Seasonal dynamics of the autotrophic community in the Lim Bay (NE Adriatic Sea)

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This work describes the dynamics of the autotrophic plankton community in Lim Bay in the north-eastern part of the Adriatic Sea from June 2008 to September 2009. There was an evident shift between microphytoplankton and picophytoplankton domination in summer and autumn periods, while nanophytoplankton contributed negligibly. Picophytoplankton dominated in June 2008 and September 2009 (contributing up to 84 % of the total phytoplankton biomass), while the micro- fraction dominated in September 2008 and June 2009 (contributing up to 97.2 % of the total biomass). The pico- fraction was dominated by Synechococccus *in terms of abundance and biomass, with the highest abundances in September of 2009. Picoeukaryotes were not that prominent in terms of abundance or biomass, but they exceeded* Synechococcus *in terms of biomass in November 2008 and February 2009, and proved to be an important and consistent component of the Lim Bay phytoplankton community. Our results indicate that seasonal variability in the community structure was more affected by specific environmental perturbations occurring in Lim Bay than by availability of nutrients.*

Key words: picophytoplankton, nanophytoplankton, microphytoplankton, flow cytometry, *Synechococcus*, northern Adriatic Sea

INTRODUCTION

Phytoplankton cell size structure strongly determines food webs, carbon pathways and energy flows, consequently moderating ecosystem functioning (LEGENDRE & RASSOULZADEGAN, 1995; MARAÑÓN, 2009). It is generally accepted that in conditions when nutrients are sufficiently high phytoplankton outcompete smaller cells (SOMMER, 1981; KIØRBOE, 1993), while small cells according to their size and related superior capacity for uptake of dissolved materials are more efficient in oligotrophic environments where nutrients are limited (FOGG, 1995; MALONE, 1980). Changes of physical, chemical or biological factors can heavily affect size

structure, concentration, biomass and distribution of the entire plankton community (MARGALEF, 1978). Those environmental perturbations are closely coupled with functional responses of the phytoplankton community and consequently affect trophic pathways in a given ecosystem. Numerous studies have shown the significance of picophytoplankton in the microbial food web and recycling of carbon and nutrients in different marine environments, seas and oceans (AZAM et al., 1983; HAGSTRÖM et al., 1988; STOCKNER, 1988). However, picophytoplankton is often considered as a background population whose biomasses remains relatively constant independently of changes in the environment (THINGSTAD & SAKSHAUG, 1990). Consequently, there is a belief that small cells dominate in stable, oligotrophic environments and are not significant in variable, eutrophic, coastal areas (CHISHOLM, 1992; LI, 2002). Nevertheless, some studies have demonstrated that picophytoplankton does respond to enrichment of the environment, but with lower magnitude than larger phytoplankton (TARRAN et al., 2006; GLOVER et al., 2007). Since they can adapt quickly to different conditions their behavior can be difficult to predict. Therefore analyzing how different size fractions of phytoplankton respond to environmental forcing in different areas is critical to understanding carbon fluxes through the microbial plankton community.

The Adriatic Sea is positioned as a northwest-to-southeast arm of the Mediterranean Sea. Its northern part has been considered as the most productive zone of the Mediterranean (DEGOBBIS et al., 2000), but recent studies revealed its certain oligotrophication (MOZETIČ et al., 2009; IVANČIĆ et al., 2010). The area of Lim Bay is a narrow embayment located on the western Istrian coast in the NE part of the Adriatic Sea (Fig. 1), and designated as a Special Marine Reserve because of its geomorphologic value. The geomorphologic features of Lim Bay, its shallowness, environmental perturbations (wind, tides, and episodic freshwater inputs) and related high physical and chemical variability may have a broad impact on phytoplankton biomass and community structure. The input of freshwater from underwater springs, stimulated

by rain (January-February), reduces salinity and provides additional nutrients into the adjacent water column (VATOVA, 1950). Another important influence on the trophic state of the bay is oligotrophic water coming from the open sea according to tidal regime changes (DADIĆ, 2009). Variability in phytoplankton biomass is consequently closely coupled with those processes that cause a shift in phytoplankton size structure. Previous research in the Lim Bay area focused on the ecology of some specific phytoplankton groups (BOSAK et al., 2009; LJUBEŠIĆ et al. 2011) while missing a detailed description of the whole phytoplankton community size structure. In the present paper, we investigated the dynamics of the phytoplankton community over its annual cycle in Lim Bay. The aims of the study were to: (1) describe seasonal variations in phytoplankton size distribution; (2) determine the relative contribution of picophytoplankton to phytoplankton abundance and biomass, and (3) evaluate environmental factors controlling phytoplankton distribution.

MATERIAL AND METHODS

Study area

The study was carried out in Lim Bay, a narrow and about 10 km long embayment situated on the western part of the Istrian peninsula (northern Adriatic Sea, Croatia) by sampling from June 2008 to September 2009, at the three distant stations LIM1, LIM2 and LIM3
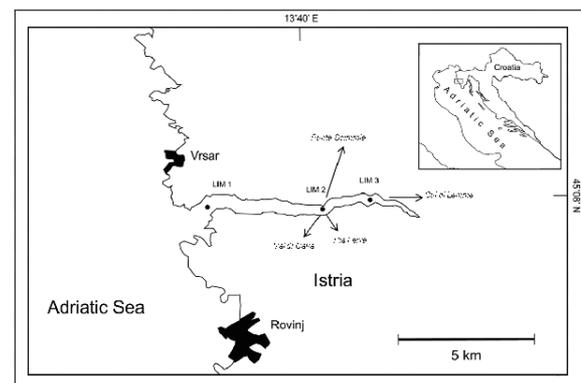
Fig. 1. Study area and sampling stations LIM1, LIM2 and LIM3 in Lim Bay, Istrian peninsula, NE Adriatic Sea

(Fig. 1). The sampling station LIM1 (45°07′55″ N, 13°37′10″ E) is located at the entrance to the embayment, while station LIM2 (45°07′51″ N, 13°41′10″ E) is located near a fish farm, which provides increased nutrient levels that are especially evident in the period of stratification (BOSAK *et al.*, 2009). The inner part of Lim Bay, represented by station LIM3 (45°08′07″ N, 13°43′00″ E; depth of 18 m), is a shallow area markedly influenced by freshwater inputs.

Sample collections

Seawater samples were collected with 5 L Niskin bottles at three depths (0 m, 5 m and 10 m) at all stations. Additional samples were collected at 2 m above the bottom (depths: LIM1-30 m; LIM2 -25 m; LIM3-16 m).

Hydrological variables

Temperature and salinity were recorded by CTD profiler SeaBird Electronics SBE 25. Dissolved nutrients (nitrate, nitrite, ammonium, phosphate and silicate) were measured spectrophotometrically (PARSONS *et al.*, 1984).

Brunt-Väisälä Frequency

CTD data were used to obtain profiles of the water column density, ρ. The Brunt-Väisälä (buoyancy) frequency (*N),* which is a measure of water column stability, was calculated for every standard-depth interval and assigned to the interval mid-point according to following equation:

$$N(Z) = \sqrt{\left(- \frac{g}{\rho(z)} * \frac{d\rho(z)}{dz}\right)}$$

where *g* is gravitational acceleration, ρ the density value at z depth and $d\rho$ the density difference over the dz depth interval (GILL, 1982).

Pigment determination

Subsamples of 500 mL for the determination of chlorophyll *a* (<200 μm =total chl *a*; <20 μm= nano chl *a*) were filtered onto Whatman GF/C filters. Subsamples were filtered directly onto GF/C filter (for total chl a), or through a 20 μm net onto GF/C filters (for nano chl *a*), and then stored at -20°C. The pigment content was measured fluorometrically after extraction with acetone (PARSONS *et al.*, 1984) using a Turner TD-700 fluorimeter.

Integrated concentrations of chlorophyll *a* (chl *a*)

The chlorophyll *a* concentration at each pair of depths was averaged, then multiplied by the difference between the two depths to get a total concentration in that depth interval. These depth interval values are then summed over the entire depth range to get the integrated chl *a* value of that particular sampling day.

Epifluorescence microscopy (EM)

Samples for epifluorescence microscopy analysis were preserved with formaldehyde (2% final concentration) and stored at +4 °C until analysis in the laboratory. The analyses were carried out using a Leitz Laborlux D epifluorescent microscope equipped with a 50 W mercury lamp and filter sets for UV, blue and green excitation. For the determination of cyanobacteria (*Synechococcus* sp.) abundance, 15 mL of sample was filtered through black polycarbonate membrane filters (0.4 μm pore size) and then at least 300 cells were counted under green light excitation distinguished by its orange autofluorescence (TAKAHASHI *et al.*, 1985). Pico- and nano phytoplankton were counted on the same filters after staining with Primulin (250 μg L^{-1} in 0.1 M Tris HCl, pH 4.0) for 15 min. These cells were detected under blue light excitation whereas heterotrophic and autotrophic nanoplankton was differentiated by the presence or absence of chlorophyll's autofluorescence (CARON, 1983).

Inverted light microscopy (ILM)

For the enumeration of phytoplankton cells, 150 mL samples were preserved with formaldehyde (2% final concentration; buffered with disodium tetraborate). Cells were identified

and enumerated using the inverted microscope (Zeiss Axiovert 200) operating with phase contrast and bright field optics in sub-samples of 50 mL after 24 h of sedimentation (LUND *et al.* 1958, UTERMÖHL, 1958). One transect along the counting chamber bottom was scanned at x400 magnification for nanoplankton and abundant microplankton, two transects at x200 magnification and at x100 magnification a total bottom count was completed for taxa greater than 30 μm. The minimum concentration that can be detected by this method is 20 cells L⁻¹. The identification of selected species was confirmed at x1000 magnification. Microalgae which could not be identified to the species or genus level were assigned to suprageneric groups as cryptophytes, coccolithophorids, prasinophytes and other phototrophic nanoflagellates. The nano- (5-20 μm), and microphytoplankton (>20 μm) size classes were determined after the measurements of the cell maximum linear dimensions. Cell biovolumes were calculated by assigning the cells to geometrical bodies and applying standard formulae (HILLEBRAND *et al.*, 1999). The phytoplankton carbon content was calculated from mean cell biovolumes (MENDEN-DEUER & LESSARD, 2000).

Flow cytometry (FC)

Samples for flow cytometry analysis were taken in duplicates. One set of samples was analyzed fresh, within 6 hours of being obtained and another set was preserved with glutaraldehyde (0.5% final concentration) for 10 minutes, frozen in liquid nitrogen, stored at -80ºC and analyzed within 10 days. Samples were analyzed using a Partec PAS (Münster, Germany) flow cytometer, equipped with an Argon laser (488 nm). Instrumental settings were standardized for all parameters each day by using fluorescence polystyrene calibration beads (Partec Calibration Beads 3 μm, ref. no. 05-4008). Data were collected in list mode files using FL3 as a trigger parameter and processed with software FloMax (Partec, Germany). The final abundance of each subgroup was obtained instrumentally, which enabled true volumetric absolute counting. The precision

of the volume measurement is defined by a fixed mechanical design, eliminating any errors related to varying beads' concentrations. The different subpopulations of phytoplankton were distinguished by their autofluorescence of the chlorophyll *a* content of the cells (FL3) and the phycoerythrin content of the phycoerythrin-rich cells (FL2) which the instrument provides as well as by the cells' forward-angle light scatter (FSC) as a proxy of their size. Those specific fluorescence signals together with size proxy allowed differentiation of *Synechococcus,* picoeukaryote and nanoeukaryote cells. In order to determine their contribution to ecosystem biomass and carbon flux flow cytometric cell counts of each analyzed group were converted to carbon units (μg C L⁻¹) using the following factors: 200 fg C cell ⁻¹ per cell for *Synechococcus* (CHARPY & BLANCHOT, 1998) and 1500 fg C cell ⁻¹ for picoeukaryotes (ZUBKOV *et al.*, 1998).

Data analyses

Statistical analyses were performed using SYSTAT 10.2 software. Differences between pico- and nanoplankton abundances and biomasses were established using parametric tests (Pearson correlations). Relationships with $p<0.05$ were taken for statistically significant.

RESULTS

Physical and chemical parameters

The vertical profiles of temperature, salinity and Brunt–Väisälä (BV) frequency (Fig. 2) showed the importance of summer stratification, with the maximum Brunt–Väisälä frequency values in the upper 10 m at all three stations. The lowest temperatures occurred on February 2009 on all stations (varying from 10.28-10.54°C) (Fig 2). The mean salinity value for the autumn/winter period of 2008 was 37.57, while for 2009 was 36.39, with exceptionally low salinity of 34.29 observed at station LIM2 in June 2008. In the summer period (June-August) with established stratification, temperature and salinity variations were much higher (Fig. 2). Freshening events in February
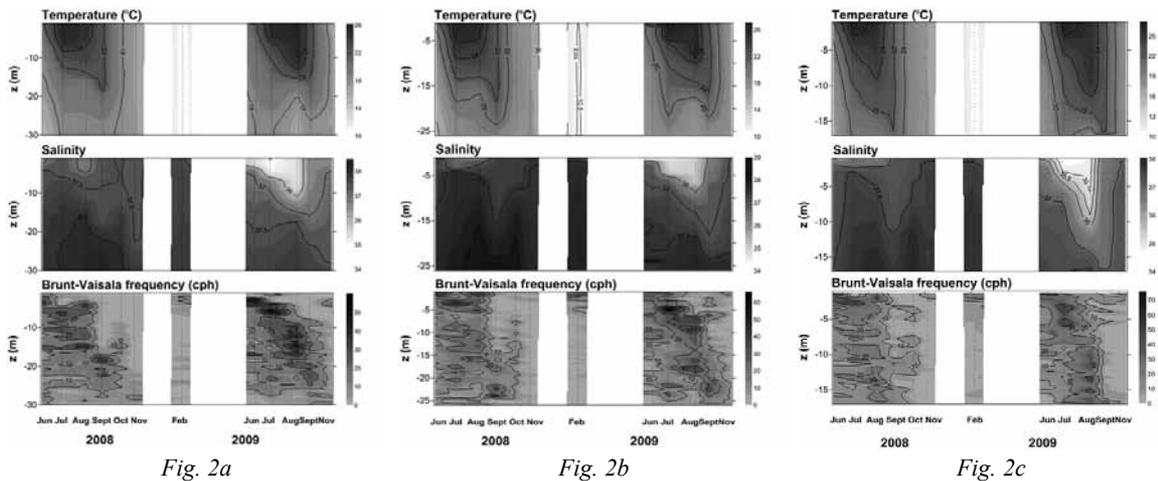
Fig. 2. Temporal and vertical distributions of temperature, salinity and Brunt–Väisäla frequency at (a) LIM1, (b) LIM2 and (c) LIM3 sampling stations

and November 2009 were followed by water column instabilities with BV frequency increasing up to 30 in the surface layer (Fig. 2C).

The integrated water column values of nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_4^+), phosphate (PO_4^{3-}) and silicate (SiO_4) for three sampling stations are given in Table 1. The highest concentrations for nitrate at all stations occurred after two freshening events in February and November 2009, while for nitrite in November 2008. The ammonia concentrations differed between stations with the highest recorded value in September 2008 at station LIM2 (Table 1). The phosphate concentrations were low in the whole area during the entire study period, sometimes below the detection limit, with the highest integrated concentrations in October 2008 at station LIM2. Silicate concentrations were generally higher than those of other nutrients, exceeding the exceptionally high integrated values of 271.29 µmol L^{-1} in November 2009.

Chlorophyll *a*

The maximum water column integrated chl *a* concentrations were recorded in August 2008 for LIM1 followed by the nano fraction maximum, while for LIM2 and LIM3 maximum chl *a* occurred in November 2009 (Table 2). For all stations the minimum integrated chl *a* values occurred in February 2009.

Phytoplankton composition and size structure

In terms of abundance picophytoplankton dominated the autotrophic community, while phytoplankton biomass was dominated by microphytoplankton. Picophytoplankton abundance was more pronounced in the autumn period, with a *Synechococcus* maximum in September 2009 (7.2 x 10^8 cells L^{-1}) and a picoeukaryote maximum in October 2008 (1.4 x 10^7 cells L^{-1}) (Fig. 3). In November 2008, the number of picoeukaryotes was drastically reduced to 2.6 x 10^5 cells L^{-1}. The nanoeukaryotes (i.e. small nanophytoplankton; measured by flow cytometry) achieved maximal abundance in June 2008 (5.7 x 10^6 cells L^{-1}; Fig. 3). Micro- and nanophytoplankton showed the highest values in the summer period (August 2008 and July 2008, respectively; Fig 4). Within microphytoplankton, diatoms were the most prominent group. The highest values for diatom abundance were observed in summer at station LIM1 (data not shown).

In terms of biomass an evident shift between microphytoplankton and picophytoplankton domination was observed during the summer and autumn of the consecutive

Table 1. Integrated values (mmol⁻²) of nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_4^+), phosphate (PO_4^{3-}) and silicate (SiO_4) in Lim Bay during the investigated period

Table 2. Integrated water column values of total and nano-chlorophyll a *(mg Chl m⁻²) in the Lim Bay during investigated period*

Station	Date	PO_4^{3-}	NO_3^-	NO_2^-	NH_4^+	SiO_4
	Jun-08	1.38	17.12	1.29	15.10	86.01
	Jul-08	0.25	6.84	0.88	6.55	66.87
	Aug-08	0.98	15.53	2.55	22.16	108.61
	Sep-08	0.95	9.42	1.99	18.36	54.67
	Oct-08	1.03	20.85	33.01	11.01	146.49
LIM1	Nov-08	2.14	60.50	35.66	11.04	167.96
	Feb-09	1.38	81.57	14.69	6.04	117.54
	Jun-09	1.23	14.39	2.51	13.60	41.54
	Jul-09	3.10	4.44	0.99	20.77	43.74
	Aug-09	1.90	16.36	2.41	31.23	140.43
	Sep-09	1.51	19.60	4.39	31.22	147.72
	Nov-09	2.87	76.85	17.19	19.02	271.29
	Jun-08	2.88	23.77	1.68	13.24	98.38
	Jul-08	1.32	14.14	1.11	7.01	92.95
	Aug-08	2.63	14.89	3.61	22.79	99.49
	Sep-08	4.31	35.52	3.68	54.16	96.74
	Oct-08	3.64	38.25	39.92	13.46	195.34
LIM2	Nov-08	3.49	52.90	42.66	6.73	164.80
	Feb-09	1.06	80.71	12.62	8.02	75.87
	Jun-09	1.93	28.86	2.39	19.60	64.22
	Jul-09	1.75	7.57	1.34	19.94	75.61
	Aug-09	2.66	27.62	2.66	35.50	106.44
	Sep-09	4.71	23.19	4.65	37.08	190.52
	Nov-09	2.80	97.78	16.99	16.99	269.52
	Jun-08	0.83	26.11	0.92	5.12	62.26
	Jul-08	0.66	11.21	0.80	5.00	56.05
	Aug-08	0.79	31.64	2.40	14.80	90.84
	Sep-08	0.40	47.04	1.98	18.23	48.72
	Oct-08	1.08	19.30	19.20	8.70	98.82
LIM3	Nov-08	3.44	58.33	47.54	6.67	164.07
	Feb-09	0.87	61.59	7.72	4.40	74.40
	Jun-09	1.34	20.77	1.57	11.01	32.08
	Jul-09	0.63	3.06	0.80	5.68	21.79
	Aug-09	1.43	25.89	2.21	22.55	57.27
	Sep-09	0.46	6.44	0.97	6.31	64.79
	Nov-09	1.60	90.94	13.32	11.48	197.88

Station	Date	Total Chl *a*	Nano Chl *a*
	Jun-08	13.35	10.52
	Jul-08	20.24	14.08
	Aug-08	33.87	21.77
	Sep-08	26.41	13.51
	Oct-08	22.91	14.23
LIM1	Nov-08	20.37	14.34
	Feb-09	8.32	7.47
	Jun-09	21.64	9.20
	Jul-09	18.33	13.77
	Aug-09	19.32	15.80
	Sep-09	16.99	16.38
	Nov-09	30.48	17.95
	Jun-08	11.90	11.14
	Jul-08	25.57	15.97
	Aug-08	16.63	11.56
	Sep-08	19.21	12.87
	Oct-08	23.41	16.72
LIM2	Nov-08	17.20	13.89
	Feb-09	7.95	6.39
	Jun-09	35.18	17.31
	Jul-09	17.18	13.64
	Aug-09	32.04	21.98
	Sep-09	21.81	19.92
	Nov-09	56.73	16.57
	Jun-08	14.47	12.63
	Jul-08	12.63	11.12
	Aug-08	12.40	8.89
	Sep-08	14.93	12.82
	Oct-08	29.53	17.53
LIM3	Nov-08	14.35	10.04
	Feb-09	5.74	4.89
	Jun-09	17.52	7.78
	Jul-09	14.20	12.20
	Aug-09	20.86	15.83
	Sep-09	23.08	20.46
	Nov-09	33.50	20.53
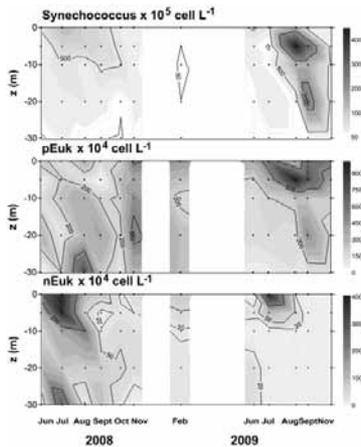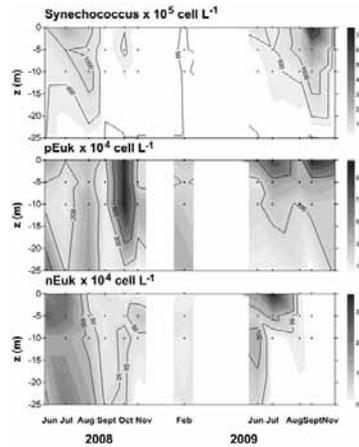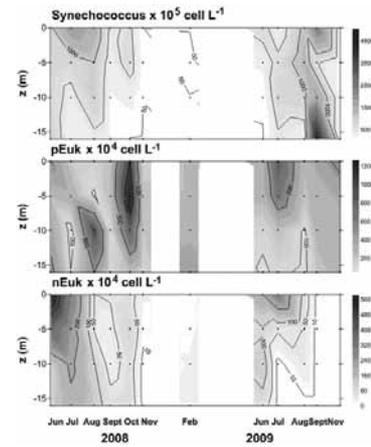
Fig. 3a   Fig. 3b   Fig. 3c

Fig. 3. Temporal and vertical distributions of Synechococcus, *picoeukaryote and nanoeukaryote abundances at LIM1 (a), LIM2 (b) and LIM3 (c) sampling stations, as determined by flow cytometry*
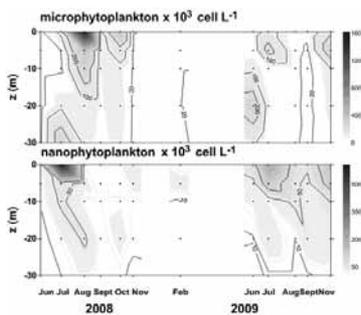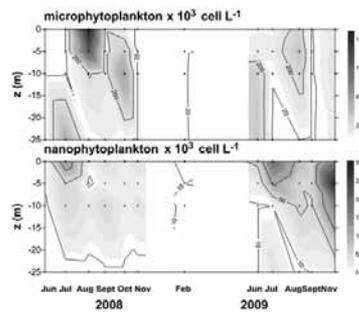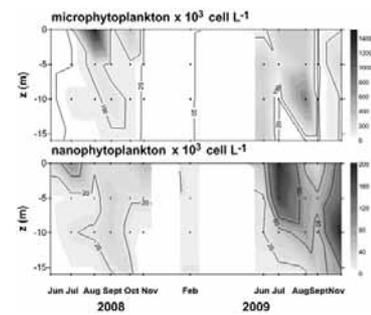
Fig. 4a   Fig. 4b   Fig. 4c

Fig. 4. Temporal and vertical distributions of microphytoplankton and nanophytoplankton abundances at LIM1 (a), LIM2 (b) and LIM3 (c) sampling stations, as determined by light microscopy

years, while nanophytoplankton provided a negligible contribution at all stations (Fig. 5). Picophytoplankton contributed on average (derived from integrated values) with 26.5 %, nano- with 6.5 % and micro- with 67.1 % to total phytoplankton biomass. Picophytoplankton dominated in June 2008 and September 2009 (contributing up to 84 % of the total phytoplankton biomass), while the micro- fraction dominated in September 2008 and June 2009 (contributing up to 97.2 % of the total biomass). Both fractions had their maximum of biomass in 2009, with microphytoplankton at LIM2 in June, and picophytoplankton at LIM1 in September 2009. In general microphytoplankton was dominated by diatoms (data not shown), while

Synechococcus dominated in picophytoplankton biomass (Fig. 6). At LIM1 and LIM3 nano-biomass was dominated by dinoflagellates, while at LIM2 coccolithophorids prevailed (data not shown). *Synechococcus* had a significant positive correlation with temperature (R=0.38, p<0.001, n=156), but negative with phosphate (R=-0.31, p<0.001, n=156).

Epifluorescence microscopy vs. flow cytometry counts

The flow cytometry counts of cyanobacteria, picoeukaryotes and nanoeukaryotes were achieved from cytograms (red fluorescence vs. forward scatter signal) of abundances. The phycoerytrin-rich *Synechococcus* was confirmed
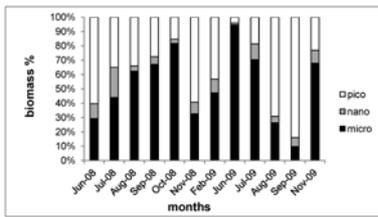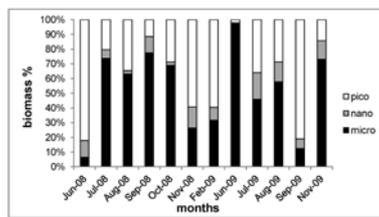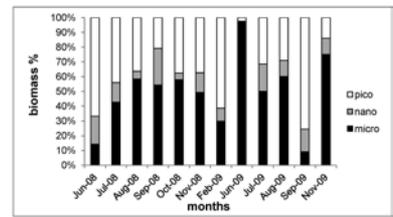
Fig. 5a   Fig. 5b   Fig. 5c

Fig. 5. Integrated carbon biomass percentage contribution of autotrophic pico-, nano- and microphytoplankton communities for (a) LIM1, (b) LIM2 (c) LIM3 sampling stations
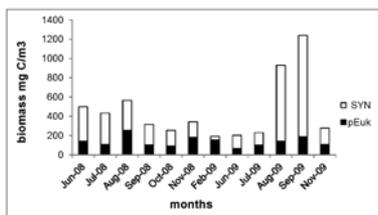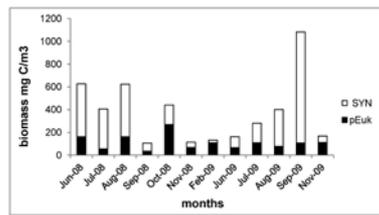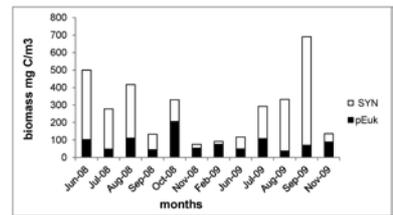
Fig. 6a   Fig. 6b   Fig. 6c

Fig. 6. Integrated biomass (mg C m-2) of different picophytoplankton groups for (a) LIM1, (b) LIM2 (c) LIM3

by specific orange fluorescence signals. Comparison of data achieved by flow cytometry of fresh and preserved sea water samples for 6 months of the investigated period (June to November of 2008) showed a high correlation for *Synechococcus* (R^2=0.83, n=77), picoeukaryotes (R^2=0.73, n=78) and nanoeukaryotes (R^2=0.74, n=77) (Fig. 7). Altogether, fixation (0.5% glutaraldehyde final concentration) and storage (-80°C for up to 10 days) of samples have not shown significant effects on *Synechococcus*,

picoeukaryote or nanoeukaryote abundance estimation (Fig. 7).

The ranges of *Synechococcus*, picoeucaryote and nanoeucaryote abundances during 6 months of the investigated period (June to November of 2008) at all three sampling stations in Lim Bay suggested the generally higher sensitivity of flow cytometry. The *Synechococcus* abundance (FC-measurements) ranged from 2.6 x 10^6 to 2.3 x 10^8 cells L^{-1} (fresh samples) and 2.5 x 10^6 to 2.2 x 10^8 cells L^{-1} (preserved samples), while
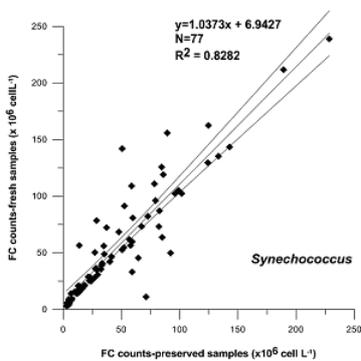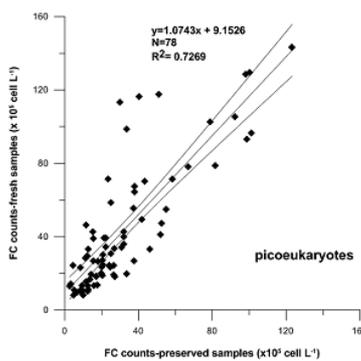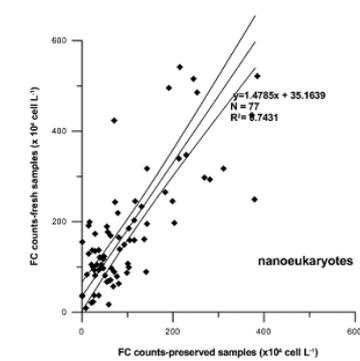
Fig. 7a   Fig. 7b   Fig. 7c

Fig. 7. Flow-cytometry data for fresh and preserved samples of (a) Synechococcus*, (b) picoeucaryote and (c) nanoeucaryote populations at Lim Bay sampling stations. Thick solid line shows the 95 % confidence limit*

their abundances obtained by epifluorescence microscope (EM) comprised from 9.9×10^5 to 2.2×10^8 cells L^{-1}. Similar to *Synechococcus*, the picoeukaryote population counted by FC varied by two orders of magnitude (fresh samples: 8.0×10^5 - 1.4×10^7 cells L^{-1}; preserved samples: 2.6×10^5 - 1.2×10^7 cells L^{-1}). The number of picoeukaryotes in samples processed by EM revealed extreme variations (6 orders of magnitude) from 0 to 3.6×10^6 cells L^{-1}. The nanoeukaryotes counted by EM (0 - 9.0×10^6 cells L^{-1}) gave slightly different values from FC values (fresh samples: 0 - 1.1×10^7 cells L^{-1}; preserved samples: 0 - 5.0×10^6 cells L^{-1}), respectively (Fig. 7). Furthermore, FC counts were compared with data gained from epifluorescence microscopy. Comparison of flow cytometry and epifluorescence microscopy counts showed moderate correlations for *Synechococcus* (R^2=0.50, n=77), picoeukaryotes (R^2=0.39, n=78) and nanoeukaryotes (R^2=0.48, n=80) (Fig. 8).

VAULOT, 2007), with the consequence of dimmer and lower phytoplankton autofluorescence. Small cells may disintegrate even during short periods of storage (DUBELAAR & JONKER, 2000). The effects of different fixatives on nano- and microphytoplankton have been investigated by many studies, but there are only a few which consider the effects that fixatives can have on smaller cells like picophytoplankton (VAULOT *et al.*, 1989; TROUSSELLIER *et al.*, 1995). In contrast to significantly lower fluorescence obtained for bacteria and phytoplankton samples when using glutaraldehyde as a fixative (TROUSSELLIER *et al.*, 1995) only a slight effect was observed in our study. However, we observed a fluorescence (FL3) decrease of *Synechococcus* in glutaraldehyde preserved samples (Fig. 9). The effect was more obvious in surface than in bottom samples (Fig. 9). However, *Synechococcus* FC counts of fresh and preserved samples were similar and significantly correlated (Fig. 7A). Fluorescence of pico- and nanoeukaryotes did not change,
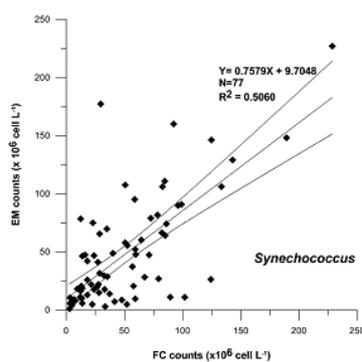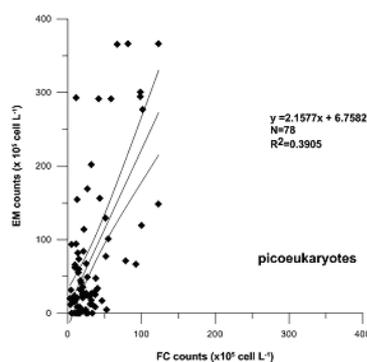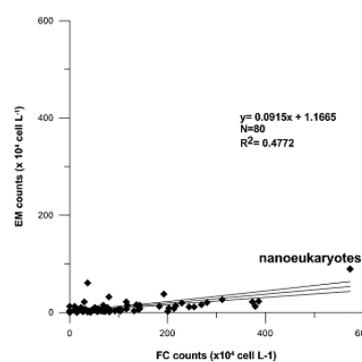
| Fig. 8a | Fig. 8b | Fig. 8c |

Fig. 8. Flow-cytometry vs. epifluorescence microscopy counts of (a) Synechococcus*, (b) picoeucaryote and (c) nanoeucaryote fixed cells at Lim Bay sampling stations. Thick solid line shows the 95 % confidence limit*

DISCUSSION

Methodological aspect

Flow cytometry has proven its efficiency and reliability in the evaluation of phytoplankton communities in many different areas (LI & WOOD, 1988; CROSBIE *et al.*, 2003), but there is still some sensitivity thresholds that need to be considered, particularly concerning

picoeukaryotes. The counts obtained by EM and FC varied throughout the sampling season and showed significant, but moderate correlation. We suspect that lower EM counts for picoeukaryotes were due to the better sensitivity of FC and the influence of fixative and storage. Chl *a* degradation in samples stored at +4ºC was already observed (CHAVEZ *et al.*, 1990; SATO *et al.*, 2006; MASQUELIER &

though twice lower nanoeukaryote counts after glutaraldehyde fixation was obtained (Fig. 7C). An interesting observation concerning glutaraldehyde as a fixative was a strange and unknown fluorescence which occurred after its addition (Fig. 10). There was no particular explanation for this effect, or some pattern between its appearances. We assume that organic components reacted with the fixative,

However, FC allows the discrimination of specific groups, recognize cell organizations and regroup organisms into size classes (MASQUELIER & VAULOT, 2007). Thus FC and EM are essential tools needed for describing both pico- and nanophytoplankton populations and should be used together to enable more complete and accurate description of the entire phytoplankton community.
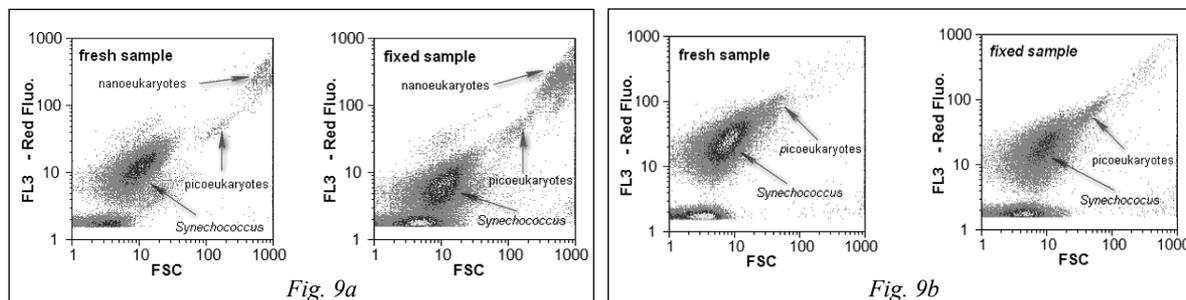
Fig. 9. Red fluorescence (FL3) vs. forward light scatter (FSC) cytograms of fresh and glutaraldehyde-fixed samples from the (a) surface (0 m) and (b) bottom (30 m) layer at the LIM3 station in June 2008

Fig. 10. Red fluorescence (FL3) vs. forward light scatter (FSC), side light scatter (SSC) and Synechococcus orange fluorescence (FL2) cytograms of (a) fresh and (b) glutaraldehyde-fixed samples from the LIM2 bottom layer (25 m) in August 2008
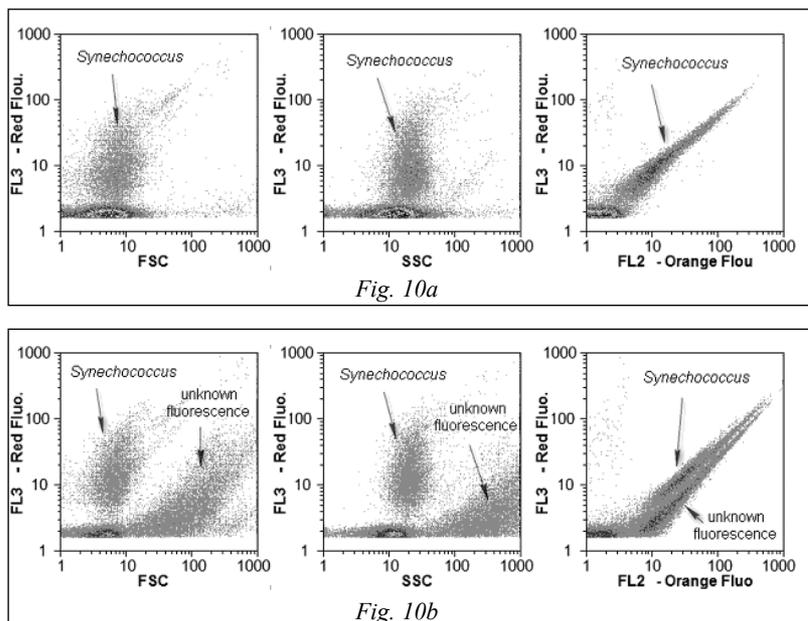
but that assumption was not explored in detail.

FC is especially suited to picophytoplankton, revealing their real importance in this system as previously reported (RADIĆ et al., 2009), while the EM can describe them partly. For nanophytoplankton FC is more accurate than EM for quantification, but lacks taxonomical differentiation and biovolume estimation.

Variability in phytoplankton biomass and community structure

The primary objective of this study was to understand how phytoplankton size dynamics associate with the physical, chemical and biological processes in the Lim Bay area of the Adriatic Sea.

This study was carried out in two seasons

(summer-autumn period) characterized by heterogeneity in water column stability that was followed by differential phytoplankton size structure. The vertical distribution of thermohaline parameters and their derivates as Brunt-Väisälä frequencies showed the change in the vertical structure of the water column from a summer stratification in June to vertical mixing in September.

Water column instabilities in February and November 2009, as a result of heavy rains, together with nutrient inputs led to certain changes in phytoplankton size structure. Observed anomalies in salinity and temperature (Fig. 2) after massive rains were expected, particularly due to the many springs present in the Lim Bay area. Massive rains increase spring water flow and consequently increase water turbidity. Water turbidity re-suspends particles from the bottom (which is mostly muddy), reducing light penetration into to water column. In the waters with fluctuating light intensities, the ability of phytoplankton communities to take up nitrogen for their growth and maintenance can vary based on their size (MAGUER *et al.*, 2011). Water instability and episodic inputs of high amounts of nutrients usually favor fast-growing phytoplankton (MARGALEF, 1978; KIØRBOE, 1993) like diatoms (PEARL *et al.*, 2003). On the contrary, in our study the turbulent mixing conditions that reduced light penetration combined with low temperatures (around 10ºC) most probably prevented microphytoplanktons larger development. Low light conditions proved to be unfavorable for NO_3^- uptake by the largest cells and one of the reasons why N uptake was dominated by small cells (MAGUER *et al.* 2011). The observed weak microphytoplankton response to nitrogen increase in February 2009 resulted in the lowest phytoplankton biomass values recorded, while picoeukaryotes maintained their highest biomass, even exceeding that of *Synechococcus* sp. Such a picoeukaryotic response to the huge supply of nitrate is in contrast to the commonly accepted preference of smaller cells for ammonium (CHISHOLM, 1992), though confirms the recent observations of GLOVER *et al.* (2007) and HUETE-ORTEGA *et al.*

(2011). A similar freshening event also occurred in November 2009. Although both freshening events (in February and November 2009) brought nutrients to the system, they provided growth of different phytoplankton size groups. This is in line with the notion that group tolerance for certain conditions could be similar, but the community size structure did not depend directly on the source of available nitrogen (SEMINA, 1968; PEÑA *et al.*, 1990; RODRIGUEZ *et al.*, 2001). During both freshening events *Synechococcus* biomass decreased at all three stations (Fig. 6). Such a unusual case may be partly explained by its preference for warmer ambient conditions, according to its weak but significant correlation with temperature, which was observed in coastal California as well (TAI & PALENIK, 2009). Another reason might be competition or grazing as speculated by ROBIDART *et al.* (2012) for an unexplainable *Synechococcus* abundance drop in the autumn period.

Otherwise, *Synechococcus* dominated over picoeukaryotes in terms of abundance and biomass which fits to previous findings of their importance and domination for mesotrophic areas (CAMBELL & VAULOT, 1993; PARTENSKY *et al.*, 1996). The evident shift in biomass dominance from picophytoplankton (in June 2008) to microphytoplankton (in June 2009), and vice versa from microphytoplankton (in September 2008) to picophytoplankton (in September 2009), we assume is partly attributable to water exchange with the Adriatic Sea. In particular, we observed a similar trend of size shift in the open sea, at station RV001 (13º61´E, 45º08´N) with microphytoplankton dominating in September 2008 and picophytoplankton dominating in August and September 2009 (ŠILOVIĆ *et al.*, unpublished results). In general, the Po river has the strongest outflow in May (up to 5000 m^3s^{-1}; SOCAL *et al.*, 2008), reducing salinity and bringing nutrients to Istrian coastal waters in the summer period and consequently supporting microphytoplankton growth (June 2009). The observed picophytoplankton mean contribution to phytoplankton biomass fits within the range reported in previous coastal studies (approximately 30%; BEC *et al.*, 2005 and

references therein). The highest observed pico-fraction contribution to phytoplankton biomass in September 2009 (still warm and stratified water column) is in accordance with their usual peak in temperate waters during warm months (IRIARTE & PURDIE, 1994; AGAWIN *et al.*, 1998). Coastal and freshwater ecosystems tend to show irregular phytoplankton biomass distribution, which reflects ecological factors affecting their size structure (RODRIGUEZ *et al.*, 1987; GASOL *et al.*, 1991). The surprising and unexpected shift in the predominant size class proved the fact that the understanding of factors shaping coastal phytoplankton structure is still incomplete (WETZ *et al.*, 2011) and that phytoplankton structure should be considered by their size and taxonomy, including all of their compartments, even the smallest one (2-20 μm). Understanding system-specific environmental conditions and group tolerances to given conditions will improve our general concept of environmental control over phytoplankton cell size.

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Sezonska dinamika autotrofne zajednice u Limskom kanalu (Sjeverni Jadran)

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SAŽETAK

Ovaj rad opisuje dinamiku autotrofne zajednice u Limskom kanalu, u sjevero-istočnom dijelu Jadranskog mora od lipnja 2008 do rujna 2009. Tijekom ljeta i jeseni jasno je uočena promjena u dominaciji biomase između mikrofitoplanktona i pikofitoplanktona, pri čemu je doprinos nanofitoplanktona bio zanemariv. Pikofitoplankton prevladavao je u lipnju 2008 i rujnu 2009 (do 84% doprinosa ukupnoj fitoplanktonskoj biomasi), dok je mikro-frakcija prevladavala u rujnu 2008 i lipnju 2009 (do 97.2% doprinosa ukupnoj biomasi). Unutar piko-frakcije prevladavale su cijanobakterije roda *Synechococcus,* kako brojnošću tako i biomasom, s najvećim vrijednostima u rujnu 2009. Međutim, u studenom 2008 i veljači 2009 pikoeukarioti su biomasom premašili populaciju cijanobakterija *Synechococcus*, čime su se pokazali kao važna komponenta zajednice fitoplanktona Limskog kanala. Rezultati ovog istraživanja su pokazali da je sezonska varijabilnost u strukturi zajednice podložnija utjecaju određenih perturbacija u okolišu nego dostupnosti nutrijenata.

Ključne riječi: pikofitoplankton, nanofitoplankton, mikrofitoplankton, protočna citometrija, *Synechococcus*, sjeverni Jadran