Distribution of phytoplankton along the thermohaline gradient in the north-eastern Adriatic channel; winter aspect*

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Abstract

The distribution of phytoplankton and its relation to the hydrographic features in the north-eastern Adriatic was investigated in February 2008. The area of interest included a thermohaline gradient in the channel situated between the coast and the islands lying parallel to the coast. The gradient is controlled by the influx of oligotrophic karstic riverine water at the south-eastern end, submarine springs in the middle part, and warmer offshore waters at the north-western end of the channel. The change of temperature and salinity in the estuarine transition zone was accompanied by abundant diatoms and dinoflagellates below the halocline, with dominant chain-forming diatoms (*Chaetoceros*, *Bacteriastrum*) in abundances reaching 5×10^5 cells dm^{-3}. The impact of coastal submarine springs detected by infrared remote sensing resulted in the growth of cyanobacteria in the nitrogen-depleted surface waters. The greater contribution of picoplankton, as well as of nanoplanktonic coccolithophorids and cryptophytes, in the outer channel system indicated their preference for oligotrophic conditions. Flow cytometric counts of nanophytoplankton were 10–30 times greater than inverted microscope counts. Cyanobacteria were about five times more abundant than picoeukaryotes. The study demonstrates how different techniques (remote sensing and in situ investigations) can be useful in understanding the biological and hydrographic set-up in the specific oligotrophic eastern Adriatic coastal environment.

1. Introduction

Phytoplankton growth is regulated mostly by nutrients, light and grazing (Reaugh et al. 2007). Transition zones in estuaries are characterised by more abundant suspended particles, increased biological production and decreased water transparency, in comparison to their upper and lower reaches (Knox 1986, Cortelezzi et al. 2007, Jay et al. 2007, Peebles et al. 2007). In contrast to macro-tidal estuaries, where turbidity maxima are considerable (McLusky & Elliott 2004), the north-eastern coastal Adriatic Sea estuaries are micro-tidal environments.

There are many elongated islands lying parallel to the eastern Adriatic coast, with channels in between. In this research, we documented the phytoplankton distribution across a thermohaline gradient in a 50 km long channel system. There is a source of freshwater at the south-eastern end of the channel, making it function like an estuary (Figure 1). The thermal front in this area was observed for the first time during a research cruise on board the US r/v 'Knorr' in winter 2003. Coincident infrared (IR) imagery indicated a strong inflow of warm water from the open sea through the Kvarnerić Gate into the Pag and Velebit Channels (Lee et al. 2004).

The karstic River Zrmanja and submarine springs (vruljas) regulate the hydrography (Orlić et al. 2000), enrichment with nutrients (Viličić et al.

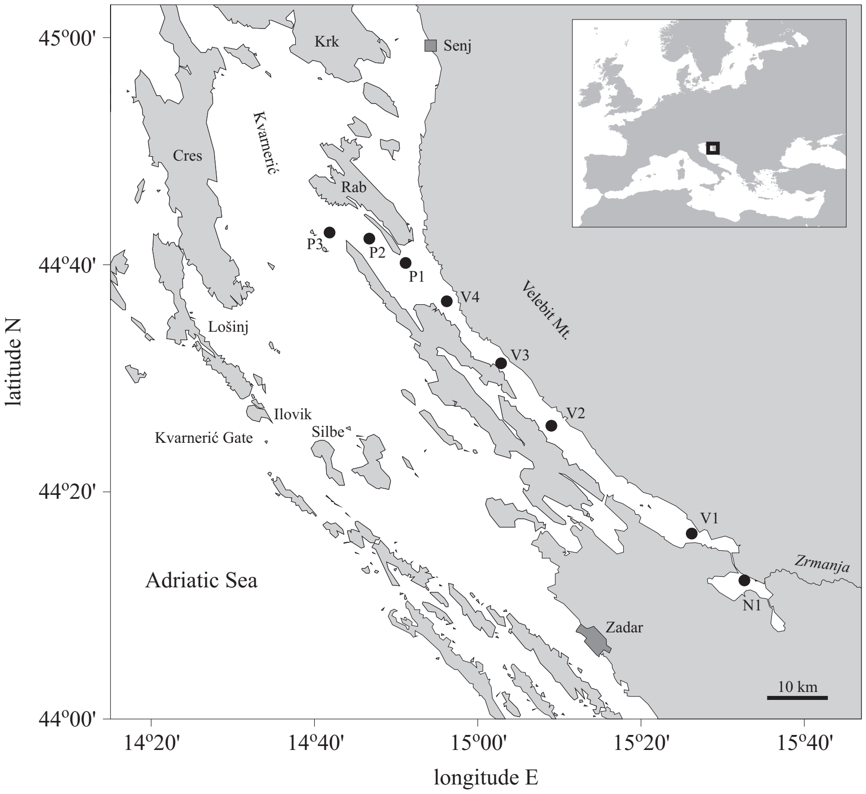

Figure 1. Positions of stations along the Pag Channel (P3, P2, P1), the Velebit Channel (V4, V3, V2, V1) and the Zrmanja Estuary (N1)

2008b) and seasonal phytoplankton growth in the Zrmanja Estuary (Burić et al. 2004, 2007a,b, 2008, Viličić et al. 2008a,b). The environment is oligotrophic, as confirmed by the pronounced deep chlorophyll maximum in summer (Viličić et al. 2008a).

This account of the impact of environmental conditions during the winter hydrological regime on phytoplankton distribution in the Pag and Velebit Channels is the first attempt to explain the trophic interrelationships and ecology of the channel system, which is oligotrophic but relatively rich in small pelagic fish and hake.

2. Material and methods

2.1. Investigated area

The eastern coastal Adriatic Sea is influenced by the oligotrophic East Adriatic Current (EAC) flowing in from the south-east (Gačić et al. 2001). The oligotrophic karstic rivers discharging along the eastern coast also support the low productivity of the eastern Adriatic Sea (Svensen et al. 2007, Viličić et al. 2008b). On the other hand, the north-western Adriatic receives large amounts of freshwater from the River Po (Raicich 1996, Vollenweider et al. 1998), which flows out to become the West Adriatic Current from the northern Adriatic down the Italian coast.

The Pag and Velebit Channel system is 50 km long and 4–14 km wide, and has a NW-SE orientation (Figure 1). It communicates with the open sea through the Kvarnerić Gate, between the outer islands of Lošinj (Ilovik) and Silba. The inner part of the Velebit Channel extends into the narrow Zrmanja Estuary. With a depth of 110 m, the Pag Channel is the deepest part of the northern Adriatic.

Besides the River Zrmanja, there are numerous freshwater sources in the channel, namely, the submarine springs known as vruljas (Benac et al. 2003). The catchment area consists of permeable carbonate rocks (Biondić et al. 1996). The springs along the coast are connected with sink holes (ponors) in the hinterland (Bonacci & Roje-Bonacci 2000), and are active in the rainy (October–December) and snow melt (March–May) periods (Penzar et al. 2001). Consequently, salinity is considerably reduced in the surface layer near the coast in the period between October and May.

The north-eastern Adriatic coastal area is strongly influenced by the catabatic bora wind in winter, with gusts reaching 60 m s^{-1}; these wind speeds surpass those observed elsewhere in the Adriatic (Makjanić 1976, Dorman et al. 2006). The circulation is largely governed by the bora.

2.2. Location of stations

The profiling was performed along a deep-to-shallow water transect, from the Pag Channel (stations P3, P2, P1) to the Velebit Channel (stations V4, V3, V2, V1) and Zrmanja Estuary (station N1) from 4 to 6 February 2008.

2.3. Methods

Temperature, salinity, density and chlorophyll fluorescence were measured with a CTD profiler (SBE 19plus, Seacat, Sea Bird Electronics Inc., USA). Chlorophyll fluorescence was calibrated in units of μg dm^{-3}. Calibration is performed regularly (once a year) in the Sea Bird Electronics Wetlab using a culture of the diatom *Thalassiosira weissflogi*, and more frequently using a spectrofluorometer (Turner TD-700, USA) and chlorophyll *a* (Sigma-Aldrich, Germany) as a standard. Transparency was estimated with a 30 cm diameter white Secchi disc.

Phytoplankton abundance was determined using an inverted microscope and a flow-cytometer. Sampling was performed using 5 litre Niskin bottles.

Microphytoplankton and nanophytoplankton were counted under a Zeiss Axiovert 200 phase-contrast inverted microscope (Utermöhl 1958, Hasle 1978a,b, Venrick 1978). Samples were preserved in a neutralised formaldehyde solution (2% final concentration). 50 cm^3 subsamples were analysed within one month of collection, following 24 h sedimentation. One transect along the counting chamber bottom was scanned at 400x and two at 200x. Recognisable nanoplankton (cells > 5 μm) and abundant microphytoplankton (cells > 20 μm and chain forming colonies) were counted and identified at 400x. At 100x a total bottom count was carried out for taxa larger than 30 μm. The minimum concentration detectable by this method is 20 cells dm^{-3}. The precision of the counting method is $\pm 10\%$. The entire phytoplankton community was identified to species or genus level following data analysis and processing using the Karl Zeiss AxioVision 3.1 System and AxioCam Camera.

A flow cytometer was used to count nanoplankton and picoplankton. Samples were treated with 0.1% glutaraldehyde for 10 minutes, frozen in liquid nitrogen, and stored at $-80°$C until analysis with a Partec PAS flow cytometer (Germany) equipped with an Argonion laser (488 nm). The different subpopulations of phytoplankton were distinguished by their autofluorescence of chlorophyll and phycoerythrin content as well as by the cells' side-angle light scatter as a proxy of their size. The data were collected in listmode files using the autofluorescence of chlorophyll as trigger parameter and processed with FloMax software (Partec, Germany).

Nutrient concentrations were measured by standard methods (Strickland & Parsons 1972, Ivančić & Degobbis 1984). The total inorganic nitrogen/orthophosphate ratio (TIN/PO$_4^-$) was calculated according to Redfield et al. (1963). The limiting nutrients for phytoplankton growth at any salinity were determined using the graphic method according to Neill (2005). Graphs were plotted for TIN vs salinity and for o-phosphate vs salinity with the vertical axes set at a ratio of N:P = 16:1 (i.e. the scales for N and P in the graphs were set so as to be proportional to the average rate at which these nutrients are absorbed by phytoplankton during growth). When these graphs are superimposed, the lowermost trendline indicates the limiting nutrient for phytoplankton growth at any salinity.

2.4. Satellite data

The NOAA 15 Advanced Very High Resolution Radiometer (AVHRR) data used in this study were received at a locally operated HRPT station

(Rudjer Bošković Institute, Zagreb). The HRPT data were processed to Level 1B format using the AAPP package supplied by EUMETSAT and the Automatic Navigation Adjustment (ANA 3.1) application provided by Meteo France, CMS. RBI processing to Level 2 is routinely done with an in-house software package (Tomažić 2006). The HRPT stream is processed with both multichannel (MCSST) and non-linear (NLSST) algorithms, at a nominal spatial resolution of 1 km. Only the NLSST product was used in this study.

3. Results

3.1. Thermohaline characteristics and water column structure

The water column was divided into three layers, distinguished according to salinity, temperature and phytoplankton biomass (Figures 2, 3). The surface layer was approximately 10 m deep, the subsurface layer extended between 10 and 40 m, and the deep layer occupied depths below 40 m.

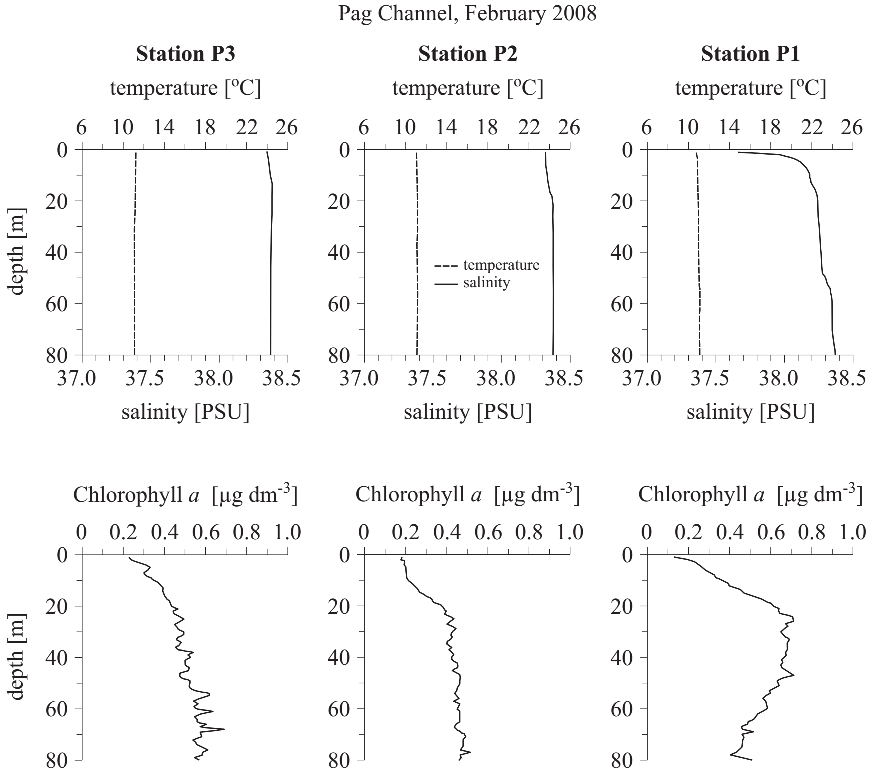

Figure 2. Vertical profiles of salinity, temperature and chlorophyll *a* concentration in the Pag Channel from 6 to 8 February 2008. For station positions, see Figure 1

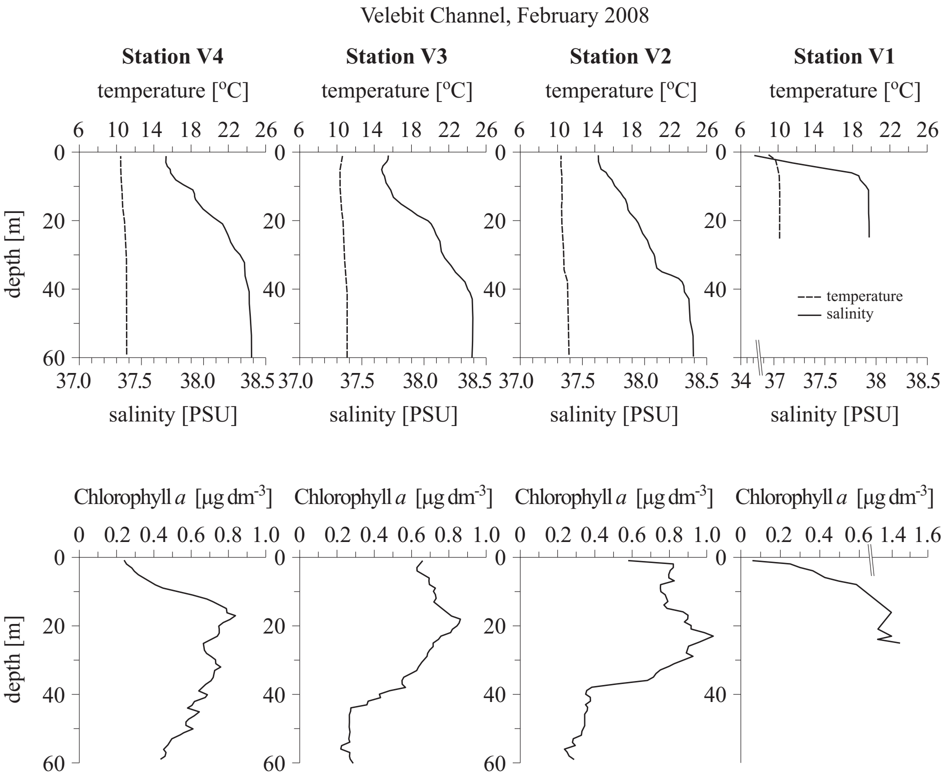

Figure 3. Vertical profiles of salinity, temperature and chlorophyll *a* concentration in the Velebit Channel from 6 to 8 February 2008. For station positions, see Figure 1

Horizontally, the data revealed three distinctive regimes, the first one in the South Velebit Channel (SVC, stations V1–V2), the second one in the Middle Velebit Channel (MVC, stations V2–P1), and the last one in the Pag Channel (PC, stations P1–P3). The SVC behaved like the outer part of a partially mixed estuary, with a 10 m deep surface low-salinity layer, obviously related to the Zrmanja River outflow, the more saline subsurface layer being influenced by conditions in the sea further north. In the MVC the existence of three layers was obvious. The water temperature varied from 10.2 to 11.6°C in the water column. In the 20 to 40 m layer, salinity increased from 37.7 to 38.4 PSU. Salinity and temperature were stable at depths greater than ca 50 m. In the PC the three layers were visible only at station P1.

IR satellite imagery from 6 February 2008 shows the surface temperature field in the broader measurement area, indicating a cooler belt closer to the coastline (Figure 4a). 6 February was rather a cloudy day. Out of 11 AVHRR data received at the RBI that day only one period (NOAA15

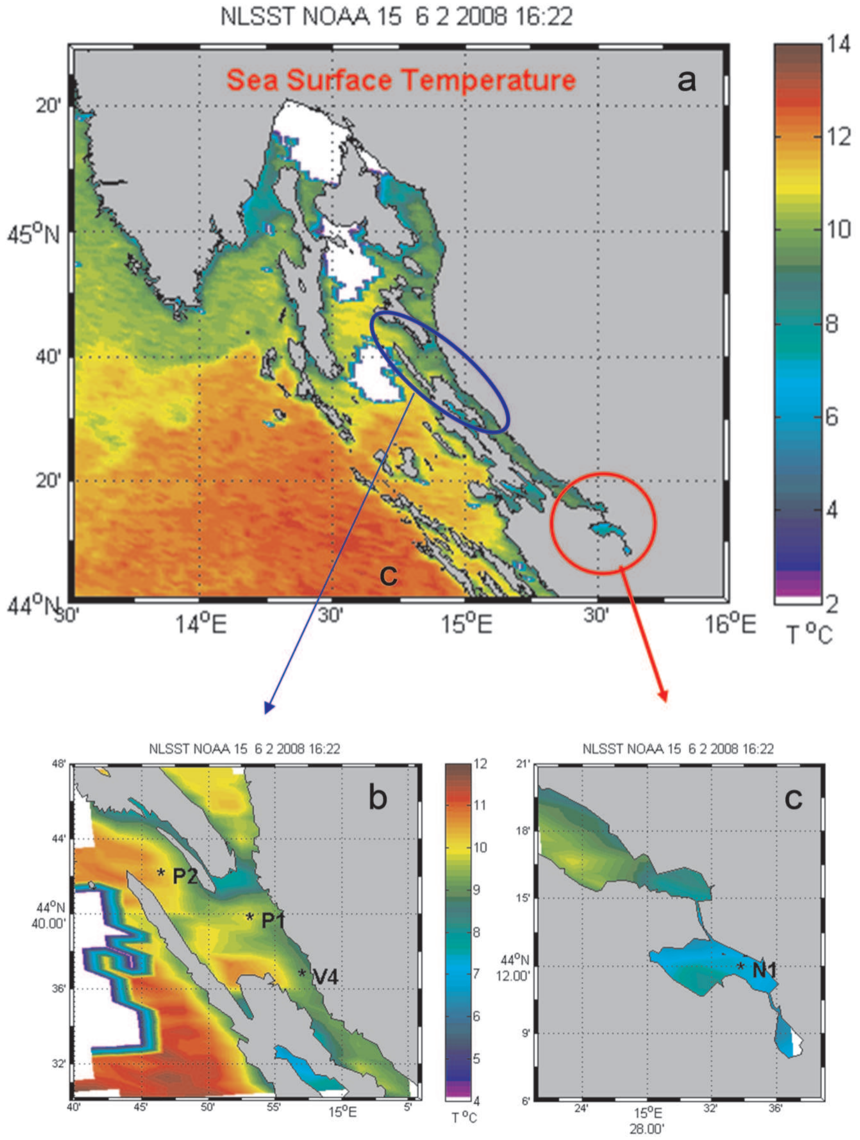

Figure 4. Satellite-derived sea surface temperature in the study area on 6 February 2008

received at 16:22:47 hrs) was relatively cloud-free over the study area. Zooming onto the measurement station areas highlights the temperature differences between the PC (Figure 4b) and the Novigrad Sea (N1, Figure 4c), which correspond very well with the field measurements (not strictly coincident; the measurements were taken from 13.30 to 23.50 hrs CET). Figure 4b also illustrates the cloud masking problem (which does not affect the present analysis). According to CTD measurements, surface temperature and salinity decreased from PC (T = 10.8–11.2°C, S = 37.5–37.7 PSU) to MVC (T = 9–10.5°C, S = 34 to 37.3 PSU) (Figure 5). There was

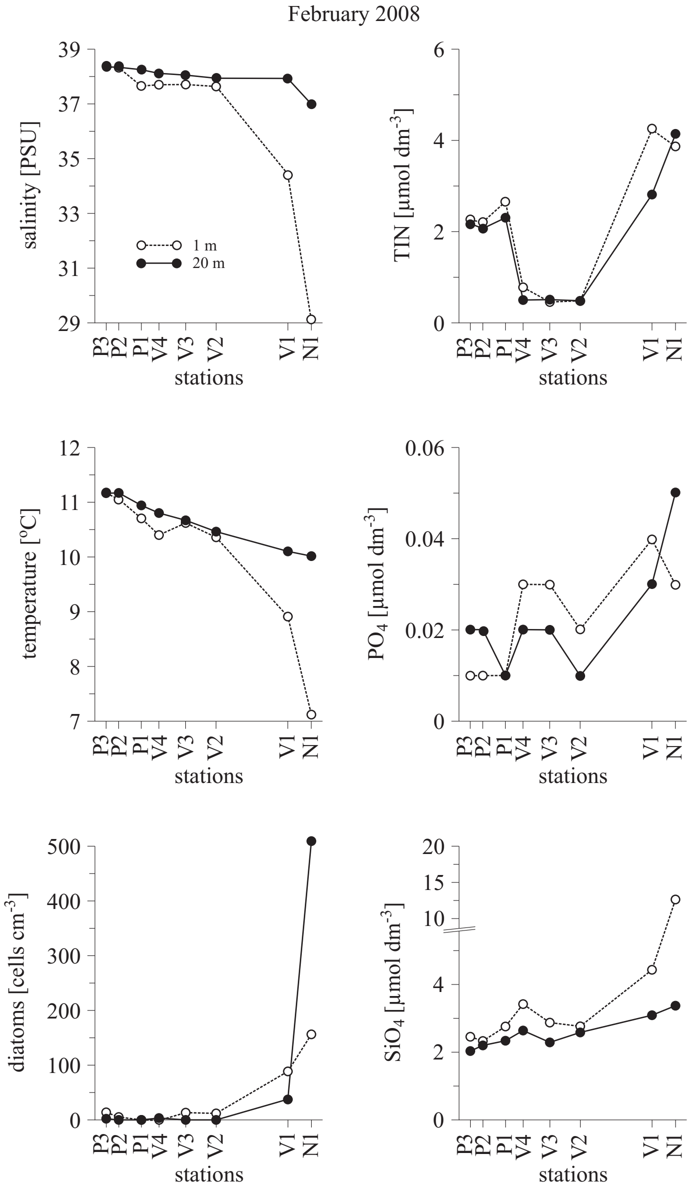

Figure 5. Horizontal distribution of salinity, temperature, total inorganic nitrogen (TIN), orthophosphates (PO_4), orthosilicates (SiO_4) and abundance of diatoms, along the Pag Channel – Velebit Channel – Zrmanja Estuary transect from 6 to 8 February 2008. For station positions, see Figure 1

an abrupt decrease in surface temperature (from 8.91 to 7.13°C) and salinity (from 34.43 to 29.12 PSU) in the innermost part of the channel between stations V1 and N1 (10.3 km apart). A slight salinity decrease was detected in the area between the Velebit and Pag Channels (between Rab Island and the coast) due to freshwater of lower temperature entering from the submarine springs there. At 20 m depth, salinity and temperature in the SVC and Zrmanja Estuary remained stable.

3.2. Nutrients

Concentrations of total inorganic nitrogen (TIN), orthophosphates (PO_4) and silicates (SiO_4) in the 0–20 m layer generally increased 2–5 fold from the outer to the inner part of the channel (Figure 5). The pattern of these nutrients was different in the area between PC and MVC (stations P1–V4), where TIN was 50% less, and PO_4 and SiO_4 slightly higher than the values at neighbouring stations; this was due to the impact of submarine springs. Orthophosphates are thought to be the limiting nutrient in the Channel; limitation by nitrogen could be detected sporadically at the more saline, outer and middle stations (P1 to V3). Redfield ratios ranged between 2.3 and 277 (Figure 6).

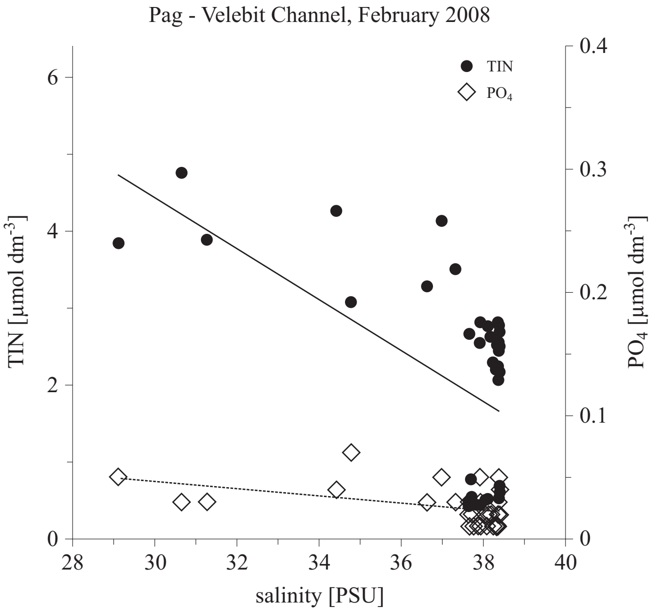

Figure 6. Demonstration of nutrient limitation at any salinity along the Pag Channel – Velebit Channel – Zrmanja Estuary transect. The lowermost trendline denotes the limiting nutrient

3.3. Chlorophyll *a*

The vertical distribution of chlorophyll was characterised by subsurface accumulation in the Velebit Channel and inner Pag Channel, in the 20–40 m layer, with a maximum at a depth of about 20 m (Figure 3). The subsurface maximum concentrations were 2–3 times greater than at the surface in the Pag Channel and MVC, and 2–4 times greater in SVC.

Chlorophyll *a* concentrations increased 10 times from the outer to the inner part of the system (0.4–5.6 μg dm^{-3}), below the halocline (Figure 7). Secchi transparency showed a 2.5 times uniform decrease from PC to the Zrmanja Estuary. The surface concentrations generally increased in the opposite direction (from 0.1 to 0.7 μg dm^{-3}), with about five times greater values in the area of surface freshwater impact (between the Pag and Velebit Channels). Chlorophyll accumulation in the MVC was due mostly to the nanoplanktonic coccolithophorids (*Emiliania huxleyii*) in the 0–20 m layer (Figures 7–9).

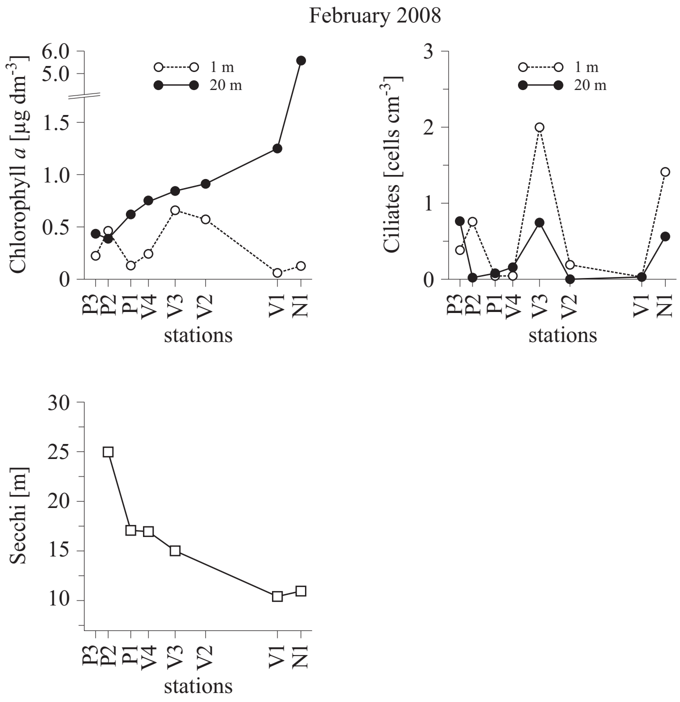

Figure 7. Horizontal distribution of surface chlorophyll *a* biomass, abundance of ciliates and Secchi visibility along the Pag Channel – Velebit Channel – Zrmanja Estuary transect from 6 to 8 February 2008. For station positions, see Figure 1

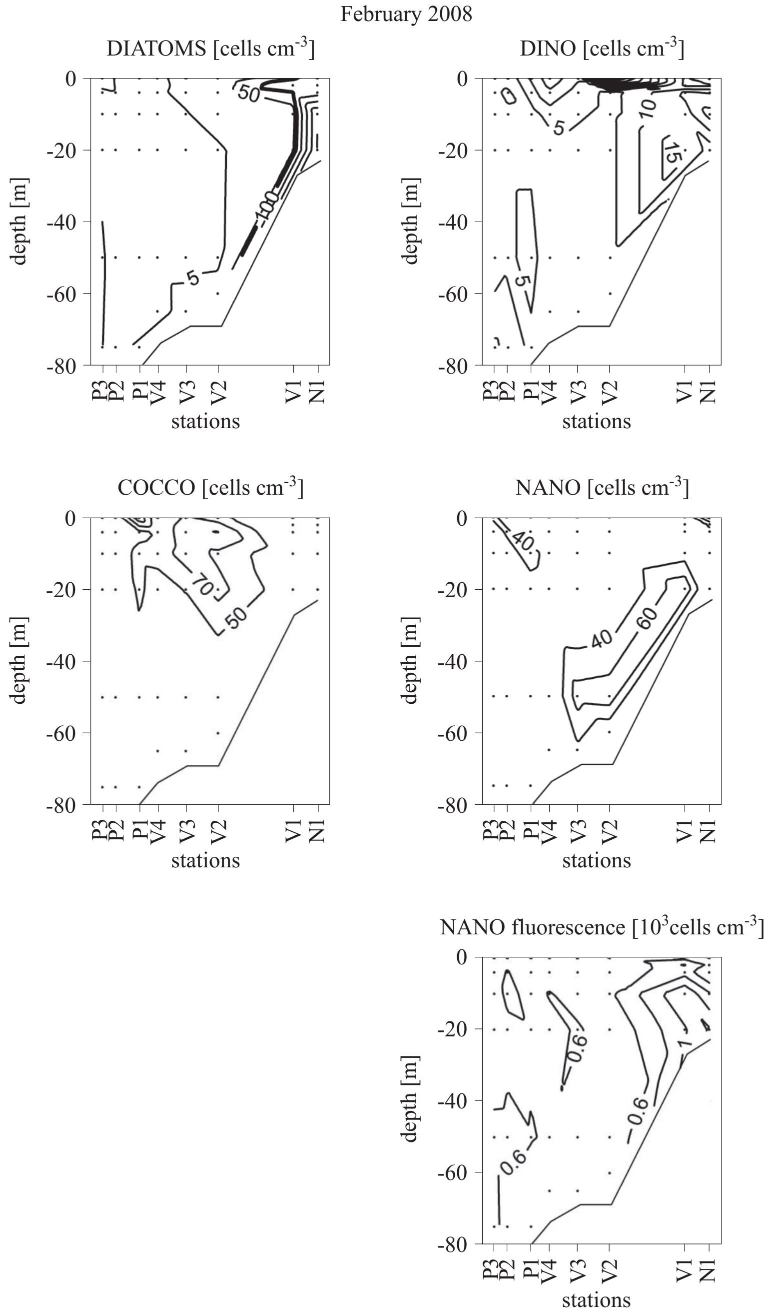

Figure 8. Along-basin vertical contour plots of the abundance of diatoms, dinoflagellates (DINO), coccolithophorids (COCCO), and nanophytoplankton determined by inverted microscope (NANO) and epifluorescence microscope (NANO Fluorescence). Dots indicate sampling depths. For station positions, see Figure 1

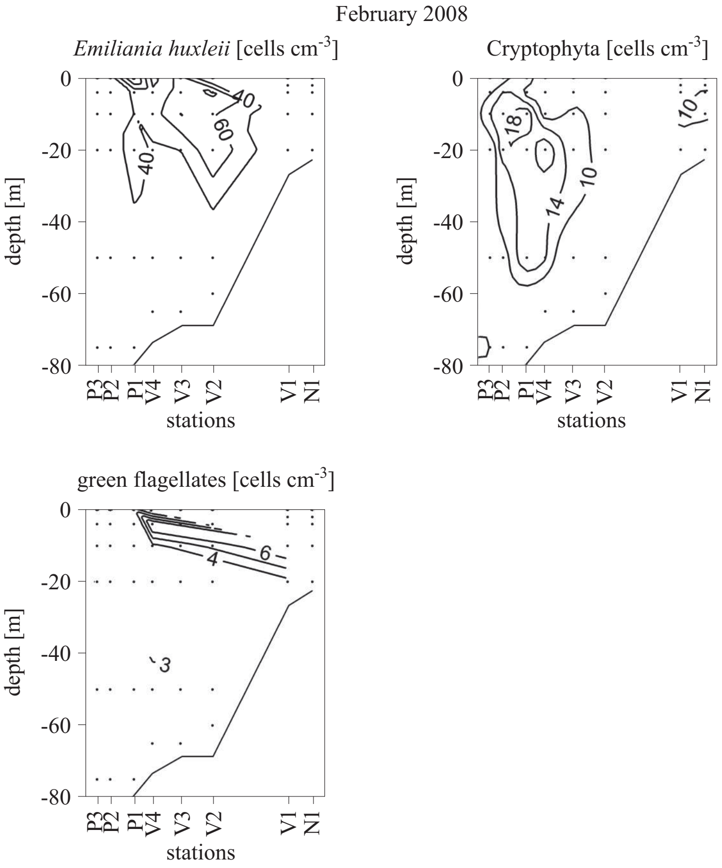

Figure 9. Along-basin vertical contour plots of the abundance of nanoflagellates: *Emiliania huxleyii* (coccolithophorid), cryptophytes and green flagellates. Dots indicate sampling depths. For station positions, see Figure 1

3.4. Phytoplankton abundance and taxonomic composition

Maximum chlorophyll biomass values (1.2–5.6 μg dm^{-3}) were contributed by diatoms, dinoflagellates and nanoflagellates in the inner Velebit Channel and Zrmanja Estuary (Figures 8, 9). Diatoms attained a five times greater abundance below the halocline than above it (5×10^5 cells dm^{-3}, in the estuary); they consisted mostly of the chain-forming species *Bacteriastrum* and *Chaetoceros* (Table 1).

Recognisable nanoplankton was composed mostly of coccolithophorids, small dinoflagellates, chlorophytes and cryptophytes.

There were one subsurface and two surface accumulations of picoplankton. The first surface accumulation was due to cyanobacteria in the central channel area (at Stations P1 and V4), and the second one

Table 1. Dominant phytoplankton species with maximum abundance (MAX) of $> 10^4$ cells dm^{-3}, and frequency of appearance (Fr) $> 10\%$ (46 samples is 100%), on 6 February 2008. AVG – average abundance, P – Prymnesiophyceae (coccolithophorids), D – diatoms, C – Chrysophyceae

	6 February 2008	**MAX**	**AVG**	**Fr**
	Fr > 10% Max > 10 000, n = 46	**[10^3 cell dm^{-3}]**		**[%]**
P	*Algirosphaera robusta* (Lohmann) Norris	14.6	1.3	17.4
P	*Emiliania huxleyi* (Lohmann) Hay et Mohler	106.1	28.0	82.6
D	*Bacteriastrum delicatulum* Cleve	224.2	10.0	28.3
D	*Bacteriastrum elongatum* Cleve	16.1	0.8	10.9
D	*Bacteriastrum mediterraneum* Pav.	45.6	3.2	19.6
D	*Cerataulina pelagica* (Cleve) Hendey	52.2	2.3	37.0
D	*Chaetoceros compressus* Laud.	381.6	12.7	30.4
D	*Chaetoceros curvisetus* Cleve	34.2	3.8	45.7
D	*Chaetoceros diversus* Cleve	17.1	1.0	21.7
D	*Chaetoceros lorenzianus* Grun.	12.3	0.5	17.4
D	*Chaetoceros socialis* Laud	36.8	1.1	34.8
D	*Dactyliosolen fragilissimus* (Bergon) Hasle	15.5	1.0	21.7
D	*Proboscia alata* (Brightw.) Sund.	11.3	0.7	39.1
D	Penatae	10.9	0.8	63.0
C	*Dinobryon* spp.	25.6	1.0	28.3
	Diatoms total	510.3	44.4	97.8
	Dinoflagellates total	30.1	3.0	60.9
	Coccolithophorids total	106.1	33.6	97.8
	Cryptophyceae	21.9	8.7	76.1
	Chrysophyceae	25.6	1.1	32.6

to photosynthetic eukaryotes in the SVC (Figure 10). Photosynthetic eukaryotes contributed mostly to the subsurface accumulation in the PC (in the 20–40 m layer). There was no significant positive correlation between total nanoplankton, counted by inverted microscope, and the flow-cytometer counted nanoautotrophs (Figure 11). The flow-cytometer counts of nanophytoplankton were about 10–30 times greater than the corresponding inverted microscope counts. The correlation between cyanobacteria and autotrophic picoeukaryotes was significant, where cyanobacteria were about four times more abundant than picoeukaryotes.

4. Discussion

The temperature and salinity distribution suggested an outflow of surface water from the Zrmanja Estuary into the Velebit Channel. On the other hand, the uniform vertical distribution of salinity in the subsurface

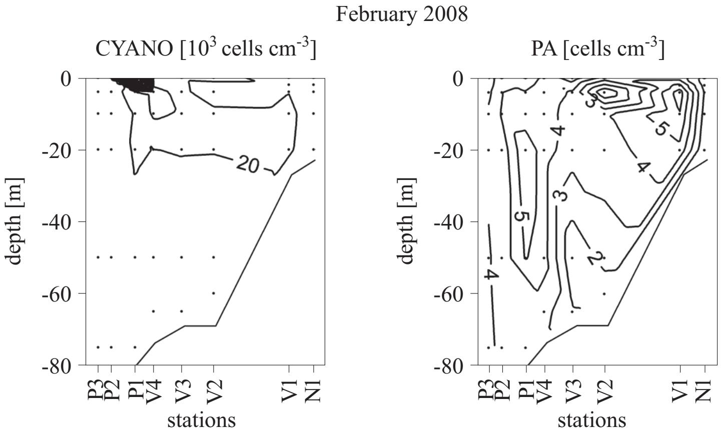

Figure 10. Along-basin vertical contour plots of the abundance of picophytoplankton (cyanobacteria – CYANO, and photosynthetic eukaryotes – PA) determined by flow cytometer. Dots indicate sampling depths. For station positions, see Figure 1

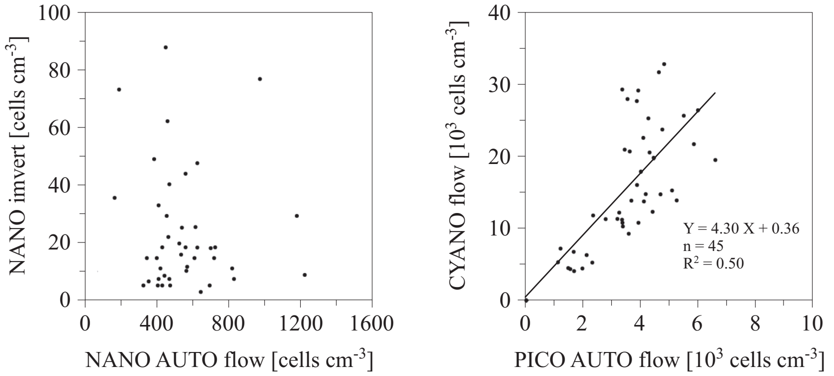

Figure 11. Relation between abundances of nanophytoplankton determined by inverted microscope and flow cytometry (upper), and between cyanobacteria and total autotrophic picoeukaryotes (lower) in February 2008. The correlation in the upper panel is statistically not significant

layer indicated an inflow of open sea water into the channel. There was also the surface spread of warmer offshore surface water into the Kvarnerić basin and Pag Channel. In the inner Velebit Channel area (upstream of station V1), there was a transition zone between marine and brackish waters. The present study has demonstrated how remote sensing and in situ investigations can be useful for acquiring an understanding of the complex hydrographic set-up. The water column structure was characterised by a thermocline more strongly defined in summer (Viličić et al. 2008a) than in February 2008, when stratification was due mostly to the freshwater influx.

The phytoplankton dynamics in the estuary is closely related to riverine water discharge, nutrient levels and water residence time (Viličić et al. 2008b). In the dry summer conditions, phytoplankton biomass decreased in the lower estuary, but increased in the upper estuary.

In January and February 2008, at the time of sampling, the amount of rainfall in the drainage area of the River Zrmanja was 50% less than average (about 40 mm) (www.dhmz.htnet.hr). Under such conditions, the estuarine input of riverine water and nutrients provoked the growth of diatoms, dinoflagellates, green flagellates and grazers (ciliates) in the stabile environment below the halocline in the Zrmanja Estuary and SVC. Orthophosphates are thought to be the limiting nutrient in the Channel, while limitation by nitrogen could be detected sporadically at the more saline, outer and middle stations (P1 to V3). The impact of coastal submarine springs between Rab and Pag islands (P1 and V4) initiated the growth of cyanobacteria in the nitrogen-depleted surface waters (0.5 μmol dm^{-3}). The low nitrogen concentration may probably be due to microbial transformations of nitrogen in the groundwater (Kroeger & Charette 2008). The greater contribution of the small size fractions – picoplankton and nanoplanktonic coccolithophorids and cryptophytes in the outer channel system – indicated their preference for oligotrophic conditions. This case study has thus provided evidence for the classical distribution of surface microphytoplankton (increased abundance of diatoms) in the nutrient-rich inner channel, and the patchiness of nanoflagellates and picoeukaryotes in the oligotrophic section of the profile. Picoplanktonic cyanobacteria usually show a broad distribution from the open ocean to the offshore and coastal waters of semi-enclosed seas and, because of their photoheterotrophy, they are responsible for a substantial fraction of both primary production and organic matter degradation/utilisation (Paoli et al. 2007). They were able to survive and grow well during nitrogen deficiency at station P1.

In this research, the chlorophyll *a* biomass was calculated from CTD fluorescence but not measured by spectrofluorometry or HPLC. Thus we could not evaluate the photoinhibition of fluorescence near the surface, especially in conditions when the profiling was performed during sunny weather.

The evidence of successful fishing activities in the oligotrophic Pag and Velebit Channels merits further scientific evaluation of the specific food web processes in this area. Transition zones could be important for the further study of frontal systems, bio-geochemical processes, regional air-sea interactions and fish biology.

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