

## Environmental drivers of zooplankton variability in the coastal eastern Adriatic (Mediterranean Sea)

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*The objectives of this paper were to determine the main environmental drivers of zooplankton variability in coastal waters adjacent to urban areas and to evaluate the differences in zooplankton abundance and population structure in relation to chemical and biological parameters in the water column. Samples were collected seasonally from May 2006 to January 2009 at 8 sampling sites in the bays and channels along the eastern Adriatic coast. Zooplankton population structure showed high similarity within the investigated region, especially evident in the homogeneity of copepod community composition, where relative importance of the individual species showed characteristic high ranking of small and medium-sized taxa. Zooplankton numerical variability primarily responded to seasonal variation in water temperature and spatial variation in salinity, but spatial distribution of the collected data showed that abundances were also linked to chemical and biological parameters generally used as descriptors of water quality. This indicates that zooplankton community size reflects the trophic status of an area and supports the use of zooplankton studies as an auxiliary method in the evaluation of the trophic state of coastal waters.*

**Key words:** coastal zooplankton, copepods, trophic state, water quality, Adriatic Sea

### INTRODUCTION

Coastal waters are among the most productive marine environments, where anthropogenic inputs from rivers, runoff and sewage systems often result in more eutrophic conditions (LEVINTON, 1995). In recent decades urban coastal settlements exert strong pressures on the environment, and coastal zones around the globe are showing increasing evidence of degradation due to human activities, which subsequently

influence the living resources and human health (MARCUS, 2004; DUARTE *et al.*, 2009). Environmental degradation includes the loss of coastal water quality through, among others, the impact of excess nutrients and the presence of organic pollutants originating from the industrial discharge and domestic sewage (AVANZINI, 2009; BOISSERY, 2009; JICKELLS, 2008).

Direct and causal relationship between phytoplankton and bioavailable nutrients is readily explored in monitoring programs aiming at

water quality evaluation or environmental status assessment (e.g. EU Water Framework Directive and Marine Strategy Framework Directive, respectively, in BORJA (2005) and BORJA *et al.* (2010)). Zooplankton are frequently used as biotic indicators of water quality in freshwater where community size and relationships among copepods, cladocerans, ostracods and rotifers provide valuable information on the existing physical and chemical conditions (SOUSA *et al.*, 2008; PATUREJ, 2009), presence of different pollutants (BALOGH, 1988; BONACINA & PASTERIS, 2001), trophic state (WHITMAN *et al.*, 2004; PATUREJ & GOŹDZIEJEWSKA, 2005) and environmental stress (PINTO-COELHO *et al.*, 2005; HSIEH *et al.*, 2011). However, although zooplankton has been extremely well studied in marine ecosystems, its potential value as indicators of alterations in the marine environmental status still needs to be assessed. Higher trophic levels in the marine plankton usually receive less attention in environmental monitoring (GISMERVIK *et al.*, 1996; PERRY *et al.*, 2004), although changes in the abundance, distribution and succession of zooplankton organisms are indicative of changes in the environmental conditions (CAPRIULO *et al.*, 2002; MARQUES *et al.*, 2008; FALCÃO *et al.*, 2011). Therefore regular monitoring of zooplankton communities in coastal waters adjacent to urban areas under increased threat or existing pressure from decrease in water quality through eutrophication or other adverse processes is a valuable aid in developing effective strategies for ecosystem management.

Within the frame of the "Coastal Sea Water Quality Control Programme" conducted by the Institute of Oceanography and Fisheries on behalf of Croatian water management company (Croatian Waters), the state of marine biological communities, as well as physical and chemical properties of the water column and sediment have been monitored in the coastal waters of the eastern Adriatic Sea since 1976 until present days (UJEVIĆ *et al.*, 1998; BOGNER *et al.*, 2004; MILUN *et al.*, 2006). During this period documented important changes in the zooplankton component, particularly those concerning copepod crustaceans, included increased densities and

reduced biodiversity caused by the progressive eutrophication of the eastern Adriatic coastal areas (REGNER, 1989, 1991, 1992). Since recent results pointed at the significant improvement in the overall state of the Croatian coastal waters (KUŠPILIĆ *et al.*, 2009; ŠOLIĆ *et al.*, 2010), in this study we present the analysis of the zooplankton community in the 2006-2009 period. The main objectives are: (1) to determine the variability of zooplankton assemblages in the coastal waters in the vicinity of larger urban settlements in relation to physical parameters of the marine environment and particularly, (2) to investigate the potential impact of chemical and biological descriptors of water quality such as nutrient concentrations and phytoplankton biomass to zooplankton abundance and population structure.

## MATERIAL AND METHODS

### Study area

Samples were collected four times per year from May 2006 to January 2009, the chosen periods corresponding to spring (May), summer (July), autumn (September-October) and winter (December-January) conditions in the water column.

The sampling stations are situated in coastal waters in the vicinity of larger settlements along the eastern Adriatic coast (Fig. 1). Stations PG1 and PG2 are located in the waters surrounding the island of Pag, which belongs to the northern Adriatic archipelago (Fig. 1A). The island extends northwest-southeast along the coast, forming the Velebit Channel. Station PG1 (44°28'1"N; 15°1'59"E, max depth 10 m) is situated in the shallow part of the Bay of Pag which is deeply incised into the coast of the island of Pag, and connected through the narrow Straits of Pag (width 780 m, depth 57 m) with the wider area of the Velebit Channel, while station PG2 (44°26'49"N; 15°2'58"E, max depth 52 m) is located in the Straits. The inner Bay area was the recipient of domestic effluents of the city of Pag and the neighbouring settlements until 2004, when the activation of the new

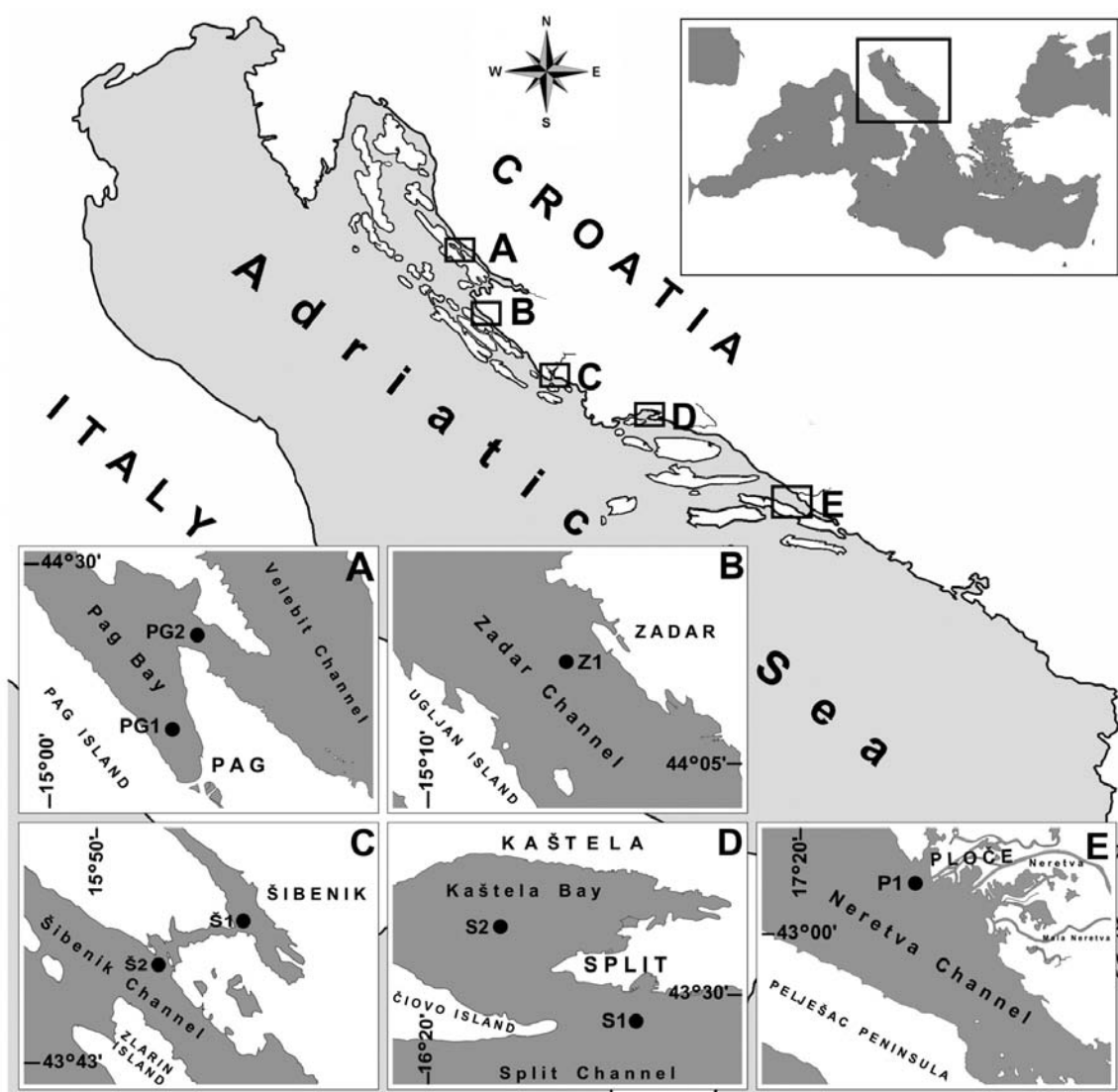


Fig. 1. Study area with investigated stations

sewage system transported the effluents in the deeper area of the Straits of Pag.

Station Z1 (44°6'36"N; 15°12'30"E, max depth 33 m) is located in the 4-7 km wide and 20 km long Zadar Channel, extending between the mainland and the island of Ugljan (Fig. 1b). Maximum depth is 50 m. In the investigated period, the Zadar Channel was the recipient of the domestic and industrial effluents of the city of Zadar and some neighbouring settlements, since the modern wastewaters treatment unit was not fully activated until April 2009.

Stations Š1(43°44'3"N; 15°53'31"E, max depth 35 m) and Š2 (43°43'7"N; 15°51'31"E,

max depth 10 m) are located in the lower part of the Krka River estuary, the former in the central part of the Šibenik City harbour, and the latter in the Šibenik Channel (Fig. 1C). Nutrient enrichment in this area comes from natural (Krka River) and anthropogenic sources (local sewage). Despite recently recorded decrease in anthropogenic input of sewage and domestic waste waters, this is still one of the most productive coastal areas on the eastern Adriatic coast (KUŠPILIĆ *et al.*, 2007).

Station S2 (43°31'6"N; 16°22'54"E, max depth 37 m) is located in the central part of the Kaštela Bay, a semi-enclosed coastal bay in

the middle Adriatic Sea (Fig. 1D). Due to the rapid urbanization and industrialization in the 1970s, Kaštela Bay has been receiving large quantities of untreated municipal and industrial effluents for decades, which adversely affected water properties and biological communities (MARASOVIĆ *et al.*, 1991). After the activation of the modern sewage system in November 2004, disappearance of nutrient and oxygen extremes as well as decrease in bacterial abundance and production were followed by the reduction of phytoplankton biomass and re-establishment of its regular seasonal cycle (BOJANIĆ & VIDJAK, 2010; ŠOLIĆ *et al.*, 2010). Station S1 (43°29'18"N; 16°26'12"E, max depth 38 m) is located out of the entrance to the Bay, in the deeper area of the Split Channel (Fig. 1D).

Station P1 (43°1'30"N; 17°24'48"E, max depth 21 m) is located in the Neretva Channel, which extends between the mainland and the Pelješac peninsula in the southern part of the Adriatic Sea (Fig. 1E). The coasts are scarcely inhabited, with the low degree of industrialization, and the area is influenced by the considerable freshwater discharge from the Neretva River.

## SAMPLING METHODS

### Zooplankton

Mesozooplankton was sampled using the 125 µm mesh size Nansen net (total length 2.5 m, mouth area 0.25 m<sup>2</sup>), towed vertically from near bottom to the surface. Net collections were preserved in 2.5% formaldehyde-seawater solution, previously buffered with CaCO<sub>3</sub>. Counting and species identification were performed using inverted microscope (Olympus). Abundances were expressed as the number of individuals per cubic meter (ind. m<sup>-3</sup>). Taxonomic identification was performed to the species or genus level. Although current copepod taxonomy places former order Poecilostomatoda into Cyclopoida (BOXSHALL & HALSEY, 2004), in the present study the term "poecilostomatoid" was retained to denote an ecologically distinct group of copepod families (Oncaeidae, Corycaeidae, Sapphirinidae) and not as a taxonomical unit.

Consecutive collections of the microzoo-

plankton samples at the same stations were performed using 5 L Niskin bottle at 0, 5, 10, 20 and 30 m depths (or to the stations' bottom depth). The samples were preserved in 2.5% formaldehyde-seawater solution, previously buffered with CaCO<sub>3</sub>, since Lugol's solution stains detritus and would reduce visibility (FONDA UMANI & BERAN, 2003). Preparation of the samples for the microscopic analysis was performed as described in BOJANIĆ *et al.* (2005). Counting and species identification were performed with an inverted microscope (Olympus) at x100 and x400 magnifications. Abundances were expressed as number of individuals per litre (ind. L<sup>-1</sup>).

### Environmental parameters

Four abiotic parameters were obtained at the time when zooplankton samples were collected, reflecting general environmental conditions and chemical water properties. Vertical temperature and salinity profiles were measured with a CTD multiparameter probe (Sea Bird 25). Oxygen concentrations were determined by the Winkler method (GRASSHOFF, 1976). Nutrient concentrations (nitrate, nitrite, ammonia, orthophosphate and orthosilicate) were determined on the Auto-Analyzer III system, using modified automated methods (GRASSHOFF, 1976).

Phytoplankton biomass was determined through chlorophyll *a* measurements, performed on the Turner 112 fluorometer following acetone extraction (STRICKLAND & PARSONS, 1972).

### Data analysis

Analysis of variance (Kruskal-Wallis ANOVA) was used to test the significance of differences among means in several data sets (nutrients concentrations, chlorophyll *a*, total mesozooplankton abundance, copepod abundance) among the sampling sites, and pairwise comparisons were obtained by applying Dunn's *post-hoc* test. Spearman rank order correlations were used to assess inter-relationships between environmental parameters and between zooplankton groups. Evaluation of trophic status in the investigated area was performed through calculation of trophic index (TRIX), representing

a linear combination of the logarithms of four variables: chlorophyll *a* concentration, dissolved inorganic nitrogen (DIN), total phosphorus (TP) and the absolute percentage deviation from the oxygen saturation (aD%O) (VOLLENWEIDER *et al.*, 1998).

Shannon diversity index was used to analyse biodiversity changes in the copepod community. In order to reveal individual species contribution to the dissimilarities among the sampling stations and to identify the seasonal pattern in the copepod population, Similarity Percentages Analysis (SIMPER) available in PRIMER5 statistical program (CLARKE & GORLEY, 2001) was performed on the species' abundances  $\log(x+1)$  transformed data matrix, with the cut off for low contributions fixed at 75%. This analysis also identified characteristic copepod species' ranking at particular sites or in particular seasons, along with their individual contributions based on their averaged abundances.

Similarities between the sampling sites were illustrated with the hierarchical Cluster analysis (CA) based on the group average clustering from Bray-Curtis similarity matrix of square root transformed abundances of the most abundant mesozooplankton groups (copepods, cladocerans, appendicularians, chaetognaths, meroplankton larvae). Application of "Similarity profile" (SIMPROOF) permutation test available in PRIMER6 (CLARKE & GORLEY, 2006) allowed for the identification of the significant internal structure within the constructed clusters, and this option was used to test the significance of the output dendrogram (at 1000 permutations). The test results are displayed by a colour convention on the dendrogram plot (the samples connected by red lines cannot be significantly differentiated) and by the test statistics ( $\pi$  value), with a significance level set at 5%.

Canonical Correspondence Analysis (CCA) was used for relating community composition to the known variation in the environment (TER BRAAK, 1986). CCA was used to evaluate the unimodal and cause-effect relations between the abundance of main mesozooplankton groups (as response variables) and environmental parameters (as explanatory variables) along the gradi-

ent. A long environmental gradient has high beta diversity, which indicates that CCA could be used (ZUUR *et al.*, 2007). Each variable was standardised to zero mean and unit variance in order to eliminate numerical differences between studied parameters, leaving only relative station-to-station changes in parameters. The CCA was performed using the software XLS-Biplot 1.1 (1999-2002) created in the Statistics Department of Virginia Tech by E.P. Smith and I.A. Lipkovich.

## RESULTS

### Thermohaline properties of the water column

During the entire investigated period sea surface temperature in the investigated area varied between 8.8°C and 26.4°C. In May samplings average water column temperature ranged between 15.6°C and 18.7°C (Fig. 2), with the beginning of thermocline formation evident at deeper stations. Highest average temperature values (19.3°C - 23.6°C) in the water column were recorded in July (Fig. 2), when further heating of the surface layer led to the establishment of thermocline, mostly between 5-10 m or 10-20 m, depending on the stations' depth. In September-October period average water column temperature ranged between 17.2°C and 19.9°C (Fig. 2), the thermocline was set deeper at deeper stations, while at shallow stations temperature conditions became uniform throughout the water column. December-January period was characterized by the lowest average water column temperatures (11.4°C - 14.1°C), and isothermal conditions (Fig. 2).

Large surface salinity oscillations were recorded in the study area, ranging from 5.96-38.44. Salinity is mostly controlled by the vicinity of freshwater sources and the lowest average values in the water column were recorded at stations Š1 and P1, both under direct riverine influences, while the highest average salinity values were generally recorded at station Z1 (Fig. 3). Seasonal fluctuations in average water column salinity were low at majority of the stations, with the exception of station Š1 where the val-

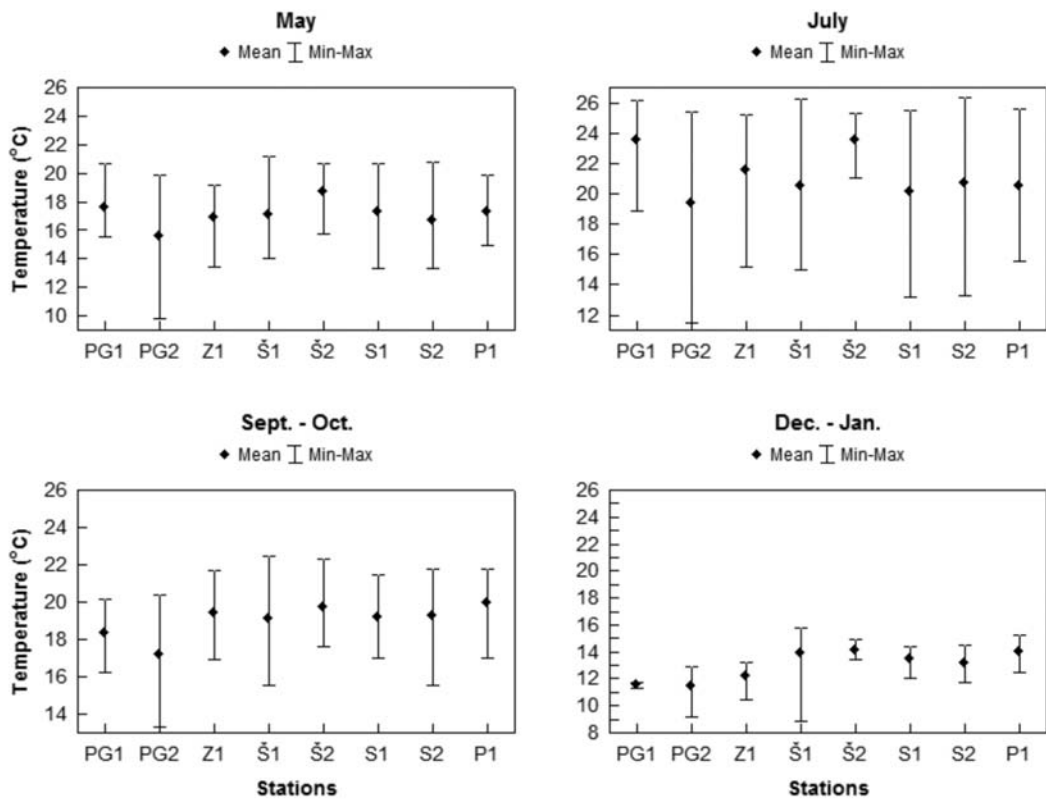


Fig. 2. Seasonal variability of average temperature at the investigated stations (vertical bars denote minimum and maximum values)

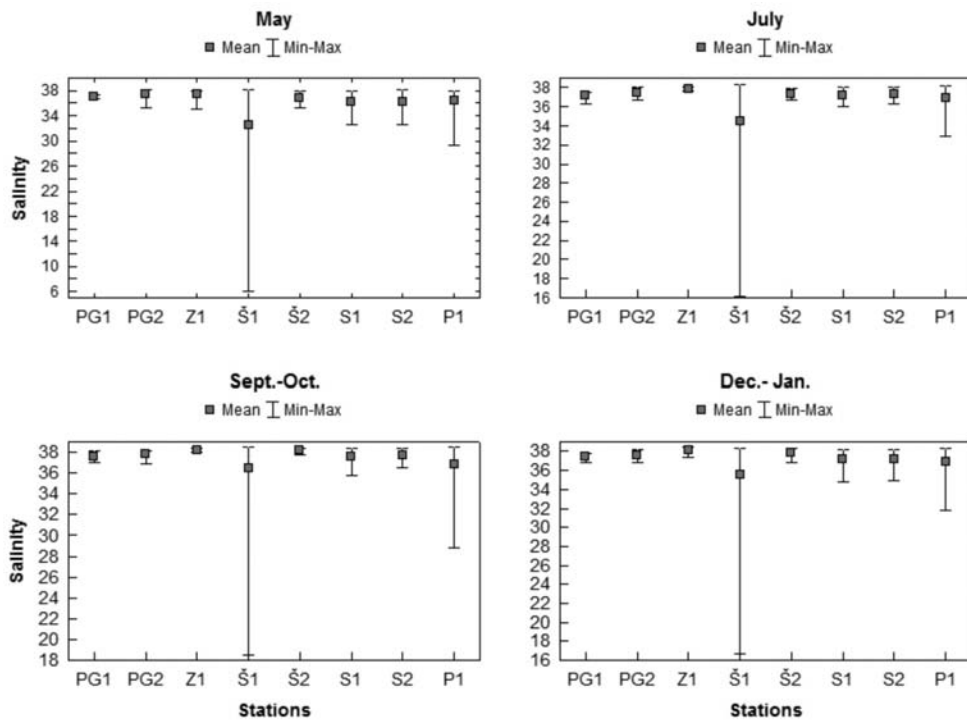


Fig. 3. Seasonal variability of average salinity at the investigated stations (vertical bars denote minimum and maximum values)

ues were noticeably lower in spring compared to other seasons, due to the increased inflow of Krka River.

**Oxygen concentration and saturation**

Water column was well-oxygenated, with concentration generally ranging over 4.27-6.75 mL O<sub>2</sub> L<sup>-1</sup>, corresponding to 85.74% and 121.59% O<sub>2</sub> saturation, respectively. Highest value of 9.31 mL L<sup>-1</sup> was recorded at station Š1 (July 2006, 0 m) (Fig. 4). Oxygen solubility was strongly negatively influenced by temperature (Table 1).

**Nutrients concentrations and phytoplankton biomass**

Highest concentrations of nitrate and ortho-silicate were determined at stations Š1, Š2 and P1 under the respective influences of rivers Krka and Neretva, coinciding with maxima in oxygen concentrations (Fig. 4). At other stations the concentrations of both nutrients did not vary greatly. Spearman correlations between nutrient concentrations and temperature and salinity confirmed the increased availability of nitrate and orthosilicate in the water column during the isothermal conditions and under the lower salinity regime (Table 1).

Table 1. Spearman rank order correlations between abiotic and biotic parameters in the environment (N=88 for ortho-phosphate, N=94 for DO, N=96 for all other data; P<0.05\*; P<0.001\*\*)

	TEMP	SAL	DIN	NO <sub>3</sub> <sup>-</sup>	HPO <sub>4</sub> <sup>2-</sup>	SiO <sub>4</sub> <sup>4-</sup>	DO	Chl <i>a</i>
SAL	-0.073							
DIN	<b>-0.419**</b>	<b>-0.255*</b>						
NO <sub>3</sub> <sup>-</sup>	<b>-0.465**</b>	<b>-0.318*</b>	<b>0.913**</b>					
HPO <sub>4</sub> <sup>2-</sup>	-0.048	0.145	0.188	0.104				
SiO <sub>4</sub> <sup>4-</sup>	<b>-0.462**</b>	<b>-0.414**</b>	<b>0.574**</b>	<b>0.641**</b>	0.053			
DO	<b>-0.688**</b>	0.180	0.196	<b>0.307*</b>	-0.026	<b>0.311*</b>		
Chl <i>a</i>	-0.173	<b>-0.440**</b>	<b>0.351**</b>	0.394**	0.043	<b>0.495**</b>	0.118	
CIL	-0.130	-0.090	<b>0.291*</b>	<b>0.293*</b>	<b>0.237*</b>	<b>0.342**</b>	0.066	<b>0.331*</b>

(TEMP=temperature, SAL=salinity, DIN=total inorganic nitrogen, DO=dissolved oxygen, CIL=ciliates)

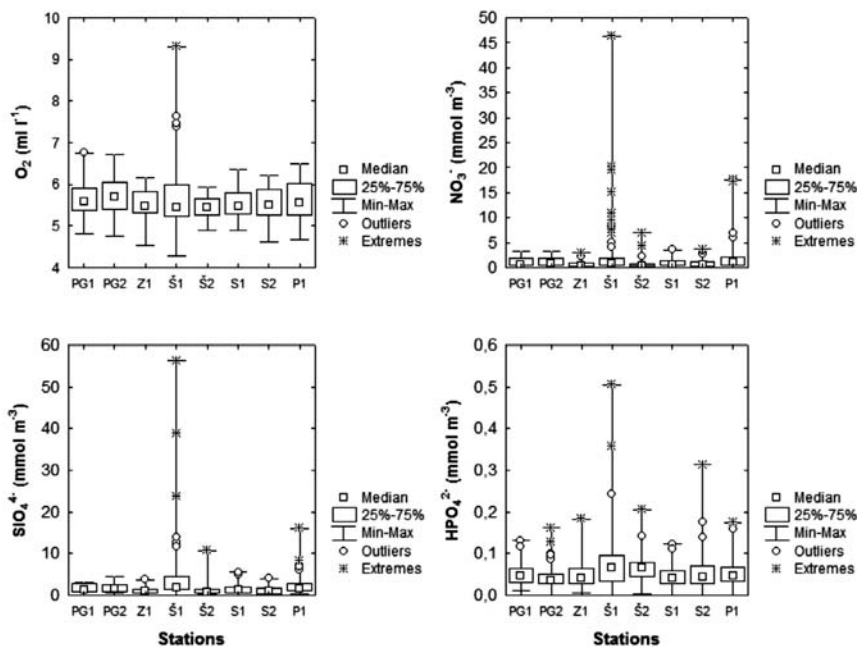


Fig. 4. Spatial distributions of oxygen and main nutrients concentrations at the investigated area

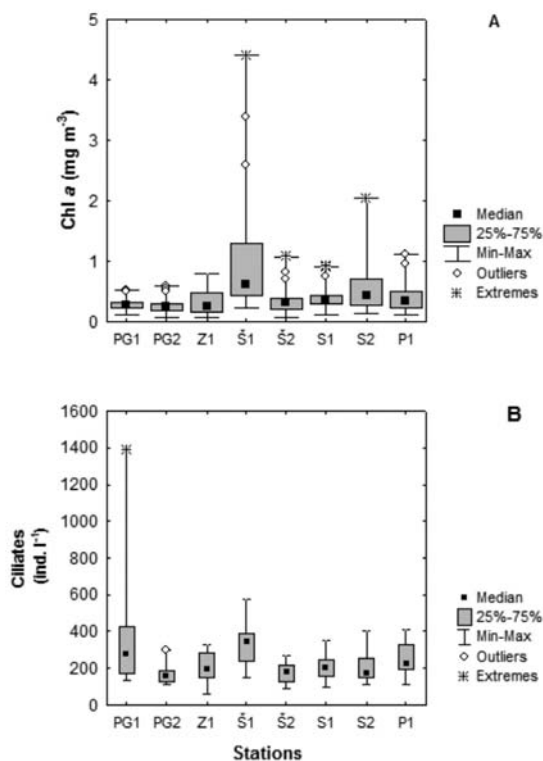


Fig. 5. Spatial distributions of a) phytoplankton biomass and b) ciliate abundances in the investigated area

Absolute orthophosphate concentrations were generally  $<0.2 \text{ mmol m}^{-3}$ , except at stations Š1, Š2 and S2 where they reached maxima of  $0.505 \text{ mmol m}^{-3}$ ,  $0.205 \text{ mmol m}^{-3}$  and  $0.313 \text{ mmol m}^{-3}$ , respectively (Fig. 4). Considering that the Spearman correlations showed no significant effect of either temperature or salinity on orthophosphate dynamics during the investigated period (Table 1), those maxima were presumably affected by the vicinity of the cities of Šibenik and Split, and their industrial and domestic effluents.

Chlorophyll *a* values at the investigated stations mostly ranged from  $0.06\text{--}2.04 \text{ mg Chl } a \text{ m}^{-3}$ . The exception was noted in September 2006, when  $4.40 \text{ mg Chl } a \text{ m}^{-3}$  was recorded at the surface at station Š1 (Fig. 5A). Apart from Š1, increased values of  $>1 \text{ mg Chl } a \text{ m}^{-3}$  were occasionally recorded at stations S2 (Kaštela Bay), at Š2 which is influenced by the Krka River, and at P1 influenced by the Neretva River. Spearman correlations showed that average Chl *a* concentrations were significantly pos-

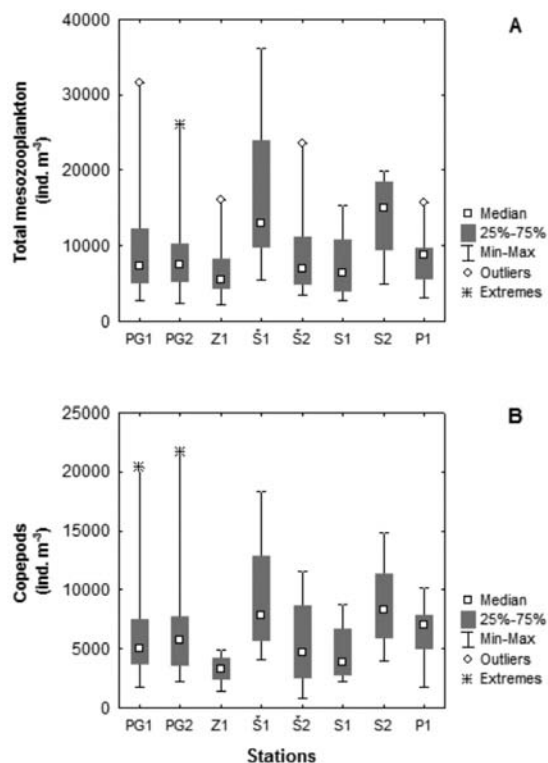


Fig. 6. Spatial distribution of mesozooplankton in the investigated area: a) total mesozooplankton abundances; b) total copepods abundances

itively correlated with average concentrations of nitrogen salts and orthosilicate, and significantly negatively correlated with average water column salinity. The correlation with the average water column temperature was negative, but not statistically significant (Table 1).

### Ciliates

Total ciliate abundances per station ranged over  $62\text{--}1389 \text{ ind. L}^{-1}$  (column average), with non-loricate ciliates (NLC) generally dominating the assemblage. The column average abundances of NLC fluctuated greatly during the investigated period, ranging from  $59\text{--}1099 \text{ ind. L}^{-1}$  (Fig. 5B). Maximum value was recorded at station PG1 in May 2006. The column average tintinnid abundances varied between  $2\text{--}290 \text{ ind. L}^{-1}$ . Spearman correlations showed no significant relationship between total ciliates and either temperature or salinity, indicating the availability of this prey group in all seasons and salinity conditions (Table 1).



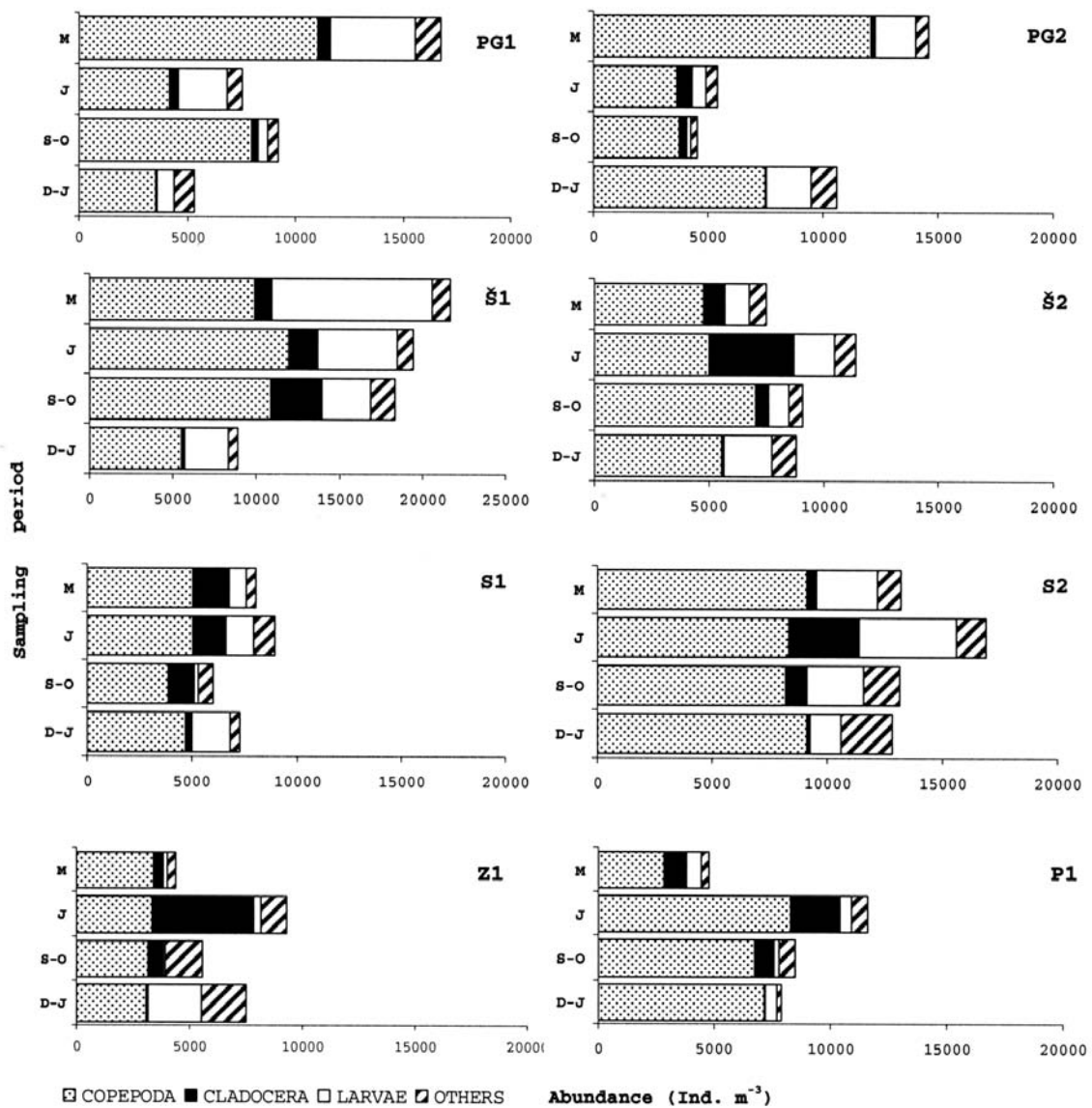


Fig. 7. Average abundances (seasonal average) of the dominant mesozooplankton groups at the investigated stations; M, March samplings; J, July samplings; S-O, September-October samplings; D-J, December-January samplings

### Metazooplankton abundance and distribution

Average abundances of copepod nauplii in the water column varied between 11-166 ind. L<sup>-1</sup>. Increased values were generally recorded in the warmer months, with maximum at station P1 in July 2007. Total mesozooplankton abundances per station ranged between 2178-36244 ind. m<sup>-3</sup> (Fig. 6A). Totals were higher in the warmer part of the investigated period, especially at stations in the enclosed bays, such as PG1

(Bay of Pag), Š1 (Šibenik Bay) and S2 (Kaštela Bay). Copepods represented on average 66.1% in all net samples, and were mostly responsible for total zooplankton fluctuations (Spearman R=0.889, p<0.001), with abundance values ranging between 826-21710 ind. m<sup>-3</sup> (Fig. 6B and 7). Larval assemblage ranked either second or third in total mesozooplankton (Spearman R=0.721; p<0.001) and were mostly dominated by bivalve larvae, followed by those of gastropods and decapod crustaceans. Cladocerans

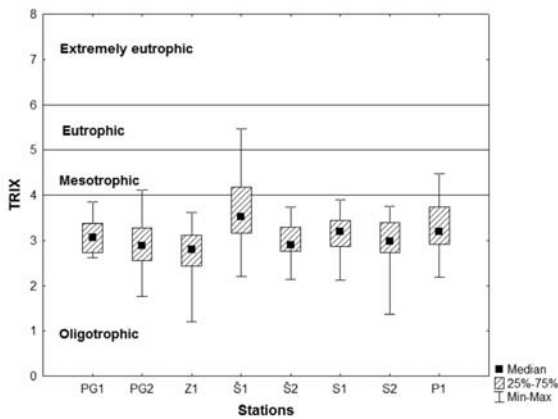


Fig. 8. Assessment of the trophic status within the investigated area based on TRIX values

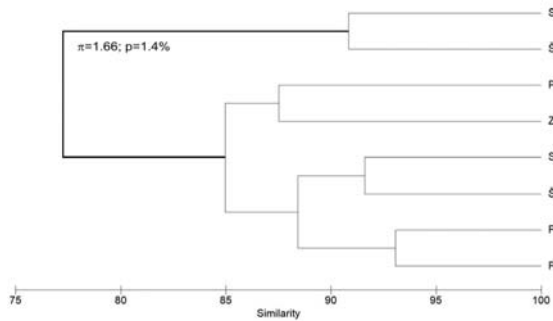


Fig. 9. Dendrogram of the hierarchical clustering (group average linking) of the investigated stations based on group average clustering from Bray-Curtis similarity matrix of square root transformed average abundances of mesozooplankton groups (copepods, cladocerans, appendicularians, chaetognaths, meroplankton larvae).  $\pi$  statistics resulting from the application of SIMPROOF test showed the only significant separation within the investigated area ( $\pi=1.66$ ;  $p=1.4\%$ ) displayed by the bold line. Other resulting subgroups are represented by gray lines, as  $\pi$  statistics indicated lack of significant internal structure in the cluster

significantly contributed to total mesozooplankton abundances (Spearman  $R=0.366$ ,  $p<0.001$ ) in the warmer periods. Among other quantitatively important mesozooplankton groups in decreasing order were appendicularians (Spearman  $R=0.487$ ;  $p<0.001$ ), chaetognaths (Spearman  $R=0.342$ ;  $p<0.001$ ), and pteropods (Spearman  $R=0.300$ ;  $p<0.05$ ), while hydromedusae, ostracods, mysids, amphipods, euphausiids and thaliaceans were present occasionally and in low numbers. Spatial and seasonal distributions of the average abundances of dominant mesozooplankton groups are presented in Figure 7.

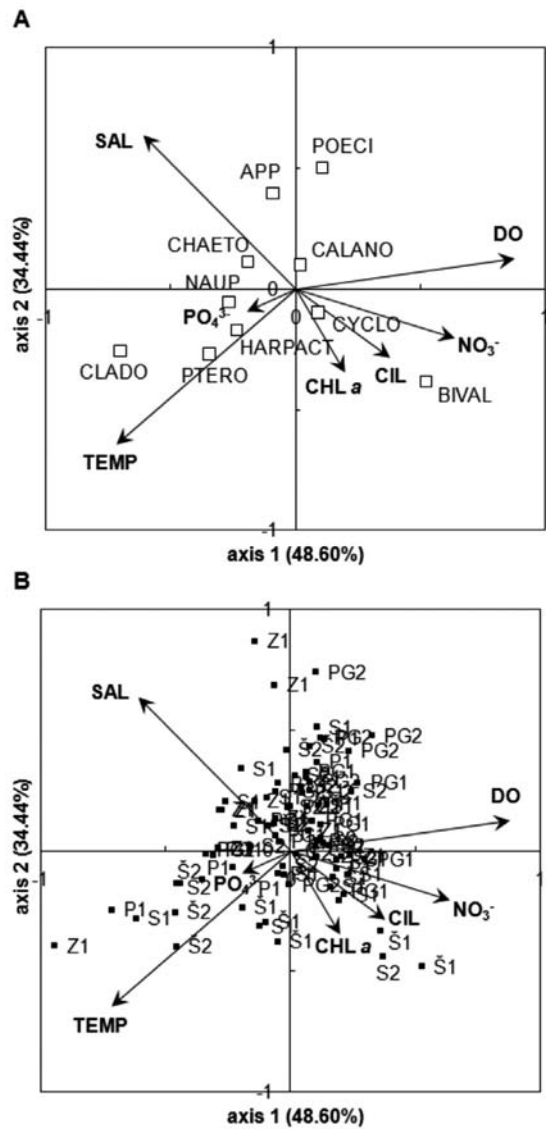


Fig. 10. Canonical Correspondence Analysis (CCA) ordination biplots of the  
 a) scores of metazooplankton groups (CALANO=calanoid copepods; POECI=poecilostomatoid copepods; CYCLO=cyclopoid copepods; HARPACT=harpacticoid copepods; NAUP= copepod nauplii; CLADO=cladocerans; APP=appendicularians; CHAETO=chaetognaths; BIVAL=bivalves; PTERO=pteropods) and  
 b) site scores and environmental variables (TEMP=temperature, SAL=salinity, DO=dissolved oxygen, CIL=ciliates)

**Spatial and seasonal variability of copepod abundance and taxonomic composition**

A total of 65 copepod species was recorded during the investigated period. Shannon diversity index ranged between 0.754 and 2.486 and was inversely related to average temperature (Spearman R= -0.222; p<0.05; N=96), while positively related to average salinity (Spearman R= -0.221; p<0.05; N=96). Additionally, Shannon diversity showed significant positive correlations with the average concentrations of nitrate and orthophosphate at p<0.05 (Spearman R=0.300; N=96 and 0.446; N=88, respectively), while the relationship with orthosilicate was not significant.

From the analysis of the average similarity performed through SIMPER within the seasons and within the stations, *Oithona nana* emerged as the most successfully adapted copepod spe-

cies at both seasonal and spatial scales. The average abundances of this species ranked first among adult copepods in spring (961 ind. m<sup>-3</sup>), summer (1194 ind. m<sup>-3</sup>) and autumn samples (1546 ind. m<sup>-3</sup>), and ranked second to *Oncaea waldemari* only in winter samples (483 ind. m<sup>-3</sup>). Spatially, it dominated at PG1, Š1, Š2, S1 and P1, and ranked second to *Oncaea waldemari* at PG2, Z1 and S2. Apart from *Oithona nana* and *Oncaea waldemari*, among the top 5 species throughout the investigated area were *Paracalanus parvus*, *Euterpina acutifrons*, *Monothula subtilis* and *Acartia clausi*. Other recorded Acartiidae (e.g. *Paracartia latisetosa*, *Acartia adriatica* and *A. margalefi*) were not among the key copepod taxa and occurred sporadically and in small abundances.

Average dissimilarity between stations based on individual copepod species abundances com-

Table 2. Comparative analysis (Kruskall Wallis ANOVA) of significance of differences among means in abiotic and biotic data sets among the sampling sites. Pairwise comparisons were obtained by applying Dunn's post-hoc test

Parameter	Kruskall-Wallis H	P	Dunn's multiple comparison test	P
NO <sub>3</sub> <sup>-</sup>	19.87	0.0059**	Z1 vs Š1	P<0.05
			Š1 vs Š2	P<0.05
NO <sub>2</sub> <sup>-</sup>	14.15	0.0486*	Z1 vs Š1	P<0.05
NH <sub>4</sub> <sup>+</sup>	6.80	ns	—	—
DIN	16.81	0.0186*	Z1 vs Š1	P<0.05
HPO <sub>4</sub> <sup>2-</sup>	7.976	ns	—	—
SiO <sub>4</sub> <sup>4-</sup>	34.87	P<0.001***	PG1 vs Š1	P<0.05
			Z1 vs Š1	P<0.001
			Š1 vs Š2	P<0.001
			Š1 vs S1	P<0.01
			Š1 vs S2	P<0.01
Phytoplankton biomass	44.10	P<0.001***	PG1 vs Š1	P<0.001
			PG2 vs Š1	P<0.001
			PG2 vs S2	P<0.05
			Z1 vs Š1	P<0.001
			Š1 vs Š2	P<0.001
			Š1 vs S1	P<0.05
Mesozooplankton abundance	22.76	P<0.05**	Z1 vs Š1	P<0.05
			Z1 vs S2	P<0.05
Copepod abundance	25.82	P<0.001***	Z1 vs Š1	P<0.01
			Z1 vs S2	P<0.01

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

puted through the SIMPER procedure ranged between minimum of 33.20% for stations Š1 and S2 (both located in enclosed bays) and maximum of 46.95% for stations PG2 and Š2 with considerable difference in depth. Further breaking of the averages down into separate contributions from each species showed that 12 copepod taxa combined accounted for 75% of the total copepod abundance at all investigated sites. Those were: *Oithona nana*, *Oncaea waldemari*, *Paracalanus parvus*, *Acartia clausi*, *Oithona similis*, *Centropages krøyeri*, *Monothula subtilis*, *Ctenocalanus vanus*, *Ditrichocorycaeus brehmi*, *Temora stylifera*, *Microsetella* spp. and *Farranula rostrata*.

Analysis based on the factor season within the same SIMPER routine showed that the average dissimilarity between the sampling periods varied in the narrow range between 40.89% (May, July) and 43.96% (May, September-October). Species identified as responsible for the 75% of the total copepod abundances in all seasons were *Oithona nana*, *Oncaea waldemari*, *Paracalanus parvus*, *Acartia clausi*, *Oithona similis*, *Centropages krøyeri*, *Monothula subtilis*, *Ctenocalanus vanus*, *Ditrichocorycaeus brehmi*, *Temora stylifera*, *Microsetella* spp., *Farranula rostrata*, *Centropages typicus* and *Clausocalanus furcatus*.

#### Comparison of investigated sites based on chemical and biological parameters and trophic index values

Comparison of the chemical and biological water properties displayed in Table 2 showed significant differences among sampling sites, but the *post-hoc* test revealed that they were generally induced by the conditions at station Š1 which differed significantly from the rest of the sampling area in chemical (total inorganic nitrogen concentrations, nitrogen salts and silicate content), as well as in biological properties (phytoplankton biomass, mesozooplankton and copepod abundances). The differences were most consistently shown between Š1 and Z1.

Values of TRIX and the trophic classes attributed to the TRIX ranges are presented in Fig. 8 (range and definition of depicted trophic

classes used for Adriatic coastal waters is available at [www.izor.hr/azo](http://www.izor.hr/azo)). At all sampling sites mean TRIX values indicated oligotrophic conditions. Occasionally mesotrophic conditions were evident at PG2 and P1, but the highest data scattering was found at station Š1, where values reached into eutrophic conditions.

Hierarchical clustering of the investigated stations based on the abundances of mesozooplankton groups separated bay stations Š1 and S2 from the rest of the investigated area at similarity level of >77.3% (Fig. 9). Application of the SIMPROOF test and the resulting  $\pi$  statistics showed that this was the only significant separation within the investigated area ( $\pi=1.66$ ;  $p=1.4\%$ ). In other resulting subgroups the  $\pi$  statistics indicated lack of significant internal structure in the clusters.

#### Analysis of environmental influences on the zooplankton community

The results of the CCA used to identify an environmental basis for community ordination are presented on the biplot (Fig. 10), showing that the first two ordination axes explained 85.29% of the data variability of most abundant zooplankton groups in the ordination of selected environmental parameters. The most important environmental variables were temperature and salinity defining the first two axes. Dissolved oxygen and nitrate concentration were strongly correlated with the first axis, and taking into account the determining effect of temperature on both latter parameters, we assumed that temperature is mainly responsible for the environmental gradient along this axis. The abundances of cladocerans, copepod nauplii and pteropods were significantly positively associated with the first axis at channel stations Z1, Š2, S1 and P1 (Fig. 10). The second axis was defined by salinity and positively affected poecilostomatoid copepods and appendicularians at stations Z1 and PG2, while negatively affecting bivalve larvae at Š1 and S2.

Additional axes explained significantly less of the overall variability in the zooplankton dataset compared to the first two axes. Third and fourth axes (not displayed) were defined

by chlorophyll *a* concentration and ciliate abundance, respectively, and can thus be regarded as the prey gradients. Zooplankton groups related to the third axis included grazers such as calanoid copepods and pteropods as well as omnivores such as harpacticoid copepods. Strong relationship with the fourth axis was displayed by cyclopoid and harpacticoid copepods, bivalve larvae and copepod nauplii.

## DISCUSSION

Despite some differences in depth and general hydrology, zooplankton population structure in the bays and channels of the eastern middle Adriatic Sea showed high similarity within most of the investigated regions. Relative importance of the individual copepod species showed characteristic high ranking of small and medium-sized taxa, such as *Oithona nana*, *Oncaea waldemari*, *Paracalanus parvus*, *Euterpina acutifrons*, *Monothula subtilis* and *Acartia clausi*. This was not unexpected, considering that *Oithona* spp. are the most ubiquitous and abundant copepods in coastal environments worldwide (PAFFENHÖFER, 1993; GALLIENNE & ROBBINS, 2001), while others are among dominant copepods in the Mediterranean (FERNÁNDEZ DE PUELLES *et al.*, 2003; RIBERA D'ALCALÁ *et al.*, 2004) and Adriatic neritic communities (HURE & KRŠINIĆ, 1998; KRŠINIĆ *et al.*, 2007). Higher diversity in the copepod population during the colder periods indicated by the Shannon diversity index values is related to the eastern Adriatic circulation pattern, characterized by the increased inflow which reinforces currents along the eastern coast in winter (ZORE-ARMANDA *et al.*, 1999), and temporary presence of oceanic species is often recorded in eastern Adriatic bays and channels during the winter isothermia (REGNER, 1985; VIDJAK *et al.*, 2007). Biodiversity of the copepod community was not adversely affected by the differences in the average nutrient load in the investigated area. Despite their overall dominance in mesozooplankton, copepod taxonomic structure revealed very little of the underlying differences in the area, stressing the relative uniformity of the copepod community in the coastal

Adriatic waters (HURE & KRŠINIĆ, 1998).

Conversely, intercomparisons of the sampling stations based on the abundances of total mesozooplankton and copepods as the most abundant constituent, showed some differentiation in the area. Differences in the abundance means were generally induced by the distinction between channel station Z1 (Zadar Channel) and bay stations Š1 (Šibenik Bay) and S2 (Kaštela Bay), designating them as the extremes in the community size, while in the rest of the sampling area a clear gradient in population magnitude could not be detected.

The lack of the significant inner structure in the Š1-S2 subgroup displayed on the cluster dendrogram showed that there are still considerable differences between the respective Bays. Besides enclosed positions and similar depths, the shared features between Šibenik Bay and Kaštela Bay include higher orthophosphate concentrations and phytoplankton biomass compared to the rest of the sampling stations, but both parameters were consistently lower in the Kaštela Bay. All through the 1980s and at the beginning of 1990s, both locations were considered as „hot spots“ of the coastal eastern middle Adriatic, subjected to the negative effects of nutrients overload on the water column properties and resident biological components (MARASOVIĆ *et al.*, 1991, 2005). However, during the last decade, Kaštela Bay has gone through a significant decrease in the antropogenically forced eutrophication, which led towards the reinstatement of the more natural conditions (KUŠPILIĆ *et al.*, 2009). On the other hand, Šibenik Bay is under considerable influence from the Krka River, and although it is one of the most pristine European rivers characterized by low concentrations of nutrients and extremely low input of terrigenous material (LEGOVIĆ *et al.*, 1994), the combined nutrient load from the riverine inflow and anthropogenic input from the town of Šibenik (GRŽETIĆ *et al.*, 1991; LEGOVIĆ *et al.*, 1994) still designate Šibenik Bay as an area of intense productivity that is apparently reflected at higher trophic levels as well.

Analysis of the main environmental influences on zooplankton abundances showed that

zooplankton primarily responded to physical parameters, such as temperature and salinity. Thus cladocerans, copepod nauplii and pteropods responded positively to temperature at group level, while for poecilostomatoid copepods and bivalve larvae this relationship was inverse. The conditioning effect of temperature on zooplankton groups and species is documented in the large part of zooplankton investigations (e.g. LICANDRO & IBANEZ, 2000; FERNÁNDEZ DE PUELLES *et al.*, 2004, 2004a; MARQUES *et al.*, 2006). Some groups were spatially controlled by local salinity variations, with for instance, bivalve larvae favouring lower salinity of Šibenik Bay, while appendicularians and poecilostomatoid copepods preferred higher salinities in Zadar Channel and Pag Straits. The CCA results suggested that chemical parameters measured during this investigation had little direct influence on the zooplankton variability, with the exception of dissolved oxygen concentration. However, considering that dissolved oxygen concentrations throughout the investigated period were favourable for zooplankton organisms, with no evidence of hypoxia ( $<2 \text{ mL}^{-1}$ ) (STALDER & MARCUS, 1997; DECKER *et al.*, 2004), strong association of this parameter with the first axis is presumably combined with the effect of temperature, which to a great extent determines the oxygen solubility in the water column. Since nitrate concentrations in the water column were also strongly correlated with temperature, it was difficult to separate its particular influence on zooplankton data variability. However, spatial distribution of the collected data showed that concentrations of the main nutrients and accompanying phytoplankton biomass, as well as ciliate and mesozooplankton abundances showed highest values in the Šibenik Bay, indicating that increased nutrients availability could be beneficial to all examined components of the food web.

Biotic parameters included in CCA such as available autotrophic biomass and ciliate prey, determined very little of the overall variability in the zooplankton data, although a number of zooplankton groups were related to CCA axes defined by those parameters. For instance, the

abundances of grazers such as calanoid copepods, cladocerans and pteropods were related to the third axis determined by chlorophyll *a* concentration. In addition, clear relationships emerged for copepod nauplii and bivalve larvae with ciliated protozoans. The nature of this relationship presumably includes predator-prey interactions since copepod nauplii are able to utilize non-loricate ciliates in their diet (BOJANIĆ *et al.*, 2006) and ciliates are also identified as the significant prey for the bivalve mollusc (KRŠINIĆ, 1987; NJIRE *et al.*, 2004). Omnivorous copepods, such as cyclopoids, mostly represented by *Oithona nana*, or harpacticoids, mostly represented by *Euterpina acutifrons*, differed in their response to the selected prey categories; while cyclopoid abundances were significantly determined by the availability of ciliates, for harpacticoids both ciliates and phytoplankton prey categories were highly important. This is in accordance with numerous studies that cite *Oithona* spp. as broadly omnivorous, but with preferential feeding for microzooplankton and motile prey (NAKAMURA & TURNER, 1997; LONSDALE *et al.*, 2000; ATIENZA *et al.*, 2006), while *Euterpina acutifrons* mainly thrives on small phytoplankton cells, but opportunistically ingests other small-sized particles, particularly when at higher concentrations (SAUTOUR & CASTEL, 1993). It is important to notice that other higher ranking copepods such as *Oncaea waldemari*, *Acartia clausi* and *Monothula subtilis* are also classified as omnivores (reviewed in TURNER, 2004), which supports the importance of heterotrophic prey items in the nutrition of dominant copepods in the coastal area (GISMERVIK, 2006).

## CONCLUSIONS

Mesozooplankton population structure and taxonomic composition of copepods as the dominant group in the bays and channels of the eastern Adriatic Sea did not reveal significant differences within the area. Copepod communities were dominated by small, ubiquitous copepods, with *Oithona nana*, *Oncaea waldemari*, *Paracalanus parvus*, *Euterpina acutifrons*, *Monothula*

*subtilis* and *Acartia clausi* dominating at both spatial and seasonal scales.

Zooplankton numerical variability in the investigated area was influenced principally by physical environmental factors such as seasonal variation in water temperature and spatial variation in salinity. However, abundances of total mesozooplankton in general, and copepods in particular were also linked to the chemical and biological parameters generally used as descriptors of water quality. This was especially evident in the Šibenik Bay, where nutrient enrichment coming from the Krka River and anthropogenic sources affected the main copepod prey categories such as available autotrophic biomass and ciliated protozoans, and consequently mesozooplankton and copepod abundances. This indicates that zooplankton community size reflects

the trophic status of an area and supports the use of the zooplankton studies as an auxiliary method in the environmental monitoring of coastal areas.

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## Utjecaj čimbenika okoliša na varijabilnost zooplanktona u priobalju istočnog Jadrana (Sredozemno more)

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### SAŽETAK

Ciljevi ovog rada su bili utvrditi glavne ekološke pokretače varijabilnosti zooplanktona u obalnim vodama u blizini urbanih područja, te procijeniti razlike u brojnosti i strukturi zooplanktonske zajednice u odnosu na kemijske i biološke čimbenike vodenog stupca. Uzorci zooplanktona su sakupljeni sezonski od svibnja 2006. do siječnja 2009. godine, na 8 postaja u zaljevima i kanalima duž istočne obale Jadrana. Struktura populacija zooplanktonske zajednice pokazala je visoku sličnost unutar čitavog istraživanog područja, što je posebno vidljivo u homogenosti sastava kopepodne zajednice, gdje je relativna važnost pojedinih vrsta svuda ukazala na karakteristično visoki udio malih i srednjih taksona. Numerička varijabilnost zooplanktona prvenstveno je bila povezana sa sezonskim varijacijama temperature i prostornim varijacijama slanosti, ali je prostorna raspodjela prikupljenih podataka pokazala da je također povezana s kemijskim i biološkim parametrima kojima se opisuje kvaliteta vode. To ukazuje da je brojnost zooplanktona povezana s trofičkim stanjem područja i ide u prilog istraživanju zooplanktona kao pomoćnog parametra u procjeni trofičkog stanja obalnih voda.

**Ključne riječi:** obalni zooplankton, kopepodi, trofički status, kvaliteta vode, Jadransko more