm weather in summer (ZORE-ARMANDA, 1980). The behavior of the eastern part of Kaštela Bay as a separate water body is evidenced by a different phytoplankton composition and increased eutrophication (MARASOVIĆ & PUCHER-PETKOVIĆ, 1991; MARASOVIĆ et al., 1991; VUKADIN et al., 1996). Seasonal fluctuations of phytoplankton, bacterioplankton, and heterotrophic nanoflagellate biomass as well as temporal variations of nutrient concentrations in the eutrophicated part of Kaštela Bay were detailed by BOJANIĆ et al. (2005, 2006) and VIDJAK et al. (2006)

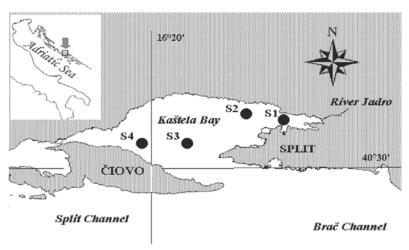


Fig. 1. The area of Kaštela Bay with sampling stations S1-S4

#### Sampling methods

Samples were collected monthly from January 1998 until November 1999 at stations S3 and S4 and from July 1998 at stations S1 and S2, with the exception of June and October 1998 and January, March, May and September 1999. Plankton was sampled at 5 m depth intervals from the water surface to the sea bottom, using 5-1 Niskin bottles. The samples were prepared for microscopic analysis as described in BOJA-NIĆ et al. (2005) and counted under an Olympus inverted microscope CK40 at x 200 magnification. The organisms were fixed with buffered formaldehyde at a final concentration of 2.5%. Formaldehyde was chosen instead of acid Lugol because it does not stain detritus (FONDA UMANI & BERAN, 2003) which can be abundant in the eutrophicated part of the bay. Since formaldehyde causes cell loss in NLC (LEAKEY et al., 1994), our data are somewhat underestimated. However, the relative values are reliable due to the consistent use of this fixation procedure throughout this study.

The biovolume of NLC was calculated by comparing the shape of the plasmatic body of each individual to one or more geometric bodies (EDLER, 1979). After measuring the dimensions with a calibrated ocular micrometer at a magnification of x 400, organisms were divided into four biovolume categories: I <10³  $\mu m^3$ , II = 10³-10⁴  $\mu m^3$ , III = 10⁴-10⁵  $\mu m^3$ , and IV >10⁵  $\mu m^3$ . We used a conversion factor of 0.14 pgC  $\mu m^3$ , as suggested by PUTT & STOECKER (1989), to transform the volumes into carbon biomass values.

Vertical temperature and conductivity profiles were measured with a conductivity/temperature/depth (CTD) multiparameter probe.

#### Data analysis

Non-parametric Spearman rank order correlations were used to assess the influence of environmental variables (temperature and salinity) on the density and biomass of the NLC. The same statistical method was used to analyze the relationships between total NLC abundance and biomass and size categories (StatSoft Statistica version 5.5). The Bray-Curtis coefficient was

used to analyze the similarity between stations, based on square root transformed NLC abundance and biomass data (Primer 5, version 5.2.9). The Abundance/Biomass Comparison (ABC) method was used to plot separate *k*-dominance curves (LAMBSHEAD *et al.*, 1983) for the abundance and biomass of NLC size categories on a single graph and comparing the curves. Since abundance and biomass are measured in different units, this plot became the basis for determining levels of disturbance of the investigated communities (WARWICK, 1986). The groups were ranked in order of importance in terms of abundance or biomass by percentage dominance (Primer 5, version 5.2.9).

#### **RESULTS**

#### Hydrography

Annual temperature oscillations were similar in all stations. The winter was characterized by temperature inversion and low average values, especially in February (~10°C; Fig. 2A). Surface layers became warmer at the end of the winter and beginning of spring, resulting in temperature stratification and formation of a thermocline in May. The vertical temperature gradient reached its maximum in July and August at depths of 10-20 m. The shallow stations (S1 and S4) warmed up faster and reached their maximums (>27°C) in July while maximum temperatures were reached in August at the deeper stations (S2 and S3). The highest temperature (27.26°C) was recorded at the surface of station S2 in August 1998. The thermocline disappeared as early as September and all layers cooled with approximately the same intensity.

Seasonal salinity fluctuations were similar in all stations. The average monthly salinity during most of the study was 37.00-37.50 psu, with salinity decreasing from April to July (Fig. 2B). The highest oscillations were recorded in the surface layer at station S1 (5.52 psu). Salinity increased with depth, except from January to March when all layers had almost equal salinity. After that, values decreased in the surface layers, having a direct impact on the vertical salinity gradient and reaching its highest in April-July 1999. The

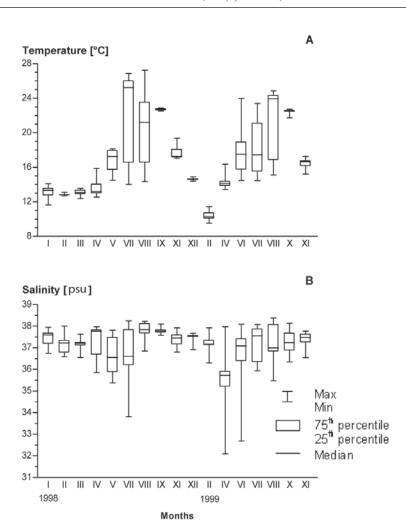


Fig. 2. Average monthly temperature (A) and salinity (B) at four stations in Kaštela Bay during 1998 and 1999 illustrated by box and whiskers column graph

highest gradient was measured at station S1 in July 1998 while the lowest salinity (32.09 psu) was recorded at station S3 in April 1999 with a vertical gradient of 5.89 psu.

#### Nonloricate ciliates (NLC)

#### **Abundance**

NLC abundance is shown in Fig. 3A. The lowest population density and seasonal oscillation were recorded in the central part of the bay (station S3), where the average monthly abundance ranged from 400±328 ind.l<sup>-1</sup> in May 1998 to 40±30 ind.l<sup>-1</sup> in November 1999. Apart from the spring peak, density peaked in autumn and remained high during January-February.

Abundance fluctuated almost identically in the western part of the bay at station S4 (r = 0.63, n = 85, p < 0.001), with high values (~600 ind. 1-1) in May 1998 and April 1999 and the lowest value in December 1998 (96±78 ind.l-1); although abundance was highest (704±260 ind.1-1) in January 1998. Density fluctuations at stations S2 and S1 were 1.3 and 2.5 times higher than at station S3, with very high abundance in summer. Density at station S2 varied from 50±38 ind.l-1 in November 1998 to 670±928 ind.l-1 in June 1999. The highest density (1430±1348 ind. 1-1) was recorded at station S1 in July 1998. In 1999, the highest abundance occurred in April (770±236 ind.1-1) and the lowest in November 1999 (20±23 ind.1<sup>-1</sup>).

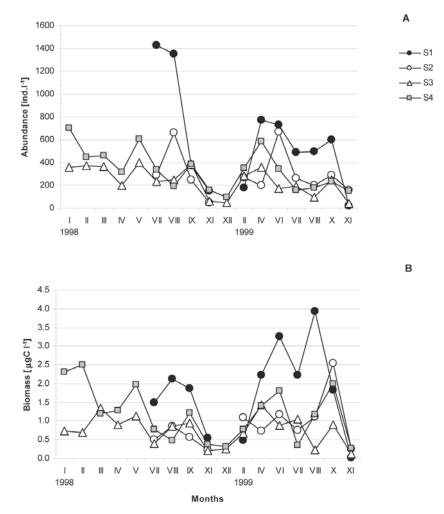


Fig. 3. Average density (A) and biomass (B) of nonloricate ciliates at four stations in Kaštela Bay

Cluster analysis showed that the highest similarity was recorded between stations S3 and S4 (90.09%). Similarity between these two stations and station S2 was 86.01%. Station S1 was identified as an outlier, significantly differing from the rest of the stations and sharing a similarity of only 76.88%.

#### **Biomass**

Average biomass values are shown in Fig. 3B. The lowest seasonal oscillations were found in the central part of Kaštela Bay (station S3) where biomass ranged from 0.13±0.13 μgC l<sup>-1</sup> in November 1999 to 1.42±0.61 μgC l<sup>-1</sup> in April 1999 and did not have clear seasonal pattern. Values were greater at the neritic stations and especially at the eutrophicated station (S1). Sea-

sonal oscillations were more pronounced in the western part of the bay (station S4) where it peaked three times in 1998: in February (2.50±2.05 μgC l<sup>-1</sup>), May (1.98±0.93 μgC l<sup>-1</sup>), and September (1.21±1.35 μgC l<sup>-1</sup>). Such fluctuations were also observed in June, august and October 1999. Similar seasonal variability was recorded at station S2 with a maximum in October 1999 (2.54±3.01 μgC l<sup>-1</sup>) and minimum in November 1998 (0.23±0.27 μgC l<sup>-1</sup>). Biomass was about 2.5 times higher at station S1 than in the central part of the bay, with a maximum average of 3.93±5.09 μgC l<sup>-1</sup> in August 1999 and minimum in November 1999, as at all stations.

There were significant differences in biomass among the stations. Cluster analysis divided the stations into two groups at a similarity

level of 76.85%. The greatest similarity was found between stations S2 and S4 (89.37%) and between these stations and station S3 (85.59%). Station S1 in the eutrophicated part of the bay was clustered separately.

#### Abundance - biomass relationship

Significant dependence between abundance and biomass was found at stations S3 and S4 (Fig. 4). At station S3, a high positive Spearman rank order correlation (r = 0.66; n = 17; p < 0.01) was conditioned by the increase in both parameters in spring and autumn, with low values in November and December. Differences were observed only in January and February 1998 when the increase in density was not accompanied by an equivalent rise in biomass due to the fact that small organisms prevailed in the plankton. Annual oscillations of both parameters at station S4 were quite similar as well (r = 0.78; n = 17; p < 0.001). Irregularities were evident in February 1998 and in August and October 1999 when a larger fraction of NLC dominated. At station S2, the correlation coefficient between abundance and biomass was significant but less pronounced (r = 0.63; n = 11; p<0.05) due to a high number of small NLC in August 1998 and June 1999. In the eutrophicated part of the bay (station S1), the coefficient of correlation between abundance and biomass data was insignificant.

#### Size categories

Organisms larger than  $10^3 \, \mu m^3$  (size I) represented less than 2.88% of the total number of NLC; the largest category (size IV, >10<sup>5</sup>  $\, \mu m^3$ ) was poorly represented (2.68% at station S2 and 5.56% at station S1). Sizes II ( $10^3$ - $10^4 \, \mu m^3$ ) and III ( $10^4$ - $10^5 \, \mu m^3$ ) were quantitatively dominant. In the eutrophicated part of the bay, the proportion of size II rose from 40.33% to 55.42% while the proportion of size III dropped from 55.22% to 37.85%. Average abundance values for all the size categories at all the stations are given in Table 1 while seasonal variability in abundance of the four size categories is shown in Fig. 5.

The quantitatively dominant size in the western part of the bay (size III) was especially

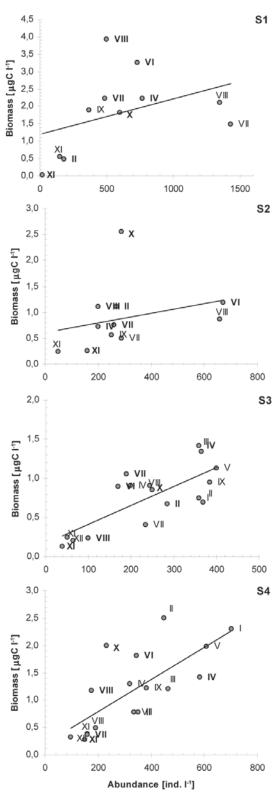


Fig. 4. Interdependence of nonloricate ciliates abundance and biomass at four stations in Kaštela Bay during 1998 and 1999 (months of 1999 are marked in bold)

Table 1. Nonloricate ciliate abundance (ind.l<sup>-1</sup>) and biomass (μgC l<sup>-1</sup>) according to size class\*, means± standard deviation

Gu - 4 i	Size I		Size II		Size III		Size IV	
Station	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass
S1	13±33	0.0015±0.0038	355±393	0.25±0.25	203±121	0.87±0.66	29±28	0.69±0.65
S2	15±42	$0.0017 \pm 0.0046$	$167 \pm 148$	$0.10\pm0.09$	109±39	0.52±0.22	8±10	$0.28 \pm 0.54$
S3	4±6	$0.0005 \pm 0.0007$	111±70	$0.08\pm0.05$	116±70	0.49±0.30	8±8	0.18±0.20
S4	3±5	$0.0003 \pm 0.0006$	129±70	$0.09\pm0.05$	194±125	0.76±0.43	10±11	0.35±0.43

<sup>\*</sup> I <103  $\mu m3;$  II = 103-104  $\mu m3;$  III = 104-105  $\mu m3;$  IV >105  $\mu m3$ 

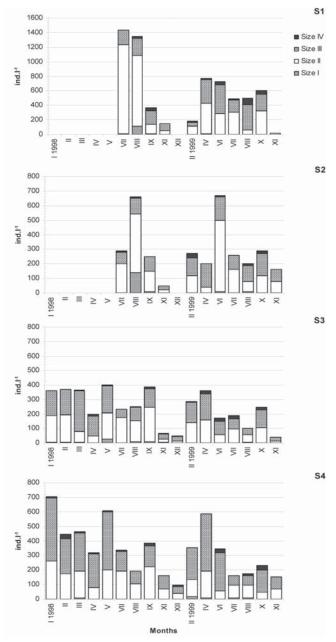


Fig. 5. Abundance of four nonloricate ciliate size categories at four stations (Size  $I<10^3 \mu m^3$ ;  $II=10^3-10^4 \mu m^3$ ;  $III=10^4-10^5 \mu m^3$ ;  $IV>10^5 \mu m^3$ )

abundant from January to June with a maximum of 432 ind.1-1 in January 1998. The abundance of small NLC increased towards the east. Variability in the middle of the bay was conditioned by changes in size II and III abundance, which represented as much as 94.09% of the total NLC. Size II prevailed in the warmer part of the year (235 ind.l-1 in September 1998) while size III was more abundant in spring (280 ind.1-1 in March 1998). Seasonal oscillations of abundance in the eastern part of the bay corresponded to fluctuations of size I and II organisms (<10<sup>4</sup> μm<sup>3</sup>) which represented 52.98% at station S2 and 56.59% at station S1. Abundance of both these sizes was extremely high in summer, with a maximum of 140 size I ind.l-1 at station S2 in August 1998 and 1220 size II ind.l-1 at station S1 in July 1998. Correlation matrices between total abundance and abundance of size categories are given in Table 2.

The proportion of individual size categories to the total biomass varied horizontally and temporally. The smallest size represented less than 1% of the total biomass. Size II ranged from 9.70% at station S4 to 23.23% at station S1. Size III represented 45.69% at station S1 and 68.21% at station S2 while size IV represented 18.54% at station S1 and 31.01% at station S2 (Table 1).

Size I did not significantly influence the annual fluctuation of total NLC biomass (Fig. 6). The highest biomass in this group did not exceed  $0.0156~\mu gC~l^{-1}$ and its share in the total biomass was no higher than 1.79% in August 1998 at station S2. Size II organisms contributed a little

more to the total biomass, especially during the summer in the eastern part of the bay (Fig. 6). In that period, size II represented 46.95% and 28.26% at stations S1 and S2, respectively. Seasonal variability in biomass was defined by temporal variability of organisms larger than 10<sup>4</sup> μm<sup>3</sup> (sizes III and IV). In the central and western parts of the bay, the highest size III biomass was recorded in spring whereas this size dominated in the eastern area in summer. The highest size III biomass values were recorded at the shallowest stations: S4 (1.60 µgC l<sup>-1</sup>in May 1998) and S1 (2.04 µgC 1<sup>-1</sup>in June 1999), corresponding to 80.57% and 62.77% of the total biomass, respectively. The biomass of size IV was extremely high in the summer and autumn with the highest values occurring in the eastern part of the bay at S1 in August 1999 (1.88 μgC l<sup>-1</sup>) and at S2 in October 1999 (1.83 µgC 1<sup>-1</sup>), when they represented 47.79% and 72.11% of the total biomass, respectively.

#### Relationship to abiotic parameters

Spearman rank order correlations identified temperature as an important abiotic variable only in the eutrophicated part of Kaštela Bay (S1), highly and significantly correlating with NLC abundance and biomass (Table 3). At other stations this parameter was weakly correlated with the number and carbon content of the NLC. Salinity significantly and negatively correlated with abundance and biomass of NLC at all stations (Table 3). The NLC abundance always more strongly correlated with salinity than with biomass.

Table 2. Spearman rank order correlations (r) between total nonloricate ciliate (NLC) abundance and biomass and abundance and biomass of the four NLC size categories\*, according to site

	S1 (n = 11)		S2 (n = 11)		S3 (n =	= 17)	S4 (n = 17)	
	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass	Abundance	Biomass
Size I	0.618	ns	ns	ns	0.551	ns	ns	ns
Size II	0.909	ns	0.881	ns	0.870	0.498	0.789	ns
Size III	0.736	0.855	ns	0.682	0.880	0.858	0.923	0.873
Size IV	ns	0.826	0.649	0.725	ns	0.548	ns	0.763

Correlations are significant at p<0.05

<sup>\*</sup> See Table 1.

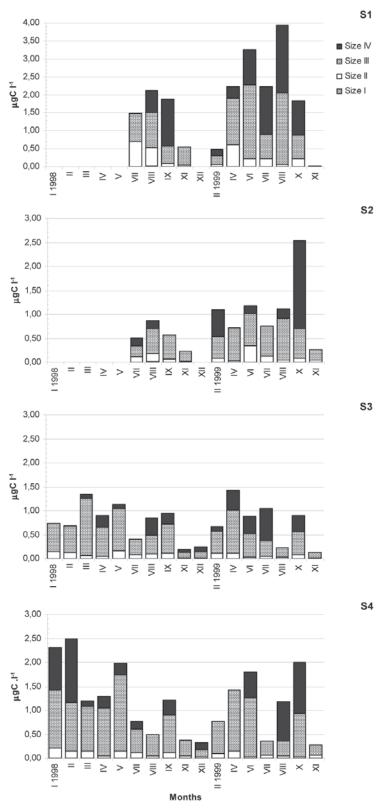


Fig. 6. Biomass of four nonloricate ciliate size categories at four stations (Size I<10³  $\mu$ m³; II = 10³-10⁴  $\mu$ m³; III = 10⁴-10⁵  $\mu$ m³; IV >10⁵  $\mu$ m³)

Table 3. Spearman rank order correlations between abundance and biomass of nonloricate ciliates (NLC) and abiotic parameters

Station	NLC	Temperature	Salinity	
S1 (n = 40)	Abundance	0.678***	-0.437**	
	Biomass	0.529***	ns	
S2 (n = 40)	Abundance	0.430**	-0.571***	
	Biomass	0.356*	-0.478**	
S3 (n = 85)	Abundance	ns	-0.476***	
	Biomass	ns	-0.369***	
S4 (n = 64)	Abundance	-0.303*	-0.524***	
	Biomass	-0.334**	-0.339**	

<sup>\*</sup>p<0.05; \*\*p<0.01; \*\*\*p<0.001; ns = not significant

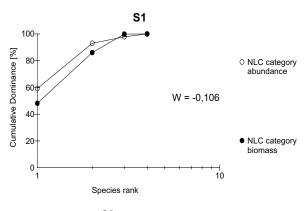
### Abundance/Biomass Comparison (ABC) method

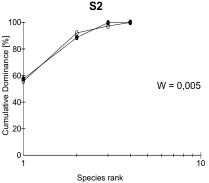
The NLC community in the eastern eutrophicated part of Kaštela Bay (station S1) had a disturbed pattern with the abundance curve plotted above the biomass curve (Fig. 7). In the less productive area of the bay (stations S2, S3, and S4), the inequality in size between the abundance and biomass dominants was less, so that the curves were closely coincident and crossed each other one or more times.

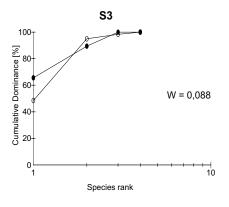
#### **DISCUSSION**

NLC in the coastal area of the middle Adriatic were first investigated quantitatively during four seasonal cruises in 1973 and 1974; NLC were of great importance only in July 1973 and February 1974, with the highest value in Marina Bay (17200 ind.m<sup>-3</sup>; KRŠINIĆ, 1982). An investigation in Kaštela Bay during 1982 indicated that NLC abundance increased in January and May, with a maximum of 357 ind.l<sup>-1</sup> in January at a depth of 1 m, less than expected considering the high production in the bay (KRŠINIĆ, unpubl. data).

More recent studies on NLC density in Kaštela Bay revealed higher values than estimated earlier and peaks that varied considerably







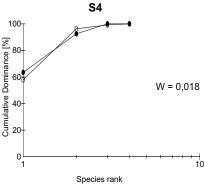


Fig. 7. Abundance/Biomass Comparison plots based on nonloricate ciliates (NLC) abundance and biomass, aggregate of four size categories at four stations in Kaštela Bay

on spatial and temporal scales (BOJANIĆ et al., 2001, 2005). Our results confirmed these findings. Abundance was highest in spring, particularly in April, while a second maximum was always less pronounced. Apart from the eastern eutrophicated part of Kaštela Bay, where favorable conditions for NLC reproduction were in summer, the second peak was in autumn-winter. These results indicate different roles of NLC in the microbial food web with respect to the trophic state of the investigated area. They also emphasize the importance of more frequent sampling.

In comparison with other neritic areas of the Adriatic Sea, the highest similarities of temporal variability of NLC abundance were found in Mali Ston Bay, Kotor Bay, and the Neretva Channel (KRŠINIĆ & MUŠIN, 1981; KRŠINIĆ & VILIČIĆ, 1989; BOJANIĆ, 2001). However, abundance values in Kaštela Bay were relatively higher than in these areas. Average NLC densities in Mali Ston Bay ranged 10-50 ind.1-1 (KRŠINIĆ & MUŠIN, 1981), and the maximum was five times lower than the value we recorded in the eastern part of Kaštela Bay (Table 4). NLC were of quantitative importance only during the cold period in the Kvarner area, when they represented 90% of the total microzooplankton (KRŠINIĆ, 1979). Nevertheless, this maximum was 7 and 18 times less than the peaks we recorded in the central and eastern parts of Kaštela Bay, respectively. In Rijeka Bay and the northern part of Velebit Channel, oligotrich ciliates were also of less importance, with densities significantly lower than in Kaštela Bay.

On the other hand, considerably higher NLC abundances were recorded in the northern Adriatic Sea. In the Gulf of Trieste, ciliated protozoa were most abundant in winter, early spring, and autumn, with over 2000 ind.1-1 (MOZETIĆ et al., 1998). Along the transect from Istra Peninsula to the mouth of the Po River, abundance increased from 1612±1396 to 5997±10092 ind.1-1 during the thermal stratification of water column, while the lowest values occurred during isothermal conditions (~480 ind.l<sup>-1</sup>; REVELANTE et al., 1985). However, the sampling method was somewhat different than ours and these abundance values were not confirmed by later regular multiannual investigations in the same area (KRŠINIĆ, 1995).

Seasonal variability of NLC abundance in Kaštela Bay resembles the NLC distribution in other marine coastal ecosystems, although the intensities of the changes differ obviously. In the Chesapeake Bay, the highest abundances of ciliated protozoa were recorded at the end of spring and beginning of summer, with values higher

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Area	Maximum abundance	Study
North Adriatic	39280*	REVELANTE & GILMARTIN, 1983
Gulf of Trieste	2070	MOZETIĆ et al., 1998
Istrian Peninsula region	1312	KRŠINIĆ, 1995
Rijeka Bay	60	KRŠINIĆ, 1981
Kvarnerić Bay	152	KRŠINIĆ, 1979
Šibenik Bay	10770	KRŠINIĆ & NJIRE, 2001
Kaštela Bay (western)	1120	This paper
Kaštela Bay (middle)	1240	This paper
Kaštela Bay (eastern)	2840	This paper
Neretva Channel	496	BOJANIĆ, 2001
Mali Ston Bay	560	KRŠINIĆ & MUŠIN, 1981
Kotor Bay	673	KRŠINIĆ & VILIČIĆ, 1989

Table 4. Maximum nonloricate ciliate abundance in other regions of the Adriatic Sea

<sup>\*</sup> sample obtained by pooling 2-l samples collected from five depths

than 15 x103 ind.1-1 in April (DOLAN & COATS, 1990) and, after the ciliate abundance dropped in late summer and early autumn, another increase was recorded in October. Such seasonal variability was explained by the occurrence of a large number of small scuticociliates in April and the appearance of large oligotrich ciliates in September (DOLAN & COATS, 1990). NLC abundance also peaked in spring in an estuary in Maine  $(45 \times 10^3 \text{ ind.}1^{-1}; \text{ SANDERS, } 1987)$ , the Gulf of Maine (6 x 10<sup>3</sup> ind.1<sup>-1</sup>; MONTAGNES et al., 1988), and Nueces estuary (25 x 103 ind.1-1; BUSKEY, 1993). The significantly higher abundance in Kaštela Bay may be attributed to the biology and physiology of the NLC species, the hydrographic and trophic states of the marine ecosystem, sampling and fixation methods, and differences in microscopic analysis that has not yet been standardized. MONTAGNES et al. (1988) confirmed the importance of ciliate size categories for better understanding their temporal variability. During summer, ciliates with a cell volume of 10<sup>2</sup>-10<sup>3</sup> μm<sup>3</sup> dominated, representing >80% of the total ciliates, while in autumn-winter, organisms in the 10<sup>3</sup>-10<sup>4</sup> μm<sup>3</sup> size category were more abundant and in late winter and spring, the 10<sup>4</sup>-10<sup>5</sup> μm<sup>3</sup> size class was most abundant. Ciliates of  $> 10^5 \,\mu\text{m}^3$  never represented more than 4% of the total (MONTAGNES et al., 1988).

Our results generally agree with these findings. Variations were found for size I oligotrichs which contributed less then 3% to the total. Size III (10<sup>4</sup>-10<sup>5</sup> µm³) dominated in number, except during summer when size II dominated. Such a size structure was probably the cause of the weak correlation between abundance and temperature, even when the total number of NLC was considered. Our results suggest that the size structure of NLC populations changes along a trophic gradient with eutrophication favoring sizes I and II and no clear seasonal pattern to the succession of size categories.

In addition to abundance, biomass helps to understand ciliate ecology. Although the seasonal distribution and oscillations of ciliate biomass are well known for many marine ecosystems, relatively little is known for the Adriatic Sea (REVELANTE & GILMARTIN, 1983;

REVELANTE et al., 1985; LIPEJ, 1992; CATALETTO et al., 1993; FONDA UMANI & BERAN, 2003). The first data concerning the distribution of ciliate biomass was published for the northern Adriatic and indicated a dominant role of naked oligotrichous ciliates, except in summer when tintinnids contributed as much to the protozoa biomass (REVELANTE & GILMARTIN, 1983). A somewhat different situation was found in Kaštela Bay where NLC always participated more than tintinnids in the total ciliate biomass, except in autumn. Significant variations were recorded at the eutrophicated station S1 where tintinnids represented over 65% of the ciliate biomass in April and July 1999 (BOJANIĆ et al., 2005).

The level of eutrophication affects total ciliate biomass as well as abundance. REVE-LANTE et al. (1985) found that eutrophication increased the biomass of a ciliated protozoan population proportionally in each size category, without changing the basic size structure of the population. Our research only partly confirmed this hypothesis because, although eutrophication increased the NLC biomass, it also changed the size structure and seasonal variability of the NLC populations. Significant differences were noticed for size III in the central (S3) and western (S4) parts of Kaštela Bay as compared to eastern station S1. NLC biomass was high from the end of winter until the beginning of autumn, when tintinnids dominated the ciliate biomass at the mesotrophic stations S2, S3, and S4 (BOJANIĆ, unpubl. data). On the other hand, the succession of both groups changed irregularly in the eutrophicated station S1 (BOJANIĆ et al., 2005), indicating a special character in the eastern part of the bay where alteration of the ciliate biomass affects the flow of energy in the marine food web.

NLC biomass in the Gulf of Trieste and Kaštela Bay was similar, with the highest values recorded during summer (Table 5). Seasonal distribution in Kaštela Bay was similar to other neritic ecosystems. In Kiel Bight, ciliated protozoa biomass was high in spring and autumn, low in summer, and lowest in the winter (SMETACEK, 1981), the same as in the central part of Kaštela Bay. In St. Lawrence Bay, ciliate biomass rose

Table 5. Ranges of				

Area	Biomass (μg C/l)	Study
North Adriatic	0.038-41.458	REVELANTE & GILMARTIN, 1983
Gulf of Trieste	0.017-~4.500	LIPEJ, 1992; CATALETTO et al., 1993
Kaštela Bay (western)	0.000-5.752	This paper
Kaštela Bay (middle)	0.000-3.297	This paper
Kaštela Bay (eastern)	0.000-11.359	This paper
Gulf of Maine	0.041-3.629	MONTAGNES et al., 1988
$10^2 - 10^3 \ \mu m^3$	0.006-0.054	
$10^3 - 10^4 \ \mu m^3$	0.011-0.877	
$10^4$ - $10^5 \ \mu m^3$	0.000-2.117	
$>10^{5}  \mu m^{3}$	~0.907	
Southern Kattegat:		NIELSEN & KIØRBOE, 1994
Oligotrich ciliates 10-15 µm	< 0.2-1.0	
Oligotrich ciliates 30-60 µm	<1-14.0	
Laboea strobilia and Tontonia spp.	<1-6.0	
Damariscotta estuary	0.019-30.400	REVELANTE & GILMARTIN, 1987

in spring and organisms over 20 µm prevailed (TAMIGNEAUX et al., 1997). Differences were found only in eutrophicated marine ecosystems where high biomass prevailed during summer (LEAKEY et al., 1992).

Our results suggest that salinity is an important abiotic variable that impacts seasonal distribution of NLC abundance and biomass. The influence of temperature is less pronounced, except in the eutrophicated eastern part of Kaštela Bay. However, relationships within the microbial food web are complex and significant correlation does not automatically imply a direct causal relationship. Negative correlations with salinity could be a consequence of the vertical distribution of the NLC, as most NLC prefer near-surface layers (JAMES & HALL, 1995; BOJA-NIĆ et al., 2001). These results should be tested on a larger data set in the future.

The Abundance/Biomass Comparison method showed that the structure of the NLC community differed in the eastern eutrophicated part of Kaštela Bay when compared to mesotrophic stations in the central and western parts. NLC smaller than 10<sup>4</sup> µm<sup>3</sup> (sizes I and II) were particularly useful in distinguishing trophic conditions in the marine ecosystem. At stations with moderate pollution (S2, S3, S4), large competitive dominants are eliminated and the size inequality between numerical and biomass dominants is reduced. Hence, the biomass and abundance curves are closely coincident and cross each other one or more times. As pollution becomes more severe (station S1), the NLC community is increasingly dominated by small species and the abundance curve always lies above the biomass curve. Although no direct measurements were made of ciliate grazing rates, given their abundance and biomass and the fact that most of the chlorophyll and primary production in Kaštela Bay is in the category less than 10 μm (NINČEVIĆ, 2000), NLC must play a critical role in secondary production in the water column.

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## Prostorne i vremenske promjene brojnosti i biomase oligotrihnih ciliata u Kaštelanskom zaljevu (srednji Jadran)

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#### SAŽETAK

Zajednica nelorikatnih ciliata (NLC) je istraživana u Kaštelanskom zaljevu od siječnja 1998. do studenog 1999. godine. Uzorci su sakupljeni na četiri postaje različitog trofičkog stanja. Istraživana je brojnost, sadržaj ugljika i veličinske kategorije oligotrihnih cilijata. Najviša je brojnost NLC zabilježena u proljeće s prosječnim vrijednostima od 400 do 608 jed.l¹, dok je drugi godišnji maksimum, u jesensko-zimskom razdoblju, slabijeg intenziteta. U eutrofiziranom su dijelu Kaštelanskog zaljeva povoljni uvjeti za razmnožavanje NLC zabilježeni u ljetnom razdoblju s najvišom prosječnom gustoćom od 1430±1348 jed.l¹ (kolovoz 1998.). Veličinska se struktura populacija ovih organizama mijenjala i duž trofičkog gradijenta. Organizmi biovolumena manjeg od 10⁴ μm³ su korisni za razlikovanje trofičkih uvjeta u morskom ekosustavu. Eutrofikacija povoljno djeluje na zastupljenost ove veličinske kategorije organizama, a sezonska sukcesija veličinskih kategorija NLC postaje nepravilna. Osim toga eutrofikacija utječe i na povećanje biomase NLC, te na njene sezonske promjene. Prosječne su vrijednosti biomase na istraživanim postajama kolebale u granicama od 0.012±0.014 do 3.925±5.094 μgC l¹¹. Visoke vrijednosti brojnosti i biomase NLC u Kaštelanskom zaljevu ukazuju na iznimnu važnost ovih organizama u sekundarnoj proizvodnji ovog područja.

Ključne riječi: Oligotrihni cilijati, brojnost, biomasa, veličinske kategorije, Jadransko more