

Dynamics of the marine planktonic diatom family Chaetocerotaceae in a Mediterranean coastal zone

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ABSTRACT

The planktonic diatoms belonging to two genera *Chaetoceros* and *Bacteriastrum*, included within the family Chaetocerotaceae, are ecologically important as they represent a constitutive component of the phytoplankton in the coastal regions and are often among bloom-forming taxa. We analysed the chaetocerotacean species composition and abundances in the coastal area of northeastern Adriatic in a biweekly study conducted from September 2008 to October 2009 with the aim of investigating seasonal dynamics and species succession on the finer temporal scale and determining the most important ecological factors influencing their distribution. The study identified seven *Chaetoceros* and three *Bacteriastrum* species as major phytoplankton components showing the clear annual succession and two types of blooms (one species/multi species) governed by differing ecological conditions. Autumn bloom was composed of 20 chaetocerotacean species with *Chaetoceros contortus* and *C. vixvisibilis* alternating in dominance. Summer period was characterized by spreading of freshwater from the Po River up to the eastern coast increasing availability of phosphate which triggered the monospecific *Chaetoceros vixvisibilis* bloom. We explained the chaetocerotacean dominant species succession pattern by the environmental parameters, with the temperature, salinity and phosphate availability as most important factors driving the species seasonality.

Keywords: *Bacteriastrum*; *Chaetoceros*; diatoms; phytoplankton; succession; Adriatic Sea

Highlights

- First coherent investigation of temporal dynamics and species succession of marine planktonic diatom family Chaetocerotaceae
- Two types of blooms (one species/multi species) are governed by differing ecological conditions
- The succession pattern of dominant chaetocerotacean species was explained by temperature, salinity and phosphate availability as the most important factors driving the species seasonality

1. Introduction

The family Chaetocerotaceae Ralfs in Pritchard (1861) include cosmopolitan diatoms, notably thriving in the phytoplankton of coastal regions (Rines and Hargraves, 1988), and play an important role in neritic food webs and biogeochemical carbon and silica cycles. The hallmarks of this family are setae, long and hollow silicate spine-like projections protruding from the valve surface (Round et al., 1990). The strong and robust setae can irritate fish gills and cause excess mucus secretion and damage, thus certain species have been characterized as nuisance and harmful to fish and invertebrates (Hallegraeff et al., 2003; Smayda, 2006). Members of this family are important model species in not only in ecology and physiology, but also in toxicology and nanomaterial studies (Nagao et al., 2010; Peng et al., 2011; Osterholz et al., 2014).

Chaetocerotaceae is one of the largest and most diverse diatom families, comprising more than hundred described species which belong to two genera, *Chaetoceros* Ehrenberg and *Bacteriastrum* Shadbolt (Hasle and Syvertsen, 1997; Rines and Theriot, 2003). The genera *Chaetoceros* and *Bacteriastrum* are often difficult to identify due to the morphological variability of their constituent species (Rines and Theriot, 2003). The main distinctions between members of these two genera are the valve shape and the number of setae per valve. *Chaetoceros* species have a bipolar/bilateral symmetry with elliptically shaped valves each adorned with usually two setae, as opposed to *Bacteriastrum* species that have multipolar/radial symmetry with numerous (6-20) setae regularly arranged around the circular valve margin (Round et al., 1990).

The genus *Chaetoceros* regularly blooms in coastal ecosystems of temperate and polar seas in the late winter/early spring and autumn period. These blooms are usually multispecies

blooms, made up from simultaneously present 15-20 different species, of which typically one to three species dominate (Rines and Hargraves, 1987). The important role in formation of this type of blooms might be the activation of resting stages (Montresor et al., 2013). Approximately one-third of the *Chaetoceros* species are reported to be capable of producing resting spores (Hargraves, 1976). These resting stages are reinoculated in the water column during upwelling events (Pitcher, 1990), and their germination may be triggered by the photoperiod (Eilertsen 1995). However, the clear cut evidence of this event sequence is lacking (Montresor et al., 2013). *Bacteriastrum* species, although frequently present in planktonic assemblages, rarely form blooms. There are several exceptions: the bloom of *B. furcatum*, recorded in the Gulf of Mexico (Fryxell, 1978), spore-forming *B. hyalinum* which regularly blooms in the summer in the North Sea (Hoppenrath et al., 2009; Kraberg et al., 2010) and the autumn bloom of *B. jadranum* in the Adriatic Sea (Godrijan et al., 2012). The ecological importance of chaetocerotacean species in the Mediterranean Sea has been previously well established in numerous occasions, such as in the late winter/early spring bloom in NW Mediterranean coastal area (Percopo et al., 2011; Arin et al., 2013) and in diatom-dominated summer/autumn DCMs (deep chlorophyll maxima) where they have been found as a rather constant feature in both the western (Arin et al., 2002; Siokou-Frangou et al., 2010) and the eastern Mediterranean basin (Boldrin et al., 2002; Casotti et al., 2003). Also, the phytoplankton studies in the Adriatic Sea found *Chaetoceros* and *Bacteriastrum* species among the dominant phytoplankton taxa together with members of diatom genera such as *Pseudo-nitzschia*, *Proboscia*, *Cerataulina*, *Leptocylindrus* and *Thalassionema* (Viličić et al., 1995; Totti et al., 2000; Viličić et al., 2002).

There have been scarce ecological investigations specifically focused on this important planktonic diatom family. Members of the Chaetocerotaceae are usually analysed in bulk with other phytoplankton (Ajani et al., 2001; Bode et al., 2005; Odebrecht et al., 2010; Widdicombe, 2010; Arin et al., 2013; Ajani et al., 2014; Du and Peterson, 2014) or only the ecology of the most important blooming species is featured (Sieracki et al., 1998; Shevchenko and Orlova, 2010; Kownacka et al., 2013). In order to fill this gap, the main objective of this study was to obtain a better understanding of the ecology of genera *Chaetoceros* and *Bacteriastrum* in the coastal areas. And specifically we aim to (i) for the first time describe and interpret seasonal dynamics and succession of *Bacteriastrum* and *Chaetoceros* species on the finer temporal scale and to (ii) investigate most important ecological factors influencing their seasonal distribution.

2. Material and methods

2.1. Sampling site

The Adriatic Sea is the northernmost part of the Mediterranean with marked west to east gradients of physical and biological properties (Cushman-Roisin et al., 2001). In the north, the Adriatic Sea is shallow (< 50 m) and the stratification/mixing regime together with the trophic state is mainly influenced by the Po River freshwater discharge, coupled with the meteorological forcing factors (north-eastern Bora wind) and the inflow of the salty, oligotrophic water brought by Eastern Adriatic Current (EAC) (Socal et al., 2008). The coastal station RV001 (45°08' N, 13°61' E) is situated in north-eastern Adriatic Sea one nautical mile from the shore of Rovinj (Figure 1.). This coastal area is mostly oligotrophic due to the EAC influence (Artegiani et al., 1997) and thus ideal as proxy for a Mediterranean oriented survey.

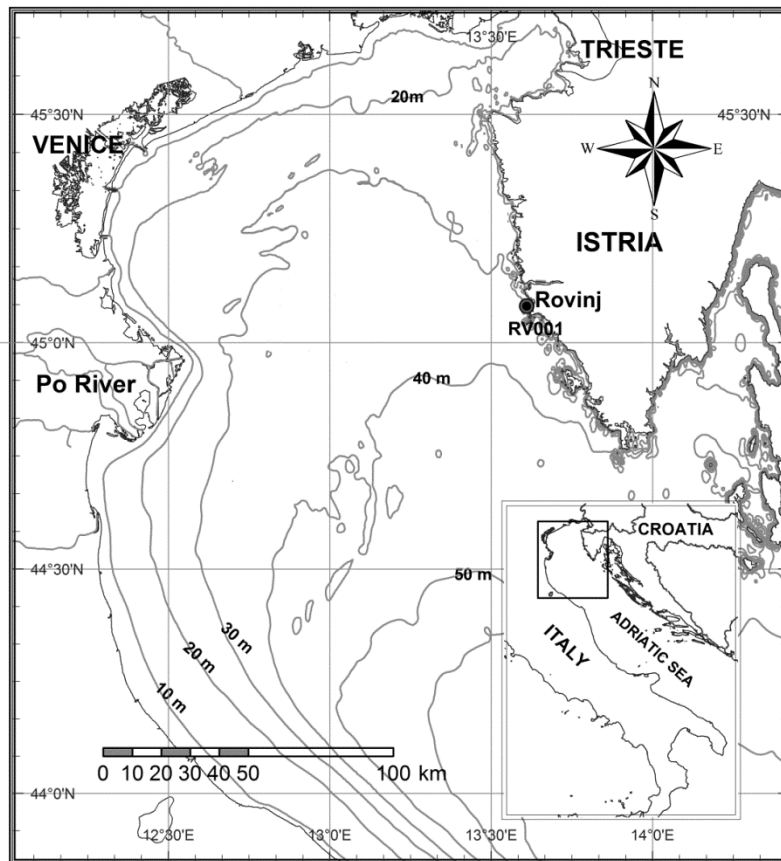


Figure 1. Map showing the location of the RV001 sampling station on the northeastern coast of the Adriatic Sea.

2.2. Sampling protocol

Seawater samples were collected with the Niskin samplers (5L) from five depths (0, 5, 10, 20, and 27 m) at the RV001 station. Additional samples were collected by plankton nets (mesh size 53 and 20 μm) vertically hauled in the euphotic layer of the water column. The sampling was performed approximately biweekly between September 2008 and October 2009. Temperature and salinity profiles were obtained with a CTD SBE 25 Sealogger probe *in situ*, while samples for nutrients and chlorophyll *a* (Chl *a*) concentration were collected in polycarbonate bottles and processed as described in Šilović et al. (2012). Subsamples for the determination of dissolved nutrients: (NO_3), nitrite (NO_2), orthophosphate (PO_4) and orthosilicate (SiO_4) were measured by spectrophotometric methods (Parsons et al., 1984). Ammonium (NH_4) was analysed by a modified technique of the indophenol method (Ivančić and Degobbis, 1984). Subsamples of 500 mL for the determination of Chl *a* were filtered onto Whatman GF/F filters, and immediately frozen at -20°C until analysis (within a week). Total Chl *a* concentrations were determined on a Turner TD-700 fluorometer (Parsons et al., 1984) after three hours of extraction in 90% acetone (in the dark, with grinding).

2.3. Phytoplankton analysis

A total number of 125 water samples (200 mL) for the phytoplankton cell counts were preserved with pseudo- Lugol's solution (0.4% final concentration) according to Verity et al. (2007). Cells were identified and counted using a Zeiss Axiovert 200 (Carl Zeiss, Oberkochen, Germany) inverted microscope operating with phase contrast and bright field optics. The variable volume (10 or 50 mL) of sub-samples depending on the cell density was sedimented in combined plate counting chambers (HydroBios, Kiel, Germany) and analysed after > 24 h according to Utermöhl (Lund et al., 1958; Utermöhl, 1958). For cells smaller than 20 μm (nanophytoplankton) which were relatively abundant, the half of transect (i.e. 1/2 diameter of counting chamber) along the counting chamber bottom was examined under 400x magnification. Individual cells with either maximum linear dimension (MLD) or equivalent spherical diameter (ESD) larger than 20 μm (microphytoplankton) and colony-forming diatom species in which chain length exceeds 20 μm were counted along two transects under

200x magnification. Very abundant species were counted on a variable number (5-20) of randomly chosen fields under either 200x or 400x magnification depending on their size. In addition, the bottom of the chamber was also examined under a magnification of 100x, to obtain a more correct evaluation of less abundant taxa. The minimum concentration of phytoplankton cells that can be detected by this method is 20 cells L⁻¹. Whenever it was possible in the light microscopy the identification was made to species level. Identifications were performed referring to general literature of phytoplankton taxonomy (Cupp, 1943; Tomas, 1997; Bérard-Therriault et al., 1999; Hoppenrath et al., 2009; Kraberg et al., 2010) and specifically for *Chaetoceros* and *Bacteriastrum*: Ikari (1927), Rines and Hargraves (1988), Hernández-Becerril (1996); Jensen and Moestrup (1998) and Kooistra et al. (2010).

2.2. Graphical and statistical data analyses

The statistical procedures were applied to investigate relationships between chaetocerotacean species abundances and environmental factors. Basic descriptive statistics for physical, chemical and biological parameters was calculated using statistical software Statistica 10 (StatSoft). For statistical multivariate analyses: nMDS – non-metric Multidimensional Scaling and Cluster Analysis, statistical software PRIMER 6 v.6.1.11. & PERMANOVA+ v.1.0.1. (Clarke and Gorley, 2006) was used. In nMDS and Cluster analysis abundance data of all *Bacteriastrum* and *Chaetoceros* species from samples collected at five different depths were integrated and averaged for each sampling date. The similarities among samples were calculated for each abundance resemblance matrix by means of Bray–Curtis similarity index (Clarke and Warwick, 2001). CCA – Canonical Correspondence Analysis was performed with CANOCO version 5. The CCA analysis was made using only species assigned as dominant (frequency of appearance $\geq 10\%$ and maximal abundance $>10\,000$ cells L⁻¹) and both abundance and environmental data from individual sampling depths were used. In CCA analysis dissolved inorganic nitrogen (DIN) calculated as sum of NO₃, NO₂, and NH₄ was used. A Monte Carlo permutation test (999 permutations) was used to test the statistical significance of each environmental variable with threshold at $p \leq 0.05$ considered as significant. For all multivariate statistical procedures data were transformed using log (x+1) overall transformation. Graphical presentations were created using the Golden Software Grapher 8.0. and Ocean Data View 4.5.6. (Schlitzer, 2011).

3. Results

3.1. Environmental conditions and diatom community

The detailed temporal distributions of physico chemical parameters, namely temperature, salinity and nutrients, were shown by Šilović et al. (2012) for the investigated period. Thus, here we present these parameters only for the surface layer and together with the integrated values over the whole column (Table 1).

Table 1. Physical, chemical and biological parameters at RV001 sampling station, measured during the study period (September 2008-October 2009) recorded at the surface (S) and integrated over the whole water column (Int. 0-30 m). min – minimum; max – maximum; st. dev.- standard deviation; N- number of samples, Lower Qu. – lower quartile, Upper Qu.-upper quartile; NO_3^- - nitrate; NO_2^- - nitrite; NH_4^+ - ammonium; PO_4^{3-} – phosphate; SiO_4^- - silicate, Chl *a* – chlorophyll *a*.

		Min.	Lower Qu.	Median	Mean	St. dev.	Upper Qu.	Max.	N
Temperature (°C)	S	9.28	13.36	18.09	17.59	5.48	22.16	27.48	25
	Int. (0-30 m)	9.26	13.36	17.17	16.55	4.6	19.45	27.48	125
Salinity	S	34.22	36.52	37.33	36.95	0.97	37.60	37.89	25
	Int. (0-30 m)	34.2	37.32	37.61	37.35	0.75	37.76	38.18	125
NO_3^- ($\mu\text{mol L}^{-1}$)	S	0.07	0.98	1.55	1.84	1.79	2.14	9.12	24
	Int. (0-30 m)	0.03	0.69	1.27	1.50	1.46	1.80	10.34	120
NO_2^- ($\mu\text{mol L}^{-1}$)	S	0.01	0.07	0.10	0.24	0.32	0.26	1.16	24
	Int. (0-30 m)	0.01	0.05	0.12	0.32	0.42	0.43	2.07	120
NH_4^+ ($\mu\text{mol L}^{-1}$)	S	0.12	0.32	0.61	0.62	0.37	0.81	1.35	24
	Int. (0-30 m)	0.12	0.36	0.55	0.68	0.59	0.80	5.1	120
PO_4^{3-} ($\mu\text{mol L}^{-1}$)	S	<0.01	0.01	0.03	0.04	0.05	0.04	0.24	24
	Int. (0-30 m)	<0.01	0.01	0.03	0.04	0.04	0.05	0.26	120
SiO_4^- ($\mu\text{mol L}^{-1}$)	S	0.47	2.50	2.95	2.96	1.19	3.98	4.99	24
	Int. (0-30 m)	0.38	2.48	3.11	3.72	2.18	4.22	11.75	120
Chl <i>a</i> (ng L^{-1})	S	0.07	0.23	0.34	0.34	0.15	0.43	0.79	25
	Int. (0-30 m)	0.07	0.25	0.34	0.38	0.17	0.47	0.85	125

Diatoms (cells L ⁻¹)	S	1.50×10 ³	8.53×10 ³	1.80×10 ⁴	1.00×10 ⁵	2.18×10 ⁵	6.56×10 ⁴	1.01×10 ⁶	25
	Int. (0-30 m)	6.10×10 ²	8.59 ×10 ³	1.57×10 ⁴	7.69×10 ⁴	1.76×10 ⁵	5.93×10 ⁴	1.37×10 ⁶	125

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Total chlorophyll *a* (Chl *a*) concentrations were below 1 µg L⁻¹ during the investigated period as shown in Figure 2A. In the phytoplankton assemblage small cyanobacteria and picoeukaryotes belonging to the picophytoplankton (size <2 µm) generally prevailed, with highest contribution in March and in August 2009. Nanophytoplankton abundances increased during the late spring/early summer months 2009 with coccolithophores and cryptophytes as the most important groups. High microphytoplankton abundances were recorded in September and October 2008 as well as in the period from April to July 2009 with the highest cell numbers (>10⁶) observed in July 2009. Diatoms were the dominant group in the microphytoplankton during the whole investigated period. Diatom cell concentrations varied considerably over time. Minimum diatom values (6.0 × 10² cells L⁻¹) were observed in January 2009, while two peaks occurred in September/October 2008 at the surface and in July 2009 at 5 m with 4.9 × 10⁵ and 1.4 × 10⁶ cells L⁻¹, respectively.

Throughout the study period 89 diatom taxa were enumerated and with the additional 24 species identified from net samples, thus adding up to 113 identified diatom taxa. Most frequently found species (present in >70% of analysed samples) were *Nitzschia longissima* and *Pseudo-nitzschia* cf. *pseudodelicatissima*, followed by *Thalassionema nitzschioides*, *Cerataulina pelagica*, *Leptocylindrus* cf. *danicus* and *Proboscia alata* (present in > 50% of samples).

The most pronounced Chl *a* peak was observed in September and October 2008 (Figure 2A) corresponding to the peak in diatom abundance (Figure 2B). The diatom peak lasted through three sampling dates. This peak was composed of 52 identified diatoms with four species, *Chaetoceros contortus*, *Pseudo-nitzschia* cf. *pseudodelicatissima*, *C. vixvisibilis*, and *Asterionellopsis glacialis*, constituting together between 43 % and 69 % of the total diatom population. During the second diatom peak in July 2009 no visible increase in Chl *a* concentration was recorded (Figure 2A). This bloom was constituted almost exclusively from a single species, *Chaetoceros vixvisibilis*. *C. vixvisibilis* made up 90 % of the total diatom abundance. There were 31 recorded diatom species during this summer peak, albeit in very low abundances.

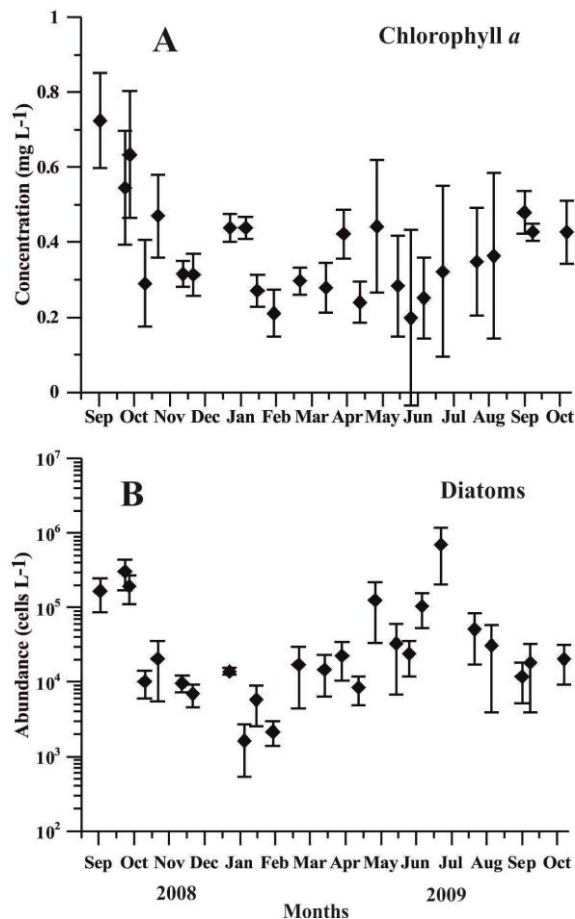


Figure 2. Temporal distribution of (A) total Chl *a* concentration, (B) diatom abundance at station RV001 from September 2008 to October 2009. Data are presented as mean values with standard deviations for the whole water column (0-30 m). Note the logarithm scale in B.

3.2. *Chaetoceros* and *Bacteriastrum* composition, bloom dynamics and species succession

During the investigated period *Bacteriastrum* and *Chaetoceros* species exhibited two distinct abundance peaks, with markedly lower numbers in between (Figure 3A). The first peak in autumn 2008 (autumn bloom) was characterized by high abundances, up to 3.02×10^5 cells L⁻¹, and it developed predominately in the surface layer. The second peak in July 2009 (summer bloom), was more evenly distributed throughout the water column, with the highest abundances recorded at 20 m of depth, 5.18×10^6 cells L⁻¹ (Figure 3A). The number of chaetocerotacean species simultaneously present in the water column at the individual sampling date ranged from 5 to 20. The highest recorded number of species occurred on the date of the autumn bloom in 2008, but was also high during June/July 2009 (Figure 3B). On few occasions, such as in December 2009, the number of identified species from the net samples was 13, while only two species were recorded in the water samples. This indicates

that they were present in the water column, but in numbers not detectable by the Ütermohl method.

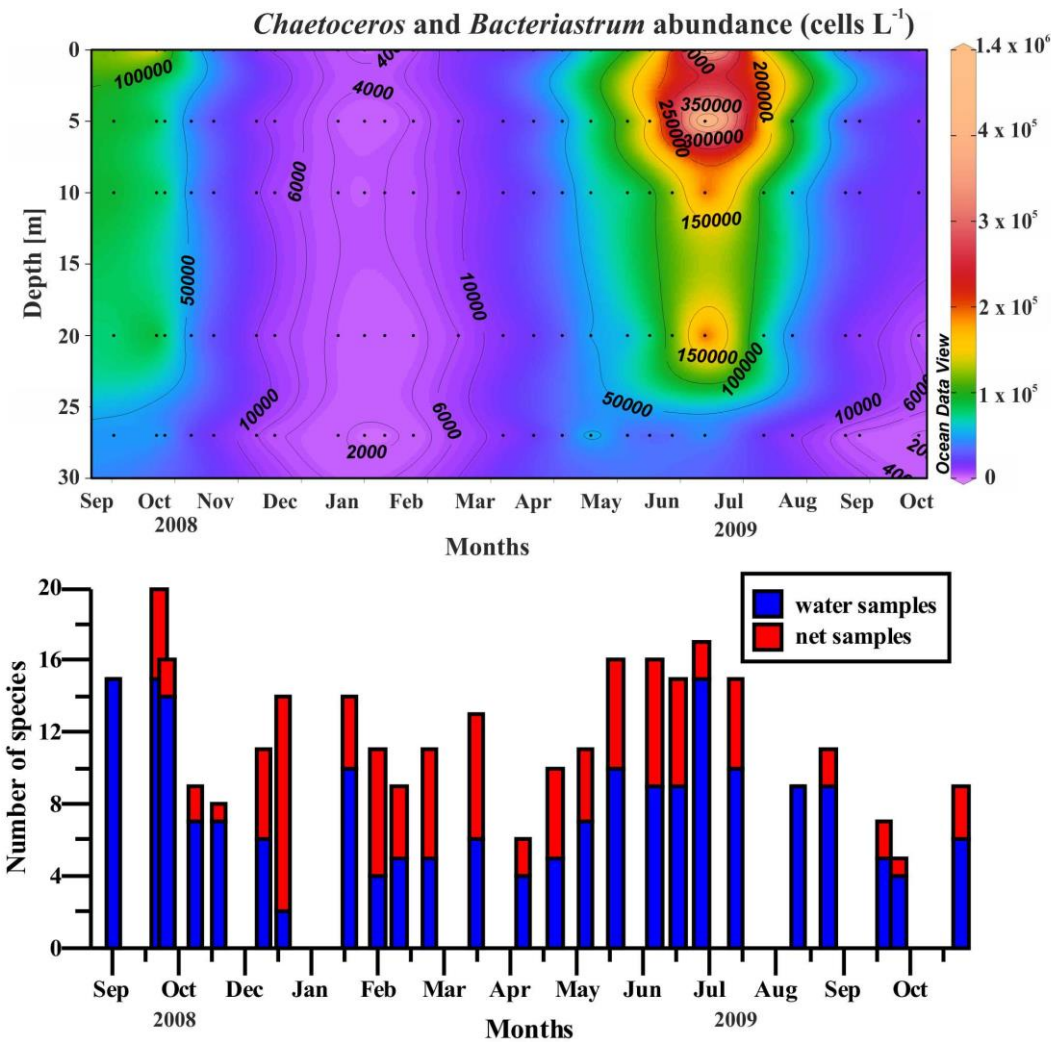


Figure 3. Temporal distribution of *Chaetoceros* and *Bacteriastrium* species at station RV001 in the period 2008-2009 A) Vertical distribution of the species abundances B) Number of species recorded in the whole water column for each sampling date.

A total of 28 *Chaetoceros* and 4 *Bacteriastrium* species were identified throughout the study (Table 3). Ten species were common in the water column, found in $\geq 10\%$ of samples and reached high abundances $>10\,000$ cells L⁻¹, and thus classified as dominant species (Figure 3). Species such as *C. curvisetus* and *C. danicus* and 5 others were frequently present in samples, but their abundances were lower than the threshold for dominant species, reaching $\sim 10^3$ cells L⁻¹. The rest of the species were considered as rare.

252 **Table 2.** *Chaetoceros* and *Bacteriastrum* species identified at the station RV001 in the period
253 2008-2009. Max- maximal abundance; Freq. - frequency of appearance. Number of samples=
254 125; + indicates species observed only in net samples. D - dominant species with Freq. $\geq 10\%$
255 and Max. $>10\,000$ cells L⁻¹ (in bold); F – species with Freq. $\geq 10\%$ and Max. $<10\,000$ cells L⁻¹;
256 ¹; R – rare and not abundant species.

Species	Max (cells L ⁻¹)	Freq. (%)
D <i>Bacteriastrum furcatum</i> Shadbolt	118 560	19
R <i>B. hyalinum</i> Lauder	640	2
D <i>B. jadrantum</i> Godrijan, Marić & Pfannkuchen	52 060	13
D <i>B. mediterraneum</i> Pavillard	50 540	12
D <i>Chaetoceros affinis</i> Lauder	18 240	21
R <i>C. anastomosans</i> Grunow	7 030	6
D <i>C. brevis</i> Schütt	10 260	17
R <i>C. coarctatus</i> Lauder	+	+
D <i>C. contortus</i> Schütt	175 180	36
R <i>C. constrictus</i> Gran	+	+
R <i>C. costatus</i> Pavillard	3 420	2
F <i>C. curvisetus</i> Cleve	7 600	17
R <i>C. dadayi</i> Pavillard	120	1
F <i>C. danicus</i> Cleve	4 180	28
D <i>C. decipiens</i> Cleve	12 920	29
F <i>C. densus</i> Cleve	5 320	12
F <i>C. didymus</i> Ehrenberg	4 560	10
F <i>C. diversus</i> Cleve	6 080	12
R <i>C. eibenii</i> (Grunow in Van Heurck) Meunier	1 520	6
R <i>C. lauderi</i> Ralfs in Lauder	1 520	6
R <i>C. peruvianus</i> Brightwell	1 140	7
R <i>C. pseudoaurivillii</i> Ikari	680	1
R <i>C. pseudocurvisetus</i> Mangin	+	+
F <i>C. rostratus</i> Lauder	6 840	14
F <i>C. simplex</i> Ostenfeld	5 680	12
D <i>C. socialis</i> Lauder	28 380	22
R <i>C. tenuissimus</i> Meunier	4 820	4
R <i>C. tetrastichon</i> Cleve	+	+
D <i>C. throndsenii</i> var. <i>throndsenia</i> (Marino, Montresor & Zingone) Marino, Montresor & Zingone	24 140	21
R <i>C. throndsenii</i> var. <i>trisetosa</i> Zingone	8 360	5
R <i>C. tortissimus</i> Gran	9 120	5
D <i>C. vixvisibilis</i> Schiller	1 349 679	17

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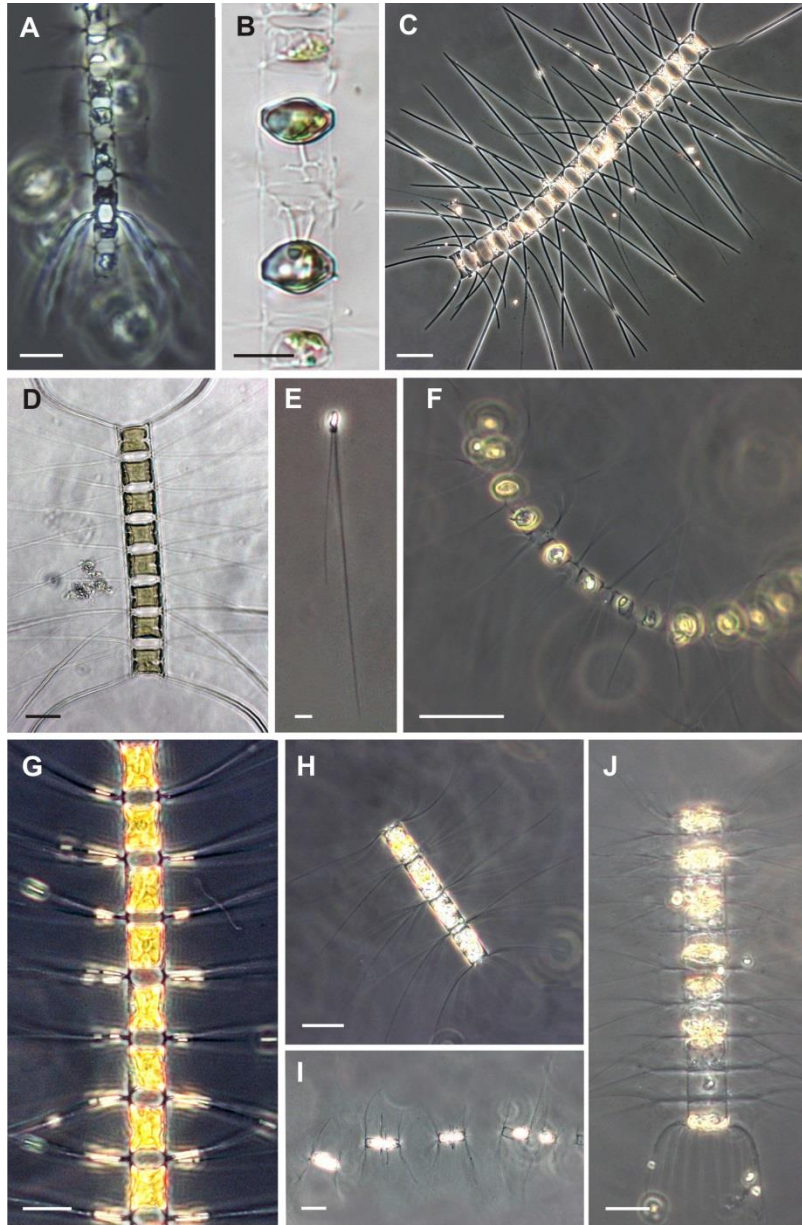


Figure 4. Light microscope micrographs of dominant chaetocerotacean species identified in the northern Adriatic: (A) *Chaetoceros contortus* (B) *C. vixvisibilis* (C) *C. decipiens* (D) *C. affinis* (E) *C. thronsdensei* var. *thronsdensei* (F) *C. socialis* (G) *C. brevis* (H) *B. furcatum* (I) *B. jadranum* (J) *B. mediterraneum*.

Analysis of temporal succession of the whole chaetocerotacean community via MDS and cluster analysis showed that the distinct species assemblages were present at the certain point in time, and indicated a successional change in the community composition (Figure 5). The group A was composed from samples collected in the first part of the study,

from September until the beginning of December 2008. In the first group species *Chaetoceros*
contortus and *C. vixvisibilis* were prevailing together with slightly less abundant
Bacteriastrum mediterraneum and *C. socialis*. In the second B group there is a single winter
sample from the middle of December, with only *C. danicus* and *Chaetoceros* sp. present. It is
followed by the third C group representing the winter assemblage from January/February
2009 characterized by high numbers of *C. danicus*, *C. eibonii* and *B. hyalinum*. Fourth group
D is the largest, describing the community that was apparently similar in composition from
end of February to the middle of July with the date corresponding to summer *C. vixvisibilis*
bloom. Group D was dominated by small single celled *C. tenuissimus*, *C. thronsenii* var.
thronsenia and *C. simplex*. Later on towards summer the intensive development of diverse
community similar to autumn was observed with addition of *C. curvisetus*, *C. danicus* and
Bacteriastrum furcatum. E group is the last including samples describing the diverse
community of late summer/autumn 2009 composed of *C. decipiens* and *C. affinis* with *B.*
jadrinum, *C. tortissimus*, *C. anastomosans* and *C. lauderi*.

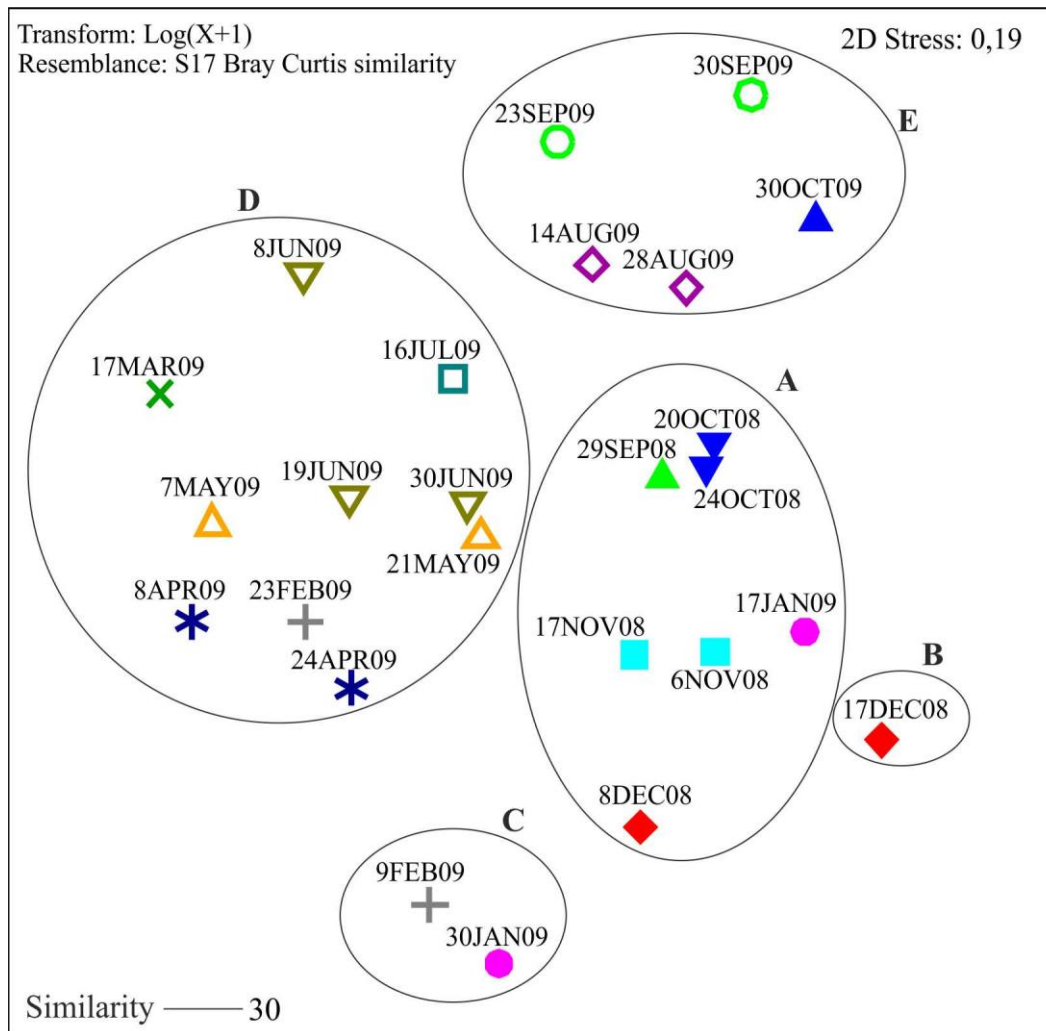


Figure 5. MDS plot for Chaetocerotaceae species abundances from different sampling dates at the station RV001 from September 2008 until October 2009. 5 distinct groups of samples are separated based on the results of the cluster analysis at the similarity level 30%, and superimposed on the MDS ordination. A- autumn/early winter 2008; B – winter 2008; C – winter 2009; D – spring/summer 2009; E – late summer/autumn 2009.

The contribution of individual *Chaetoceros* and *Bacteriastrum* species was investigated in detail on the two peak occasions, September–November 2008 and July 2009. Generally, the autumn bloom was composed of similar species on all sampling dates, however the contribution of the dominant taxa markedly varied between them (Figure 6 A-C). On 29th September 2008, the chaetocerotacean community was composed of 15 species, with 5 species contributing over 5% to the chaetocerotacean abundance. Of these species, *B. mediterraneum* and *C. socialis* contributed with 27% and 21%, respectively. *C. contortus* contributed with only 14% which changed three weeks later, when this species was

dominating with 63% of contribution. Only six days later, contribution of *C. contortus* fell to just 18% and *C. vixvisibilis* took over the dominant position, with 45% of contribution. The chaetocerotacean community was composed of 20 and 16 species, on 20th and 26th October, respectively. The end of the bloom was recorded after additional 10 days, when chaetocerotacean population markedly decreased in numbers (Figure 3A) and change in the species composition occurred (Figure 6D). The community was then composed of only 9 taxa, with dominant *B. mediterraneum*, *C. curvisetus* and *C. densus*. The summer 2009 bloom was composed of one species (99%), *C. vixvisibilis*, which was not recorded on the dates preceding the bloom (not shown). Fifteen other species contributed all together only 1% of overall chaetocerotacean numbers (Figure 6E).

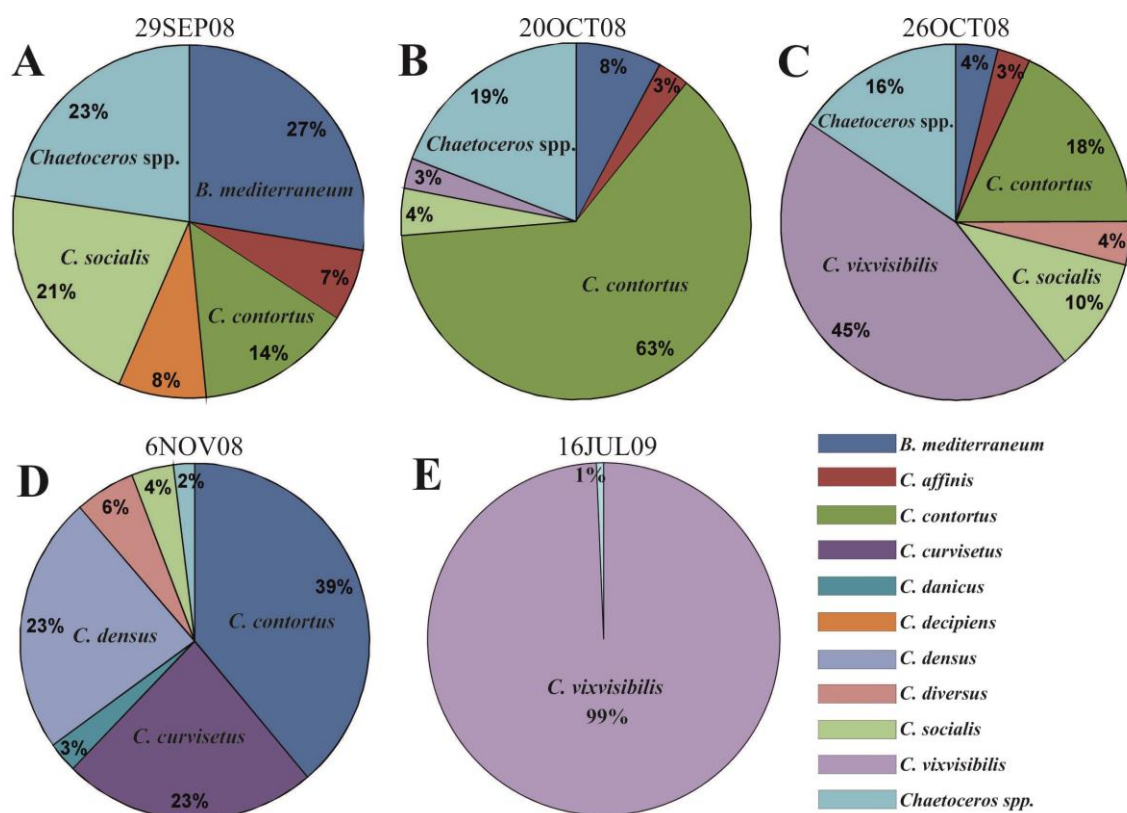


Figure 6. Relative percentage contribution of individual species to the Chaetocerotaceae community during the selected dates of autumn bloom A) 29th September 2008, B) 20th October 2008, C) 26th October 2008, post-bloom D) 6th November 2008 and E) summer bloom on 16th July. Species with more than 3% contribution are listed, others are combined in

group *Chaetoceros* spp. Data are calculated from mean values for the whole water column for each sampling date.

3.3 Ecology of dominant *Chaetoceros* and *Bacteriastrum* species

CCA ordination analysis was used to visualise the relation of individual chaetocerotacean species to the influencing environmental factors (Figure 7). The first two ordination axes from the CCA explained 84% of the species–environment relation. The first axis had an eigenvalue of 0.34 and explained 57%, while the second had an eigenvalue of 0.16 and explained 27%. Temperature ($F = 11.50$, $p = 0.001$), salinity ($F = 5.70$, $p = 0.001$), and PO_4 ($F = 2.70$, $p = 0.006$) were the most important factors influencing dominant chaetocerotacean species while DIN ($F = 1.40$, $p = 0.219$) and SiO_4 ($F = 1.40$, $p = 0.219$) were not found significant.

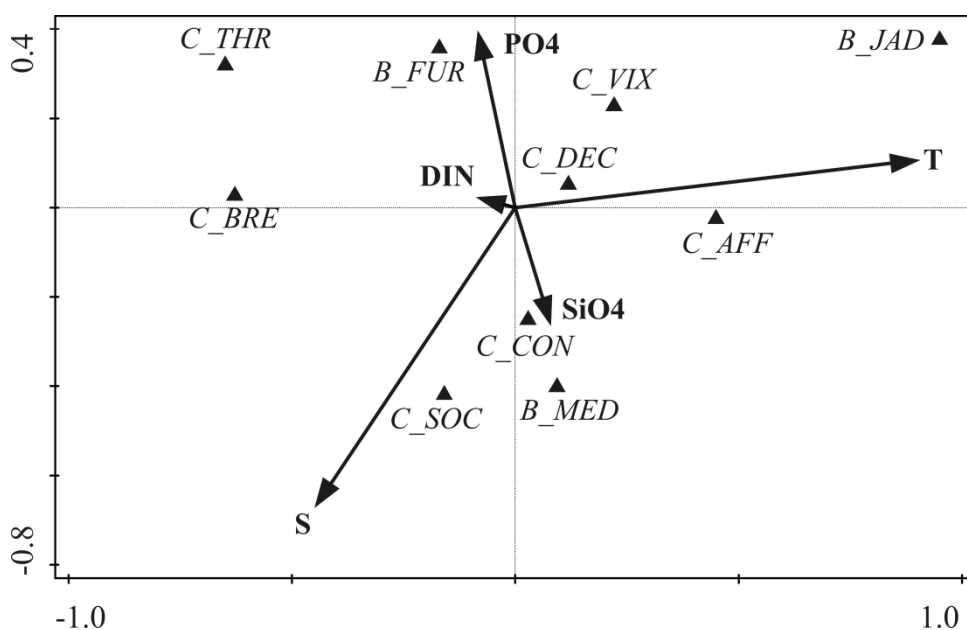


Figure 7. Results of CCA analysis of dominant chaetocerotacean species and environmental data. Triangles give the position of species plotted on the ordination diagram. Length and direction of environmental parameter arrows indicate their importance in terms of influence on selected species. Abbreviations: T - Temperature; S – Salinity; DIN – dissolved inorganic nitrogen; SiO_4 – orthosilicate; PO_4 – orthophosphate; B_FUR - *Bacteriastrum furcatum*; B_JAD - *Bacteriastrum jadranum*; B_MED - *Bacteriastrum mediterraneum*; C_AFF - *Chaetoceros affinis*; C_BRE - *Chaetoceros brevis*; C_CON - *Chaetoceros contortus*; C_DEC

- *Chaetoceros decipiens*; C_SOC - *Chaetoceros socialis*; C_THR - *Chaetoceros throndsenii*
var. *trisetosa*; C_VIX - *Chaetoceros vixvisibilis*.

The distribution of dominant species abundances over time (Figure 8) showed that the majority reached high abundances during the autumn bloom in 2008. *Chaetoceros contortus*, *C. vixvisibilis* and *C. decipiens* all had three peaks of abundance during the investigated period. Of those, *C. contortus* was situated near the SiO₄ arrow, however the relationship was not considered as significant. On the other hand, *C. vixvisibilis* and *C. decipiens* were situated in the upper right quadrant of CCA plot (Figure 7) and found to be positively related to temperature and phosphate and negatively with salinity. Also close to the temperature arrow were *C. affinis* and *B. jadrinum* (Figure 7), which had abundance peaks in late summer/autumn 2009 (Figure 8 C, E). *B. mediterraneum* and *C. socialis* each had a single peak in the autumn 2008 (Figure 8 D, E). *B. mediterraneum* was situated near the SiO₄ arrow, whereas *C. socialis* was positively related to salinity (Figure 7). *B. furcatum*, *C. throndsenii* var. *trisetosa* and *C. brevis* had only one distinct peak of abundance during 2009 (Figure 8 C, D, E). *B. furcatum* was positively related to PO₄, while other two species were not significantly related to any environmental factors (Figure 7).

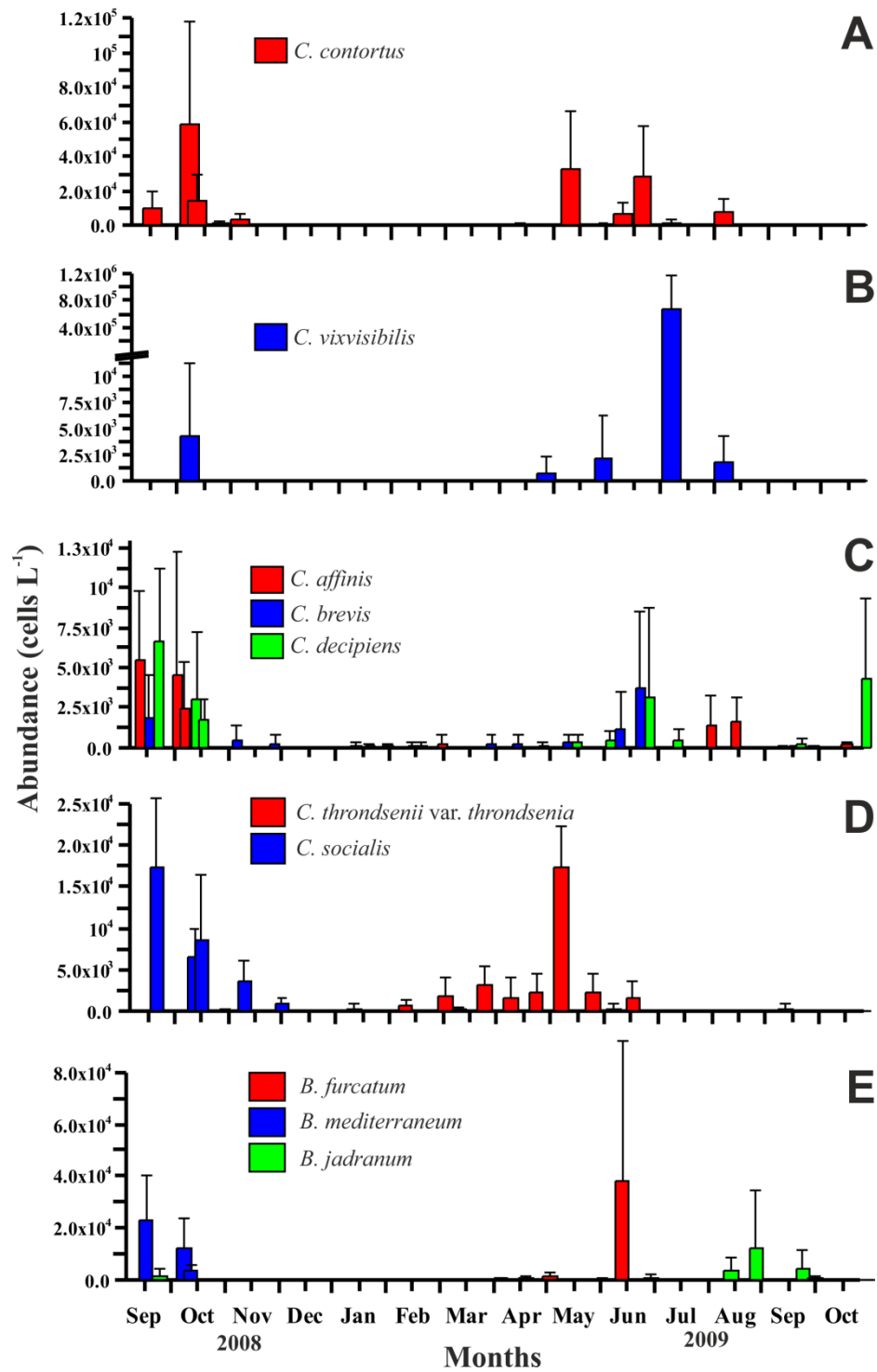


Figure 8. Temporal distribution of dominant *Chaetoceros* (A-D) and *Bacteriastrium* (E) species abundances at the station RV001 from September 2008 to October 2009. Data are presented as mean values with standard deviations for the whole water column (0-30 m).

4. Discussion

Results of this study focused specifically on the diatom family *Chaetocerotaceae* and allowed us to clarify aspects of the seasonal dynamics and succession of chaetocerotacean species in the coastal environments. The taxonomical affiliation of identified species generally agreed with the species lists provided in recent northeastern Adriatic Sea studies (Viličić et al. 2009; Cabrini et al., 2012; Godrijan et al., 2013). The study by Viličić et al. (2009) identified four typical species for the phytoplankton assemblages in the northern Adriatic area, namely *C. socialis*, *C. vixvisibilis*, *C. curvisetus*, *C. decipiens* and *C. affinis*. These taxa were also identified as dominant components in our study, but we added five more *Chaetoceros* species to the list, together with three species belonging to the genus *Bacteriastrum*. Generally our results showed that chaetocerotacean species were consistently present in the diatom community, and can be divided into four successional groups composed of different taxa. Interestingly, we did not find that any of the species persisted throughout the year, as Rines and Hargraves (1988) in their study about the seasonal distribution of the genus *Chaetoceros* in Narragansett Bay. However, we did find that major bloom-forming species *C. vixvisibilis* and *C. contortus* were present in both summer and subsequent autumn pulse probably persisting in between in low numbers, thus not detectable with our research method.

4.1 Seminal chaetocerotacean species

In our study *Chaetoceros contortus* was recognized as the most frequently found species. It is a very robust, eurythermal species (Rines and Hargraves, 1987) capable of surviving very low temperatures (Shevchenko and Orlova, 2010). *C. contortus* is very often synonymised with the morphologically very similar *C. compressus* (Cupp, 1943; Hasle and Syvertsen, 1997). Both species are easily recognizable in the water samples due to the common presence of heavy, contorted setae (Rines, 1999). Recent study by Chamnansinp et al. (2015) resolved the status of both species and described morphological characters used for their delineation, and specifically indicated that many records of *C. compressus* outside the tropics seem to be due to taxonomic confusion. In our study all specimens had almost circular valves, a typical *C. contortus* character. However, majority of the studies conducted in the northern Adriatic area (Revelante and Gilmartin, 1976; Bernardi Aubry et al., 2012) recognized *C. compressus* as one of the most significant phytoplankton species. We suggest that this is a case of the misidentification, as *C. compressus* appears to be confined to tropic areas (Chapmannship et al., 2015; Rines, 1999). In the northwestern Adriatic Sea, *C. contortus* (noted as *C. compressus*) was recorded as a key species responsible for the summer

diatom peak in July together with *Proboscia alata* (Bernardi Aubry et al., 2012). We recorded *C. contortus* in early summer, but we identified it as a much more significant component of the autumn bloom. Nevertheless, we are unable to draw conclusions based on our data on the relation of this species with the temperature and can only hypothesize that this environmental parameter is a trigger for the species seasonal occurrence in the plankton.

The second important species was *Chaetoceros vixvisibilis*, a very frequent and abundant diatom species in the phytoplankton assemblage of the eastern Adriatic Sea (Viličić et al., 1995; Viličić et al., 2009). The species environmental preferences have been described by Hernández-Becerril et al. (2010), and generally agree with the results in this study. *C. vixvisibilis* is a thermophilic species forming summer/autumn blooms related to higher seawater temperatures, but more importantly, with higher concentrations of orthophosphate (0.02 - 0.05 $\mu\text{mol L}^{-1}$) and low salinity down to 33, directly corresponding to the Po River inflow.

Chaetoceros affinis and *C. decipiens* are both generally widespread and frequently reported species in temperate environments (Hasle and Syvertsen, 1997; Kooistra et al., 2010; Kraberg et al., 2010), including the Adriatic Sea (Viličić, et al. 2009). These two species reached relatively high cell concentrations in the water column in late summer/autumn. The same seasonal distribution was found for *Chaetoceros affinis* in Narragansett Bay (Rines and Hargraves, 1987), while *C. decipiens* was reported all year round in both Narragansett Bay and the Baltic Sea (Jensen and Moestrup, 1998). However, there are difficulties in correctly separating *C. decipiens* from a very similar species *C. lorenzianus*. This is due to the apparent transitional morphologies (Rines and Hargraves, 1988; Kownacka et al., 2013), which may account for the reported irregularities in the species seasonal dynamics. Recent studies in the morphology of *C. lorenzianus* (Ishii et al., 2011; Kownacka et al., 2013) emphasize the importance of resting spores and two elevations extended into branching spines on the spore primary valve face. In our study we did not record any resting spores, therefore until more clearly defined taxonomic criteria are set, we consider all Adriatic specimens to belong to *C. decipiens sensu lato*. Ecologically, the development of both *C. affinis* and *C. decipiens sensu lato* was related to the temperature and lower nutrient levels of nitrogen and silicate in accordance with their seasonal distribution. Their occurrence in nutrient limiting environment indicates an existence of adaptive physiological mechanism to these conditions, possibly including enzymatic activity like active degradation of β -1,3-d-glucans present in these species (Myklestad et al., 1981).

Chaetoceros socialis is another widely distributed diatom (Rines and Hargraves, 1988), reported to form dense blooms (Sieracki et al., 1998). Recently, Degerlund et al. (2012) showed evidence on physiological separation between two varieties from the cold-water and temperate regions. Chamnansip (2013) raised the northern, cold-water variety to a species level named *Chaetoceros gelidus* and established synonymy of warm-water species *C. socialis* and *C. radians*. Species characters found in Adriatic specimens match the morphology of the *C. socialis* temperate strains found in the Tyrrhenian Sea (Degerlund et al., 2012; Kooistra et al., 2010), and therefore we retain the name *C. socialis* for the species found in our study. *C. socialis* is very common in the Adriatic, more abundant and frequent in the northern than in the southern part (Viličić et al., 2009). Although in the northwestern part of the Adriatic it is recognized as a typical spring diatom (Bernardi Aubry et al., 2004), it forms autumn blooms in the central area (Viličić et al., 2009) and along the northeastern coast (Godrijan et al., 2013) which is in accordance with our findings.

In recent literature *Chaetoceros brevis* was reported as a truly oceanic Antarctic diatom species (Timmermans et al., 2001; Van Oijen et al., 2004; Janknegt et al., 2008). Rines and Hargraves (1988) pointed out that this species is probably a taxonomical “collecting ground” containing atypical forms of other taxa. There is a similar species, *C. pseudobrevis* (Jensen and Moestrup, 1998) which both Hustedt (1930) and VanLandingham (1968) considered as a synonym of *C. brevis*, but further studies are required in order to establish the valid delineating characters. In our study, temperate *C. brevis* morphotype showed one distinct abundance peak during summer, coinciding with higher temperatures and lower silicate levels in the water column.

Chaetoceros thronsdensei var. *throndsenia* is a small- single celled species (Marino et al., 1987) reported as common and at time abundant, in the late spring and autumn in the Gulf of Naples (Marino et al., 1991). The same seasonal distribution was found in our study, with maximal abundance of this species recorded in May, related to increased concentrations of orthophosphate and ammonium. The higher concentration of ammonium are easily explored by the small-celled diatom species and bacterioplankton (Šilović et al., 2012).

The seasonality of three dominant *Bacteriastrum* species has been already described in northeastern Adriatic habitats by Godrijan et al. (2012) and their results are generally in accordance with our findings. *Bacteriastrum delicatulum* was previously reported for the same area (Bosak et al., 2009; Viličić et al., 2009; Cabrini et al., 2012), and the name was used by Godrijan et al. (2012). However, subsequent reports based on

morphological/molecular information identified *B. furcatum* from the cultured strains isolated from the Adriatic Sea (Bosak et al., 2015). Moreover, in our study all observed complete colonies were heteropolar with the morphological attributes more similar to *B. furcatum* (Fryxell, 1978; Sarno et al., 1997; Bosak et al., 2015) and the true *B. delicatulum* isopolar chains (Tomas, 1997) were not found in any of the investigated samples. *B. furcatum* showed one abundance peak in summer, similarly to *C. brevis*, in agreement with Godrić et al. (2012) who detected *B. furcatum* (noted as *B. delicatulum*) abundance peak during June–August period. *Bacteriastrum mediterraneum* maximal abundances were recorded in autumn within the same study, and we also observed this species as a component of autumn multispecies diatom bloom. Another autumn species was *Bacteriastrum jadrinum*, which maxima occurred at the same time as *C. affinis*, in the period of higher temperature and low nutrient levels in the water column. The high cell concentrations of *B. jadrinum* were recorded previously in the northeastern Adriatic in September (Godrić et al., 2012) and November (Šupriha et al., 2011), in similar environmental conditions with low levels of nutrients. *B. jadrinum* has a unique way of colony formation where cells are held together in regular filaments by the cell jacket, an extruded organic structure (Bosak et al., 2012). The exopolymers are highly absorptive substances and can readily sequester and concentrate nutrients from the surrounding water (Decho, 1990). Therefore, in addition to colony formation, it is possible that this organic material can allow for efficient uptake of nutrients when concentration in nearby water is low, acting as an active nutrient trap as suggested for the gel structure of the marine diatom *Berkeleya rutilans* (Drum, 1969).

4.2. Bloom dynamics

After establishing the seminal chaetocerotacean species we will discuss the two recorded types of blooms: the autumn 2008 multispecies bloom and summer 2009 monospecific one. This might not only be important for the chaetocerotacean family, but also for the general bloom ecology. The autumn diatom bloom recorded in 2008 was triggered by the water column mixing event which happened at the end of September (Šilović et al., 2012). This enriched the upper layers with nutrients stimulating the growth of autumn diatoms among which were the 20 chaetocerotacean species. The major blooming species were *Pseudo-nitzschia* cf. *pseudodelicatissima* and *Asterionellopsis glacialis*, but significant contributors were also two dominant chaetocerotacean species. These were *Chaetoceros contortus* and *C. vixvisibilis* and they had alternating peaks of abundances between different sampling dates. In 2009, the following year, the autumn bloom shifted towards end of

November as the mixing event did not occur until the end of October (Godrijan et al., 2013). The great autumnal diversity of *Chaetoceros* species found within this study, was previously recorded for the nearby area of Lim Bay (Bosak et al., 2009) but no detailed account on the temporal development of the bloom was given. In other temperate coastal marine areas such as Narragansett Bay (Rines and Hargraves, 1988) and Danish waters (Jensen and Moestrup, 1998) the autumn bloom is also simultaneously composed of up to 15-20 different *Chaetoceros* species with a few species making up the majority of the population.

The trigger for the summer diatom bloom observed in July 2009 was the surface spreading of the Po River plume which occurred along the whole Istrian coast (Godrijan et al., 2013). These summer Po River freshwater spreading events, as a thin surface layer over the northern Adriatic basin, have been occurring as a regular phenomenon in recent years (Djakovac et al., 2012; Viličić et al., 2013). The lower salinity coincided with the slightly increased concentrations of nutrients, mostly orthophosphate, which probably influenced the development of monospecific *Chaetoceros vixvisibilis* bloom, recorded not only at our investigated station but also along the whole northeastern Adriatic coast (Godrijan et al., 2013). For the northern Adriatic four major peaks in phytoplankton abundance are reported (February, May, July and September) (Bernardi Aubry et al., 2012). But Marić et al. (2012) report a change in the timing and composition of these blooms in the recent decade on this station. As the July bloom intensified and is dominated by a single *Chaetoceros* species, it resembles a typical spring bloom. Usually the spring and autumn blooms differ in both terms of abundance and biodiversity, spring peaks are higher and have only one-two taxa. The autumn peaks are lower in abundance, but with higher biodiversity (Cabrini et al., 2012). The two blooms have the different implications on the carbon cycling in the ecosystem. In particular, the autumn bloom is mainly consumed by the intense grazing activities and the energy transported further in the food-web (Fonda Umani et al., 2012). On the other hand, the intense winter blooms are mostly exported to bottom and only partly grazed by zooplankton (Fonda Umani et al., 2012) so we can assume that this was the fate of the *C. vixvisibilis* summer bloom. This is corroborated with the high abundances recorded at 20 m of depth and large proportion of resting spores within the cells, which presumably sank to the sea floor during/after the bloom. We hypothesize that the seasonal dynamics of *C. vixvisibilis* can be attributed to its biological traits, mostly to its spore formation ability.

Although this study was done on the data collected from only one year, our results indeed showed that it is possible to distinguish a species succession on a fine scale. As chaetocerotacean species are among the most important components in the coastal seas, they can be viewed as important determinants of the ecological status of coastal waters as determined by biological quality element phytoplankton according to the European Directive 2000/60/EC (European Community, 2000). It is therefore very important to have the basic knowledge about the species temporal and bloom dynamics and the ecological conditions that govern these features. Such diverse interactions of individual chaetocerotacean species 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 coastal ecosystem dynamics.

Acknowledgements

We wish to thank prof. Damir Viličić on numerous useful and constructive advices and his tremendous support during this study. We thank the captain and crew of “Burin” and “Vila Velebita”, particularly Paolo Krelja, Margareta Buterer and Paolo Paliaga for their help during the field work and in the laboratory. The presented research was supported by the Ministry of Science, Education and Sport of the Republic of Croatia Projects No. 119-1191189-1228 and 098-0982705-2729.

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