Cryptophyte bloom in a Mediterranean estuary: High abundance of *Plagioselmis* cf. *prolonga* in the Krka River estuary (eastern Adriatic Sea)

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Summary: During the June 2010 survey of phytoplankton and physicochemical parameters in the Krka River estuary (eastern Adriatic Sea), a cryptophyte bloom was observed. High abundance of cryptophytes (maximum 7.9×10^6 cells l^{-1}) and high concentrations of the class-specific biomarker pigment alloxanthine (maximum 2312 ng l^{-1}) were detected in the surface layer and at the halocline in the lower reach of the estuary. Taxonomical analysis revealed that the blooming species was *Plagioselmis* cf. *prolonga*. Analysis of the environmental parameters in the estuary suggested that the bloom was supported by the slower river flow as well as the increased orthophosphate and ammonium concentrations. The first record of a cryptophyte bloom in the Krka River estuary may indicate that large-scale changes are taking place in the phytoplankton community. Such changes could have a major impact on the natural ecosystem dynamics and the mariculture production in the area.

Keywords: estuarine ecosystem; phytoplankton bloom; cryptophytes; chemotaxonomy; Mediterranean Sea; Krka River estuary.

Proliferación de una criptófita en un estuario mediterráneo: alta abundancia de *Plagioselmis* cf. *prolonga* en el estuario del río Krka (Adriático)

Resumen: Se observó una proliferación de una criptófita durante el estudio del fitoplancton y de los parámetros fisicoquímicos en el estuario del río Krka (Adriático) en junio de 2010. La abundancia más alta de criptofitas (máximo 7.9×10^6 células l^{-1}) y las mayores concentraciones del pigmento marcador específico de la clase, la aloxantina (máximo 2312 ng l^{-1}), fueron detectados en la capa superficial y en la haloclina en el tramo inferior de la ría. El análisis taxonómico reveló que la especie que proliferó fue *Plagioselmis* cf. *prolonga*. El análisis de los parámetros ambientales del estuario sugirió que la proliferación estaba favorecida por el caudal lento del río, así como el aumento de las concentraciones de ortofosfato y amonio. El hecho de registrar por primera vez una proliferación de una criptófita en el estuario del río Krka podría indicar cambios a gran escala en la comunidad de fitoplancton. Tales cambios podrían tener un impacto importante en la dinámica de los ecosistemas naturales y la producción de la maricultura en la zona.

Palabras clave: estuario; proliferación de fitoplancton; criptófitas; quimiotaxonomía; mar Mediterráneo; estuario del río Krka.

INTRODUCTION

Nanoplanktonic cryptophyte flagellates (Phylum Cryptophyta, Class Cryptophyceae) are widely distributed in most aquatic habitats (Barlow and Kug-rens 2002, Barone and Naselli-Flores 2003, Novarino 2005). Current taxonomy of the group recognizes more than 200 validly described species, mostly based on light and electron microscopical observations (Cerino and Zingone 2007). Ecological investigations of cryp-

tophytes are often hampered due to the identification issues caused by their small size (usually <20 μm), cell fragility in common fixatives and key taxonomic characters that are difficult to observe under the light microscope. Investigations of marine cryptophyte communities with a dilution cultures method (Cerino and Zingone 2006) and molecular techniques (Metfies et al. 2010) revealed a pronounced seasonality of cryptophyte species, suggesting that the commonly used class-level approach is over-simplifying. Furthermore, species-specific aspects of cryptophyte ecology, such as mixotrophy (Tranvik et al. 1989, Roberts and Laybourn-Parry 1999), vertical migrations (Erata et al. 1995), high tolerance to environmental changes (Klaveness 1988) and interactions with other organisms such as dinoflagellates (Hackett et al. 2003, Park et al. 2006) and ciliates (Hansen and Fenchel 2006) indicate the ecological and physiological complexity of the group.

Reports of cryptophyte blooms and cryptophyte-dominated communities in oceanic (Gieskes and Kraay 1983, Buma et al. 1992), freshwater (Barone and Naselli-Flores 2003) and estuarine (Mallin 1994, Gameiro et al. 2004) environments show the potentially high ecological importance of cryptophytes. Furthermore, red tides caused by cryptophyte blooms were observed in natural environments (Laza-Martínez 2012 and the references therein) and enclosed oyster ponds (Pastoureaud et al. 2003). However, cryptophyte blooms in the Mediterranean Sea are very rare and exceptional events. The only reports to date were given by Andreoli et al. (1986) and Bazin et al. (2014), who investigated the red tides in the Po River delta (northern Adriatic Sea) and the Segura River estuary (western Mediterranean Sea), respectively. Cryptophytes that dominated these blooms were identified under light and electron microscope as the members of *Plagioselmis* (Butcher 1967), a common and widespread genus in the Mediterranean Sea (Novarino 2005).

Here we present the new report on the cryptophyte bloom in the Mediterranean Sea dominated by *Plagioselmis* cf. *prolonga* Butcher ex G. Novarino, I.A.N. Lucas and S. Morrall. The bloom is described in detail, contributing to the knowledge on the ecology and physiology of the species. Furthermore, increased cryptophyte abundance is discussed in the context of possible changes in phytoplankton communities of the Krka River estuary that might have a major impact on natural ecosystem dynamics and mariculture production in the area.

MATERIALS AND METHODS

Study area

The highly stratified Krka River estuary is situated in the central part of the eastern Adriatic Sea (Fig. 1). The estuary is 23.5 km long and relatively narrow except for the two larger pools, Prokljan lake and Šibenik harbour. The influence of the sea on the hydrographic properties is limited due to the low tidal range in this part of the Adriatic Sea. A sharp halocline caused by freshwater inflow is present throughout the year (Žutić and Legović 1987). The vertical gradient of salinity causes mortality and accumulation of freshwater planktonic organisms at the halocline, and the accumulated organic matter supports high bacterial production and regeneration processes (Žutić and Legović 1987, Fuks et al. 1991). The whole system is considered phosphorus-limited, except for the anthropogenically-influenced Šibenik harbour pool (Legović et al. 1994). The phytoplankton community in the estuary is dominated by diatoms and the annual phytoplankton production reaches maxima in February-March and October (Viličić et al. 1989, Cetinić et al. 2006). The exceptionally large phytoplankton blooms occur either in the marine layer of the estuary or in the upstream-situated freshwater lake Visovac, and are often followed by hypoxic conditions and mass mortality of marine benthic organisms in the lower reach of the estuary (Legović et al. 1991a, Legović et al. 1991b, Petricioli et al. 1996). Owing to the favourable hydrographical parameters, the lower reach of the Krka estuary is an important area for mariculture, with the yearly production of bivalves exceeding 2000 t (Jukić et al. 2007).

Sampling and measurements

The sampling was performed in June 2010 along the lower reach of the Krka River estuary (stations E3, E4a and E5) and at the marine station (C1) near Zlarin island (Fig. 1). Six depths were sampled at each station with 5-L Niskin (Hydro-Bios, Kiel, Germany) bottle (0.5, 1.2, 2, 2.8, 10 and 20 m at E3; 0.5, 1, 1.8, 2, 10 and 30 m at E4a; 0.5, 1, 1.5, 4, 10 and 20 at E5; 0.5, 2, 5, 10, 20 and 25 m at C1). The sampling depths were selected after the examination of the temperature, salinity and chlorophyll *a* fluorescence vertical profiles, measured using SeaBird 19 plus CTD probe (SeaBird Electronics, Inc. Washington, USA).

Standard colorimetric methods were used to determine orthophosphate (Murphy and Riley 1962), orthosilicate (Mullin and Riley 1955), nitrate, nitrite (Wood et al. 1967) and ammonium (Ivančić and Degobbis 1984) concentrations at the sampled depths.

Samples for the light microscopy (LM) analysis of phytoplankton abundance were fixed in situ in 1.4% hexamine-buffered formaldehyde (Kemika, Zagreb, Croatia) and cells were counted under the Zeiss Axiovert 200 inverted microscope (Carl Zeiss, Oberkochen, Germany) using the Utermöhl (1958) protocol. Subsamples of 10 or 50 ml (depending on the cell density) were sedimented in Utermöhl combined plate-counting chambers (Hydro-Bios, Kiel, Germany) for >24 hours. Nanoplankton (cells <20 μm) were counted at 400x magnification on half of the transect (i.e. 1/2 diameter of the counting chamber), and the larger cells were counted at 200× magnification (two full transects). Very abundant species were counted on a variable number (5-20) of randomly chosen fields of view at either 200 or 400× magnification depending on their size. In addition, the bottom half of the chamber was examined at a magnification of 100× to obtain a more correct evaluation of less abundant microphytoplank-
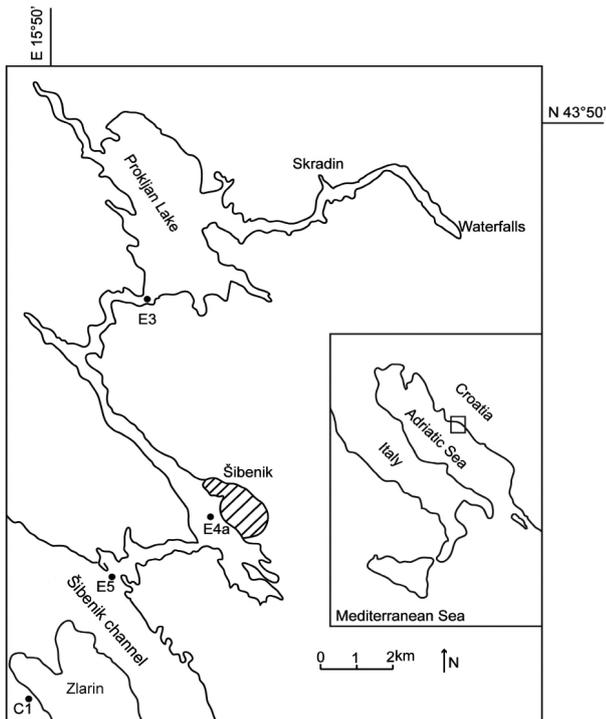
Fig. 1. – Map of the investigated area showing estuarine (E3, E4a, E5) and coastal (C1) sampling stations.

ton taxa. The minimum concentration of microphytoplankton cells that can be detected by this method is 20 cells l^{-1}. Cryptophyte cells were photographed under the phase contrast and differential interference contrast filters at 1000× magnification with the Zeiss MRc digital camera and analysed in Zeiss AxioVision 4.8.2. software.

For the scanning electron microscopy (SEM) analysis of field samples, 30 mL of 1.4% hexamine-buffered formaldehyde fixed sample from the 1-m depth of station E4a was gravity filtered on a 1- to 3-μm pore size Nucleopore polycarbonate filter (Nucleopore, Pleasanton, CA). The filter was rinsed three times with 20 ml dH_2O and dehydrated in a series of ethanol dilutions (25, 35, 50, 75, 80, 90 and 100%). After that, the filtered material was dried in a series of ethanol : hexamethyldisilazane (HMDS, Sigma-Aldrich, Seelze, Germany) dilutions (3:1, 1:1, 1:3 and 100% HMDS). Finally, filters were air dried for 1 hour at 60°C, sputter-coated with gold-palladium and observed using JEOL JSM-6500F (JEOL-USA Inc., Peabody, MA, USA) and Mira II FE LMU (Tescan, Brno, Chech republic) scanning electron microscopes. The cryptophyte *Plagioselmis* cf. *prolonga* was identified using the standard literature for the identification of cryptophytes (Hill 1992, Novarino et al. 1994, Novarino 2003, 2005).

Samples of 1-L volume for the high performance liquid chromatography (HPLC) analysis of phytoplankton pigments were filtered in situ on 0.7-μm pore size Whatman Glass Fibre Filters (GF/F) and preserved in –80°C liquid nitrogen until the analysis. The extraction in 4 ml of cold 90% acetone was performed by sonication, and the extract was clarified by centrifugation. Pigments were separated by reversed phase

HPLC following the protocol of Barlow et al. (1997). Extracts were mixed 1:1 (v/v) with 1 M ammonium acetate and injected into an HPLC system with the 3-mm Thermo Hypersil-Keystone column MOS2, C-8, 120 A pore size, 150×4.6 mm (Thermo Hypersil-Keystone, Bellefonte, PA, USA). Pigments were separated at a flow rate of 1 mL min^{-1} using a linear gradient program with a duration of 40 min. Solvent A consisted of 70:30 (v/v) methanol:1 M ammonium acetate and solvent B was 100% methanol. Chlorophyll and carotenoids were detected by absorbance at 440 nm (SpectraSYSTEM, Model UV 2000, Thermo Fischer Scientific, USA). Qualitative and quantitative analyses of individual pigments were performed by external standard calibration using authentic pigment standards (VKI, Denmark). The retention time of the cryptophyte-specific pigment alloxanthine was confirmed by the analysis of the monoculture of cryptophyte *Rhinomonas* cf. *reticulata* (Lucas) Novarino isolated from the Krka estuary.

Principal component analysis (PCA) of the environmental data with the subsequent overlay of alloxanthine concentration data was performed in Primer 6 software (Clarke and Gorley 2006). Statistica 8.0 software was used to calculate the Pearson's correlation between the alloxanthine concentration and the environmental parameters, with the significance recognized at $p<0.05$. Sigma Plot 12.5 software was used for the graphical presentation of the data.

RESULTS

Environmental parameters

The sharp halocline was detected at station E3 in the 1.5- to 3-m layer and at station E4a in the 1- to 2-m layer (Fig. 2). Salinities of 32.5 to 33 were observed in the top 1.5 m layer at the E5 station, while the coastal marine station C1 had no vertical gradient of salinity. The surface temperatures ranged from 20 to 24°C, gradually decreasing to 13 to 16°C in deeper layers. Minimum temperature was detected at the bottom of station E4a (14.71°C at 36.2 m) while the highest temperature (24.17°C) was measured in the surface layer of the same station (Table 1).

Chlorophyll *a* (chl *a*) concentrations at station E4a were 1484 to 5028 ng l^{-1} in the upper 2-m layer, indicating the on-going phytoplankton bloom. A strong significant correlation of alloxanthine with chl *a* (0.968) confirmed that cryptophytes were the dominant component of the bloom. High chl *a* values were also observed at station E3 (up to 1384 ng l^{-1}), while the overall chl *a* concentrations decreased downstream from station E4a, with maxima of 832 ng l^{-1} at station E5 and 276 ng l^{-1} at station C1. Vertical distribution of chl a at station C1 was fairly uniform, with slightly higher concentrations in the surface layer.

Concentrations of total inorganic nitrogen (TIN = $NH_4^+ + NO_2^{2-} + NO_3^-$) and orthosilicates decreased along the estuary, from stations E3 to E5. Concentrations were higher in the surface layer and at the halocline (Fig. 3, Table 1). The highest values of TIN and orthosilicates were detected at the halocline of station
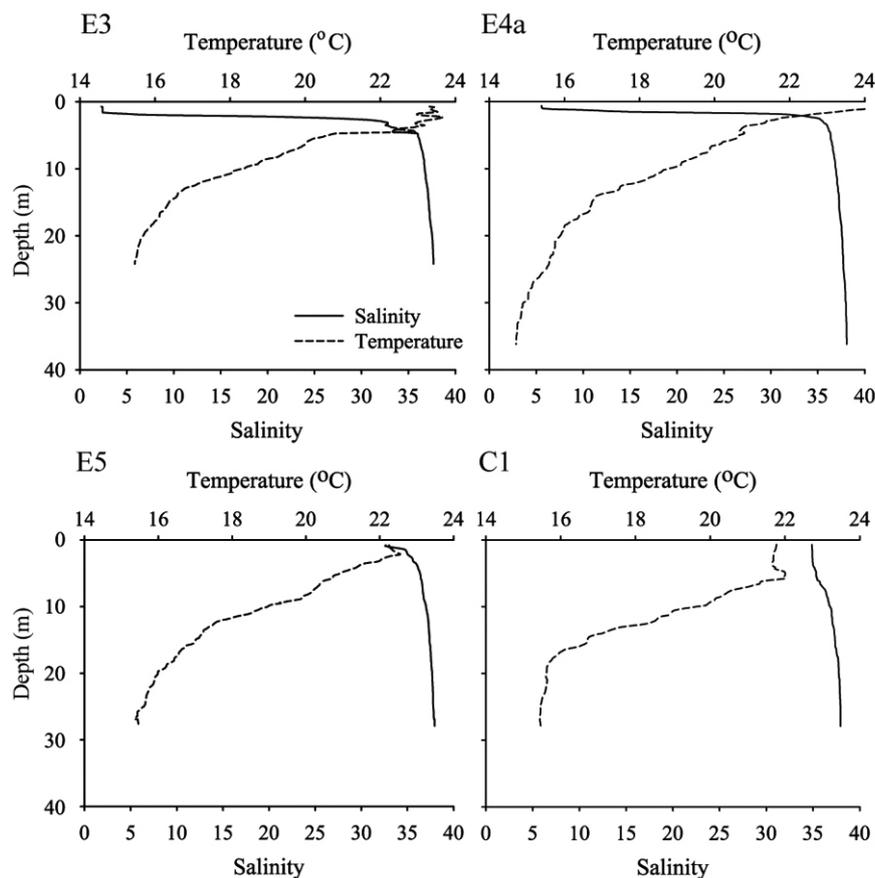
Fig. 2. – Vertical profiles of temperature (dashed line) and salinity (solid line) at the sampled stations.

E3 (13.9 and 44.1 µM l^{-1}, respectively). The distribution of orthophosphates (PO$_4$$^{3-}$) did not follow the same pattern. The highest concentration of orthophosphates was detected at the halocline of station E4a (0.3 µM l^{-1}), and the average concentrations of orthophosphates in the surface layer and the halocline of station E4a were more than three times higher than the average concentration in the area. Phosphorus limitation of the area was confirmed by the high Redfield ratio (TIN/PO$_4$$^{3-}$), which approached values of 21.7 to 31.3 at the halocline of station E4a, where the PO$_4$$^{3-}$ concentration was the highest (Table 1). Station C1 was extremely oligotrophic, with the Redfield ratio reaching 1318 at the surface and with an average silica concentration of only 3.35 µM l^{-1}.

Plagioselmis cf. *prolonga* bloom

High concentrations of alloxanthine were detected in the sub-surface layer of station E4a, with peak val-

ues between 1 and 2 m depth (1434-2311 ng l^{-1}, Fig. 4). The abundance of cryptophytes reached maximum values at the same depth (7.9×10^6 cells l^{-1}). A high concentration of alloxanthine was also detected at station E5 at 4 m depth (240 ng l^{-1}), where cryptophyte abundance was 2.6×10^5 cells l^{-1}. At both E4a and E5 cryptophyte abundance was high in the surface layer and at the halocline, where cryptophytes accounted for 40 to 49% of total phytoplankton, decreasing sharply in deeper layers. A lower concentration of alloxanthine indicated a lower abundance of cryptophytes upstream from Šibenik harbour (at station E3) and at the marine station C1, with the contribution to total phytoplankton ranging from 2 to 15%. Validity of LM counts was tested and confirmed by strong and statistically significant positive correlation of alloxanthine concentration with cryptophyte abundance (0.991).

The PCA of environmental parameters was conducted to link the distribution of alloxanthine concentrations with the physicochemical parameters. The

Table 1. – Descriptive statistics of physicochemical parameters and cryptophyte abundance. Pearson's correlation of alloxanthine concentration with the physicochemical parameters is highlighted in bold when values are statistically significant (p<0.05)

|	Temperature (°C)	Salinity	TIN (µM l^{-1})	NO$_3$$^-$ (µM l^{-1})	NO$_2$$^{2-}$ (µM l^{-1})	NH$_4$$^+$ (µM l^{-1})	PO$_4$$^{3-}$ (µM l^{-1})	SiO$_4$$^{4+}$ (µM l^{-1})	Redfield ratio (TIN/PO$_4$)	Chlorophyll *a* (ng l^{-1})	Cryptophytes (cells l^{-1})
Minimum	14.71	2.29	1.07	0.18	0.03	0.86	0.01	1.86	21.71	112.61	8.5×10^3
Maximum	24.17	37.93	35.04	11.70	0.37	2.48	0.30	44.31	1317.73	5028.75	7.9×10^6
Arithmetic mean	15.69	29.08	6.61	2.67	0.14	1.56	0.07	15.93	153.33	1070.00	8.98×10^5
Standard deviation	2.98	12.78	7.76	3.14	0.11	0.51	0.08	15.15	256.34	1307.88	2.1×10^6
Pearson's correlation	0.395	–0.296	0.242	0.182	**0.557**	**0.490**	**0.950**	0.376	–0.218	**0.968**	**0.991**
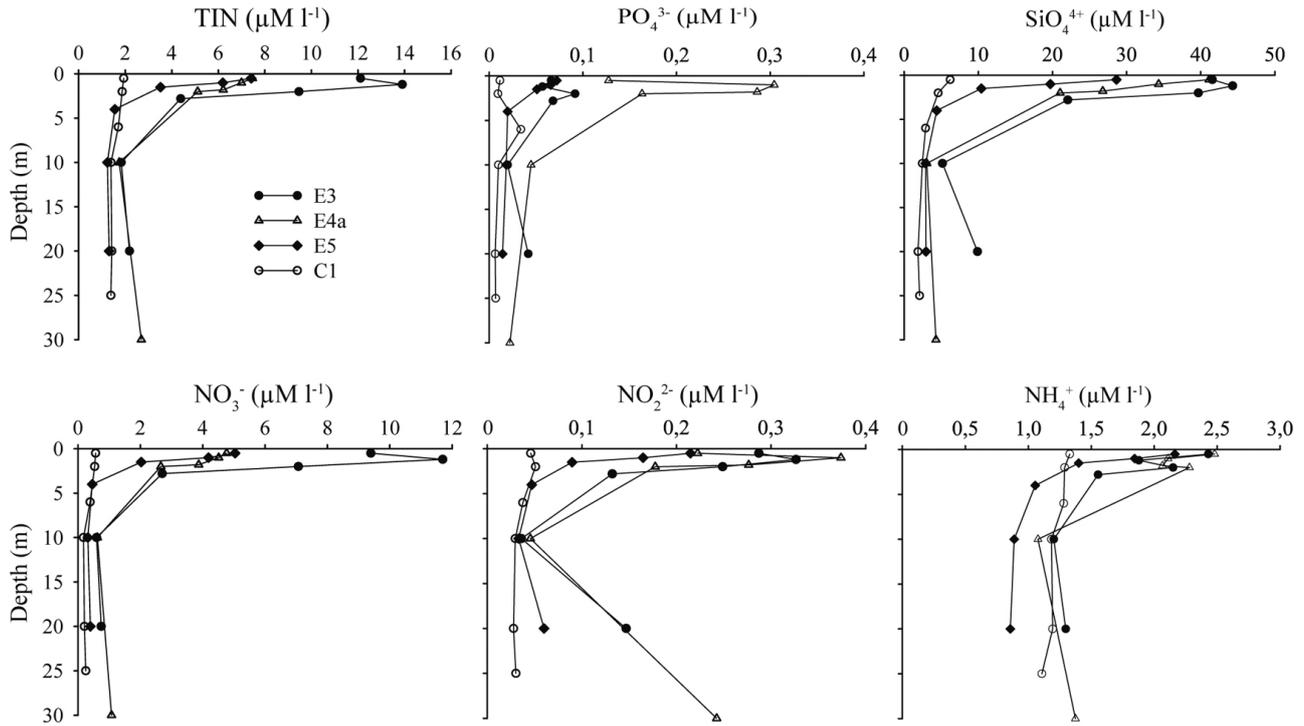
Fig. 3. – Vertical profiles of total inorganic nitrate (TIN), orthophosphate (PO_4^{3-}), orthosilicate (SiO_4^{4+}), nitrate (NO_3^-), nitrite (NO_2^{2-}) and ammonium (NH_4^+) concentrations at the sampled depths.
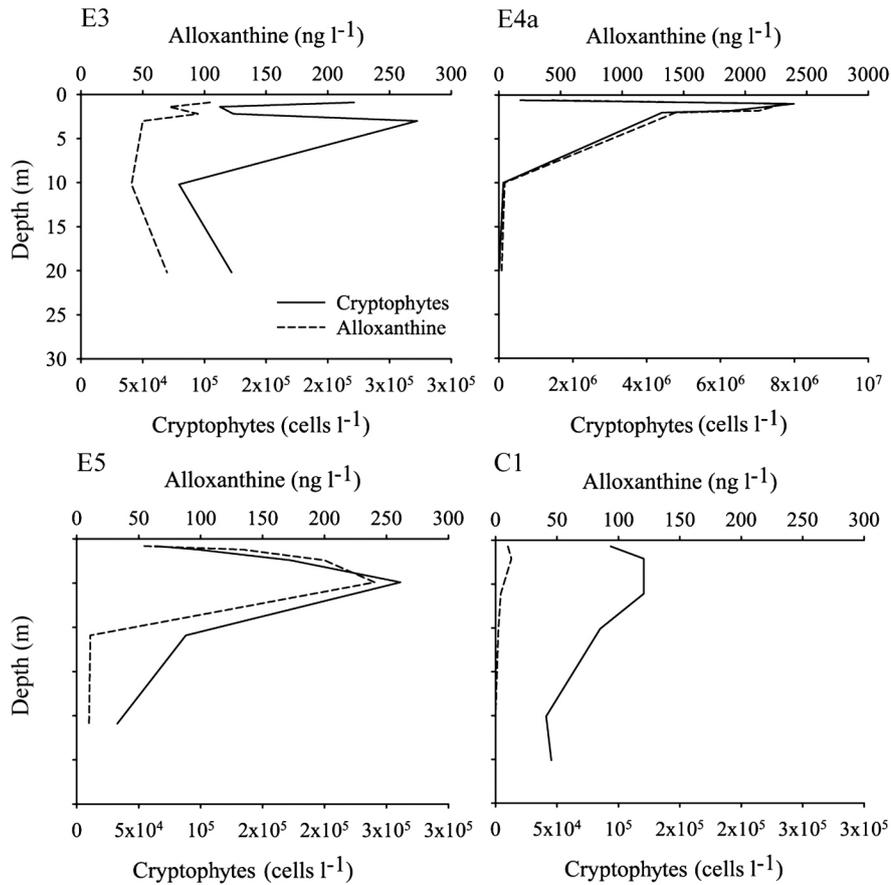

Fig. 4. – Abundance of cryptophytes determined by the LM counts (solid line) and the concentration of the class-specific pigment alloxanthine (dashed line) at the sampled depths. Note the larger scale on the graph representing station E4a.

Table 2. – First two principal components generated by the principal component analysis of physicochemical parameters

Variable	PC1	PC2
NH_4^+	−0.122	−0.088
NO_2^{2-}	−0.059	−0.012
NO_3^+	−0.522	−0.152
PO_4^{3-}	−0.030	−0.041
SiO_4^{4+}	−0.681	−0.476
Temperature	−0.074	−0.053
Salinity	0.489	−0.859

first two principal components accounted for 98% of the variance, and were both primarily defined by orthosilicates, nitrites, ammonium and salinity (Table 2). The scatter plot of the first two principal components showed three main groups of the samples (Fig. 5): A) low salinity and nutrient rich samples from the surface layer; B) intermediate salinity and nutrient-rich samples from the halocline; and C) marine samples defined by high salinity and a low nutrient concentration. An overlying plot of alloxanthine concentrations showed that cryptophytes were most abundant in the nutrient-rich samples from the halocline and in the freshwater
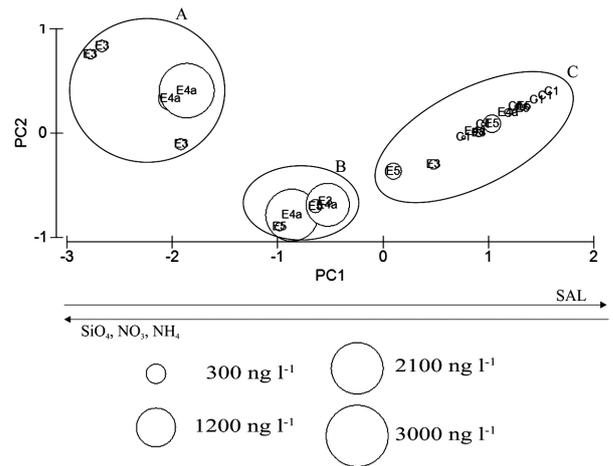

Fig. 5. – Scatterplot of the first two principal components generated by principal component analysis of physicochemical parameters. Overlaid are the alloxanthine concentration values: A, low salinity and high nutrient samples from the surface layer; B, samples from the freshwater/seawater interface (halocline); C, high salinity and low nutrient samples from deeper layers of estuarine stations and all samples from station C1.
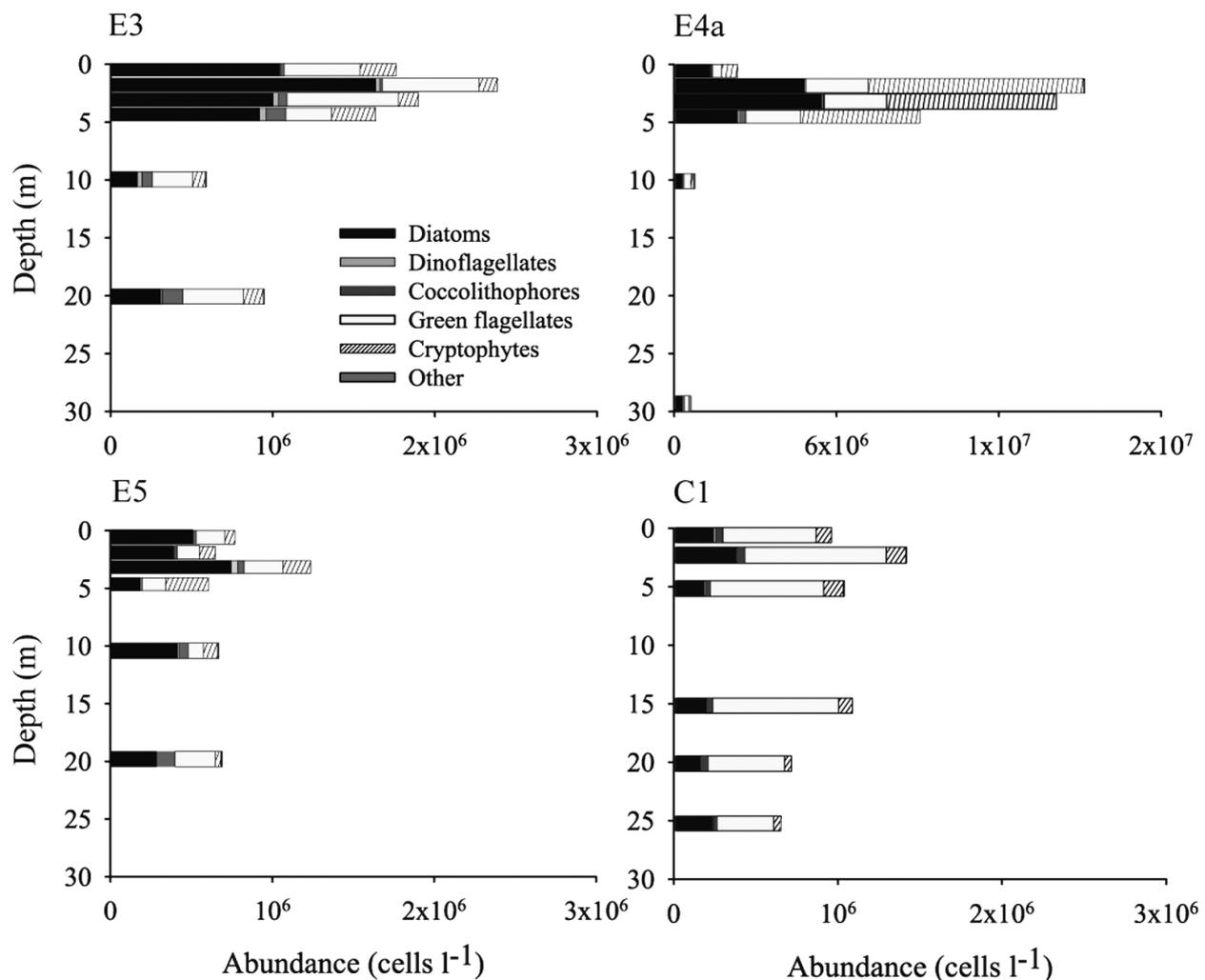

Fig. 6. – Bar chart presentation of the abundance of main phytoplankton groups at the sampled depths. Note the larger scale on the graph presenting station E4a.
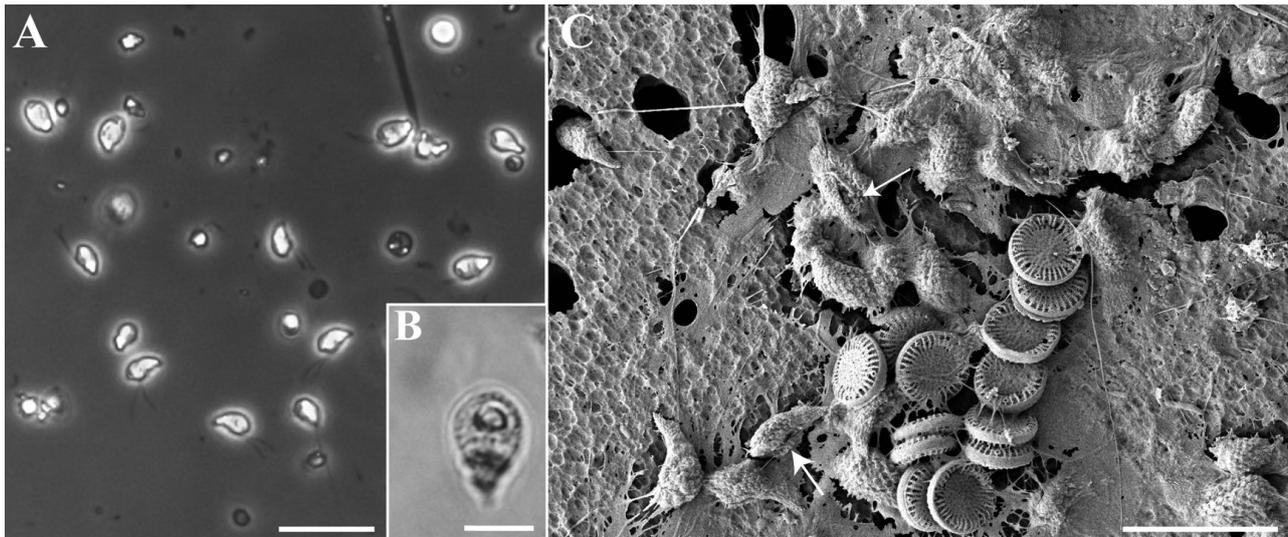
Fig. 7. – Images of *Plagioselmis* cf. *prolonga* taken with the light (A, B) and scanning electron (C) microscope. A, dominance of characteristic tear drop-like cells in the samples from station E4a (phase contrast); scale, 20 µm. B, cell with the centrally placed pyrenoid (differential interference contrast); scale, 10 µm. C, aggregates containing *Plagioselmis* cf. *prolonga* and nanoplanktonic centric diatoms. Hexagonal plates are visible on all cells. Furrow is visible on cells that are lying on dorsal side (arrows); scale, 10 µm.

layer. At the time of the bloom, cryptophytes were present at very low salinity values (2.5 and 5.5 at the surface layer of stations E3 and E4a, respectively), which indicates their high tolerance to decreased salinity. Furthermore, alloxanthine concentration showed a significant positive correlation with phosphates (0.950), ammonium (0.490) and nitrite (0.557) (Table 1).

Along with cryptophytes, the samples were rich in green flagellates, the most abundant group at station C1 (maximum 8.6×10^5 cells l^{-1} at 2 m depth) and co-dominant at station E4a, and in diatoms, which dominated stations E3 and E5, and were very abundant (5.4×10^6 cells l^{-1}) below the halocline of station E4a (Fig. 6). The diatom assemblage at all stations was dominated by marine nanoplanktonic single-celled *Chaetoceros* species (*C. subtilis, C. throndsenii* and *C. tenuissimus*) and small colonial centric diatoms belonging to the *Cyclotella* genus. The freshwater layer and the halocline at the estuarine stations were also abundant in terms of the large freshwater diatom *Synedra acus*. A very low abundance of dinoflagellates, coccolithophores and other groups was observed throughout the estuary.

Cryptophytes were identified at the class level during LM counts, and no quantitative taxonomic composition was determined. However, most of the cells counted throughout the estuary represented a distinct morphotype (Fig. 7A). The LM and SEM analysis of field samples revealed that the blooming cryptophyte belonged to the genus *Plagioselmis*. Cells were teardrop-shaped, 6-10 µm long and 4-6 µm wide, with two long flagella (Fig. 7A, B). Under the LM, the large central pyrenoid was visible (Fig. 7B). Due to the formaldehyde fixation, cells lost their characteristic red-brownish colour and chloroplasts were poorly visible. Hexagonal plates were clearly visible under the SEM (Fig. 7C). A prominent furrow, extending up to 2/3 of the cell length from the anterior to the posterior end was also observed (Fig. 7C). Cells were often associated with large amounts of sticky mucous matter that was visible under both LM and SEM, forming aggregates that contained cells of other phytoplankton (Fig. 7C). Taxonomical analysis using the available taxonomic literature (Hill 1992, Novarino et al. 1994, Novarino 2003, 2005) suggested that the blooming species was *Plagioselmis* cf. *prolonga* Butcher ex G. Novarino, I.A.N. Lucas and S. Morrall.

DISCUSSION

Although the riverine inflow determined the depth of the halocline, vertical distribution of temperature was regulated by the solar radiation. Distribution of orthosilicates was defined by the river inflow, while high concentrations of TIN and orthophosphates at station E4a (Šibenik harbour) probably originated from both anthropogenic input and microbial regeneration. Nutrient enrichment, slower river flow and the increased temperature supported the cryptophyte-dominated bloom.

Novarino (2005) hypothesized that *Plagioselmis prolonga* is the key primary producer in the pelagic ecosystems of the Mediterranean Sea. The association of the species with sticky organic exopolymers presented in his study was also observed during the bloom of *Plagioselmis* cf. *prolonga* in the Krka estuary. It is not clear whether the organic exopolymers were excreted naturally by *Plagioselmis* cf. *prolonga* or the cells were attracted to the organic exopolymers excreted by other phytoplankton cells. Since all *Plagioselmis* species are free-swimming and do not excrete organic exopolymers in culture (Novarino et al. 1994), observed exopolymers may also be a fixation artefact such as discharged ejectisomes (Booth et al. 1982). The species blooming in the Krka estuary was highly tolerant of low salinity. Similar euryhaline characteristics were reported for other cryptophyte species that form blooms in estuarine ecosystems (Mallin 1994, Gameiro et al. 2004, Laza-Martínez 2012).

Cryptophyte blooms are rarely reported in marine and estuarine phytoplankton communities of the Mediterranean Sea. As a result, detailed ecological data linked with particular species are scarce. Furthermore, a high degree of species-specific and strain-specific seasonality in cryptophytes further complicates research on their ecological preferences and possible triggers of blooms. Available reports on the ecology of *Plagioselmis* spp. shed more light on the ecological preferences of the genus. Cerino and Zingone (2006) found that the species *Plagioselmis prolonga* is present throughout the year in Mediterranean coastal waters, and the peak abundance (6.8×10^5 cells 1^{-1}) was detected in April. The bloom of *Plagioselmis* sp. detected by Andreoli et al. (1986), and probably misidentified as *Chroomonas* sp. (Novarino 2005) in the eutrophic Po River delta reached an abundance of 50×10^6 cells 1^{-1}. The bloom reported by Andreoli et al. was detected in winter, and characterized by discolouration in form of a red tide. Such discolouration was not detected in our study, possibly due to the almost seven times lower abundance of cryptophytes during the bloom in the Krka estuary. The green discolouration observed in the Krka estuary was probably caused by the other groups (e.g. green flagellates) in the surface layer, while cryptophytes dominated at the halocline. Another bloom of the cryptophyte identified as *Plagioselmis prolonga* was recently given by Bazin et al. (2014). The bloom was detected in the early spring at the halocline of the Segura River estuary, with cell counts reaching 156×10^6 cells 1^{-1}. High phosphate concentrations (up to 6 μM 1^{-1}) and stratification of the water column contributed to bloom development. The blooms of *Plagioselmis* spp. were also observed outside the Mediterranean Sea, as reported by Seoane et al. (2012) in bays and estuaries of the Cantabrian coast (Bay of Biscay, Spain). Seasonal differences between reported blooms of *Plagioselmis* spp. detected in the same water body (Mediterranean Sea) and at a similar latitude indicate that the genus comprises a high diversity of physiologically and ecologically different strains and species.

Statistical analysis suggests that the cryptophyte bloom at station E4a was linked to the higher concentration of orthophosphates at the halocline. The main sources of orthophosphates in Šibenik harbour are anthropogenic eutrophication and bacterial regeneration of the organic matter (Fuks et al. 1991, Legović et al. 1994). Orthophosphate concentration in this oligotrophic estuary was high enough to trigger the bloom, and low enough to favour the growth of small-sized phytoplankton taxa (nanoplanktonic diatoms, cryptophytes and green flagellates).

Investigations conducted in other anthropogenically-influenced estuaries have detected a shift in the dominant communities due to increased levels of ammonium. High concentrations of ammonium (usually higher than 4 μM 1^{-1}) inhibit the uptake of nitrate, thus limiting the growth of larger diatoms and favouring smaller primary producers such as cryptophytes and green flagellates (Dugdale et al. 2012). The concentrations of ammonium during our study were highest (2.8 μM 1^{-1}) at the depths where cryptophytes bloomed, and

showed a significant positive correlation with the alloxanthine. It could be hypothesized that ammonium was the primary nitrogen source at station E4a, suggesting much higher initial concentration before the bloom that could have been limiting for larger diatoms.

Previous investigations of the phytoplankton in the Krka estuary (Cetinić et al. 2006, Svensen et al. 2007) reported that diatoms dominate the summer phytoplankton community, with peak abundances upstream from Šibenik harbour. The shift from diatom-dominated summer blooms in the upper reach of the estuary (station E3) to the cryptophyte-dominated blooms in the anthropogenically-influenced Šibenik harbour (station E4a) observed in our work supports our hypothesis that the phytoplankton community is undergoing changes due to eutrophication.

High abundance of cryptophytes may affect the mariculture production. Although cryptophytes are considered a "high-quality" food, lacking hard exoskeleton structures or toxic metabolic products (Brett and Müller-Navarra 1997, Sterner and Schulz 1998), their higher content in bivalve food causes the red colouration of the tissue, making the mariculture products unfavourable for commercial needs (Pastoureaud et al. 2003). Furthermore, cryptophytes are essential for the development of toxic or harmful blooms of some dinoflagellates (Adolf et al. 2008) and ciliates (Peterson et al. 2013). Such diverse interactions of cryptophytes with their environment and their importance for the marine food web emphasize the necessity for future monitoring of phytoplankton communities and environmental parameters in the area in order to detect the changes that might have a profound effect on the estuary's ecosystem dynamics and mariculture production.

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