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Morphology and ecology of the diatom *Chaetoceros vixvisibilis* (Chaetocerotales, Bacillariophyceae) from the Adriatic Sea

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The chain-forming diatom *Chaetoceros vixvisibilis* is one of the most abundant and frequent diatoms in the northeastern Adriatic Sea. This species had not been previously studied by electron microscopy. Its general morphology is similar to those species allocated in the subgenus Hyalochaete: cells and chains (straight, of variable length, usually 4–8 cells per chain, but can be longer) of delicate appearance, valves thinly silicified, with slightly eccentric annulus and costae radiating from it, a single rimoportula present only at terminal valves, long, thin and delicate setae perforated by tiny poroids with no spines, and one plate-like chloroplast per cell. Setae with no spines are not common among members of Hyalochaete (and the whole genus *Chaetoceros*), except *C. socialis*. The most characteristic feature is, however, the resting spores commonly found, which also show morphological variability, from solitary to paired, both valves convex to domed and surface smooth or with small granules, and one to four strong spines often branching dichotomically. Abundances of *C. vixvisibilis* are positively correlated to the Po River inflow. Maximum abundances ($>10^6$ cells L^{-1}) were found in the period April–July, in the temperature range between 12 and 16°C, salinity between 33 and 38, when total phosphorus concentration is higher than 0.4 μmol L^{-1}, but occasionally also in nitrogen limited conditions.

KEYWORDS: phytoplankton; diatoms; *Chaetoceros vixvisibilis*; morphology; ecology; Adriatic Sea

INTRODUCTION

The diatom *Chaetoceros vixvisibilis* Schiller *in* Hustedt was originally described by Schiller (Hustedt, 1930) from the plankton of the Adriatic Sea. This is one of the most abundant (1.5×10^6 cells L^{-1}) and frequent (present in 22% samples) diatoms in the northeastern Adriatic (Viličić *et al.*, 2009). Cells can be easily distinguished by resting spores and appear to be restricted in distribution to the Mediterranean Sea (Marino and Modigh, 1981; Viličić *et al.*, 1995), and one single paper reports it from the Gulf Stream (Gould and Fryxell, 1988).

This species has not been yet studied in detail (e.g. by electron microscopy) and also its ecological impact has not been investigated. In this paper, we study both morphology and ecology of this species in the northeastern Adriatic Sea, on the basis of samples taken during 2001 to 2007, in periods with different hydrological regime.

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Study area

The Adriatic Sea is the northernmost section of the Mediterranean Sea (42–46°N) (Fig. 1), where specific hydrodynamic conditions (circulation, stratification and mixing) result from the Po River discharge and wind regime. The Adriatic Sea is longitudinally and transversely asymmetric, as revealed by its hydrography (Orlić *et al*., 1992; Polimene *et al*., 2006; Jeffries and Lee, 2007) and phytoplankton distribution (Smodlaka, 1986; Polimene *et al*., 2006). Longitudinally, there are three distinctive sections, the northern, mid and southern parts; transversely there are eastern and western parts with different coastal geomorphology. The shallow northernmost part is <50 m deep. Freshwater input from the largest Italian and Mediterranean river, the Po, regulates the general distribution of nutrients (Degobbis *et al*., 1986; Degobbis and Gilmartin, 1990; Raicich, 1996; Zavatarelli *et al*., 1998; Socal *et al*., 2008), productivity (Chiaudani *et al*., 1980; Smodlaka, 1986; Giordani *et al*., 1997) and circulation (Gačić *et al*., 2001). There are two distinctive water masses influencing the northeastern Adriatic area, viz., the less saline Northern Adriatic Water (NAW), and the higher salinity Eastern Adriatic Current (EAC). The northeastern region is mostly oligotrophic, due to the influences of the northwesterly ingoing EAC (Artegiani *et al*., 1997; Poulain, 1999; Gačić *et al*., 2001; Viličić *et al*., 2009) and the oligotrophic karstic rivers (Viličić *et al*., 2008).

Advection of the EAC influences biological characteristics in the northern basin, where biomass is frequently accumulated in frontal zones, close to the Po (Mangoni *et al*., 2008) or further towards the eastern coast, along the Istrian front (Zore-Armanda *et al*., 1983; Kršinić, 1995; Lee *et al*., 2005).

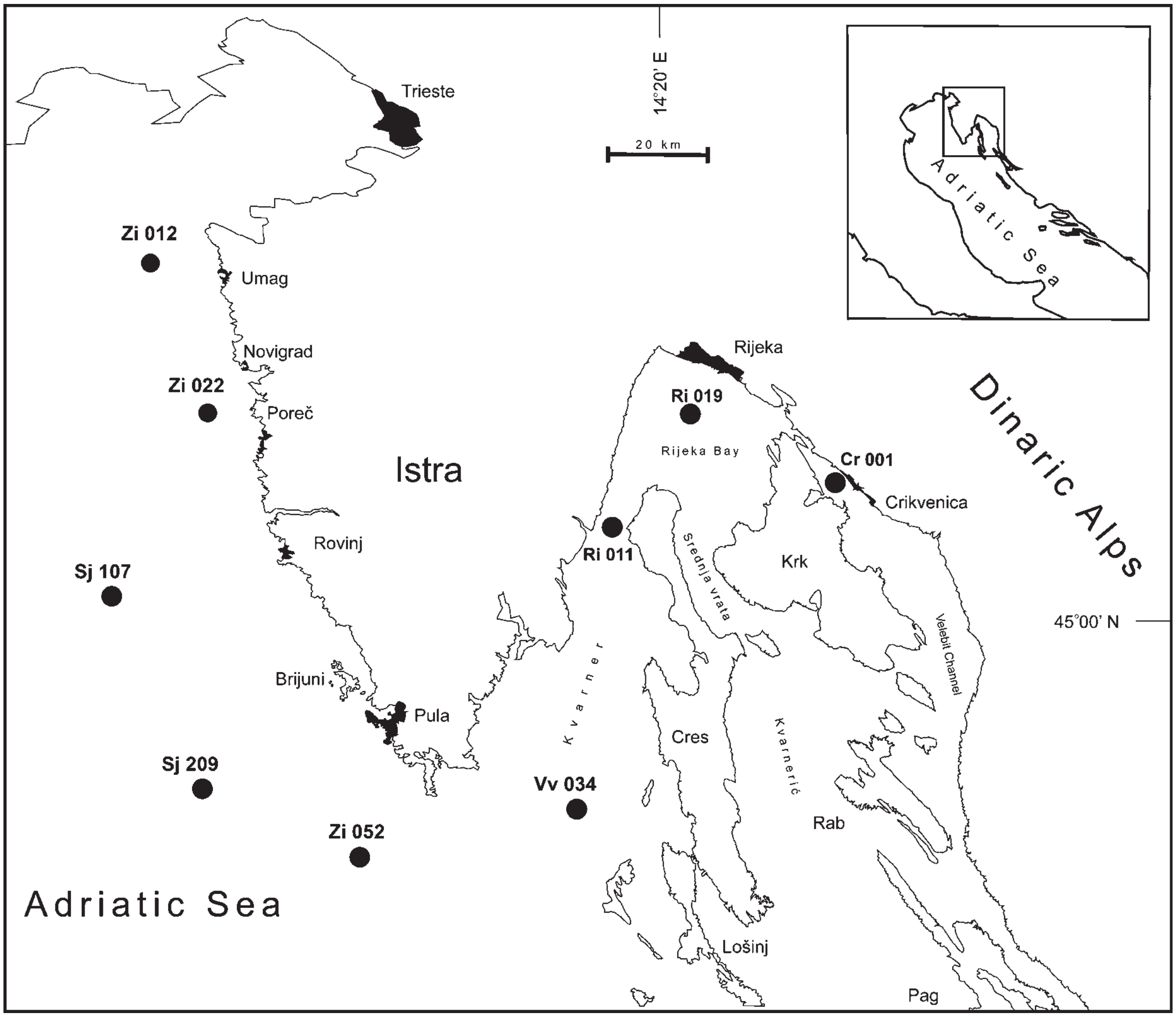

Fig. 1. Position of the northeastern Adriatic sampling stations.

METHOD

Phytoplankton composition and abundance were analyzed in 1114 samples collected by Niskin samplers in the 0–20 m layer at six stations along the Istrian peninsula and at three stations in the Kvarner region during 2001–2007 (Fig. 1). The frequency of sampling was approximately monthly. Longitudinal and seasonal phytoplankton distributions were constructed using data from stations along the "Istria profile", distinguishing northern stations (Zi 012, Zi 022), western stations (Sj 107, Sj 209), southern stations (Zi 052, Vv 034) and Kvarner (easternmost) stations (Ri 011, Ri 019, Cr 001).

Samples were preserved in a 2% (final concentration) neutralized formaldehyde solution and analysed within a 1 month period. The taxonomic list was prepared after counting cells using a Zeiss Axiovert 200 (Oberkochen, Germany) inverted microscope (Utermöhl, 1958; Hasle, 1978a, b; Venrick, 1978). Sub-samples of 50 mL were analyzed microscopically after 24 h of sedimentation. Enumeration was carried out using phase contrast and bright-field illumination at magnifications of ×400, ×200 and ×100. One transect along the Wild Row counting chamber bottom was scanned at ×400, two transects at ×200 magnification. Larger nanoplankton (>5 μm) and abundant microphytoplankton (>20 μm) were counted and identified at ×400. Identification was checked using ×1000 magnification. At ×100, a total bottom count was completed for taxa >30 μm. The minimum concentration that can be detected by this method is 20 cells L^{-1}. The phytoplankton community was identified to species or genus after image analysis and processing using the Karl Zeiss AxioVision 3.1 System and an AxioCam Camera.

Conventional methods for studying morphology of planktonic diatoms were followed, including acid-cleaning (Simonsen, 1974; Hasle, 1978b) and critical point-drying methods. The material (either rinsed or cleaned) was also studied for scanning electron microscopy (SEM), using a JEOL JSM6360LV, and only cleaned material for transmission electron microscopy (TEM), employing a JEOL-1200 EXII. Terminology for diatoms and the genus *Chaetoceros* follows classical and more recent proposals (Anonymous, 1975; Ross *et al.*, 1979; Rines and Hargarves, 1988; Round *et al.*, 1990; Hernández-Becerril, 1996).

Nutrients and oxygen concentrations were determined according to standard methods (Strickland and Parsons, 1972). Temperature and salinity were measured by CTD probe (Seabird SBE25, Bellevue, WA, USA). Nutrient limitation was assessed using a combination of nutrient concentrations and ratios (Fisher *et al.*, 1988; Dortch and Whitledge, 1992; Justić *et al.*, 1995). According to Dortch and Whitledge (Dortch and Whitledge, 1992), when dissolved inorganic nitrogen (TIN: nitrate, nitrite, ammonium), phosphate and silicate concentrations are less than 1.0, 0.2 and 2.0, respectively; they are considered limiting. To estimate which nutrient would be depleted first, the molar ratios of the nutrients were calculated. Based on the findings of Dortch and Whitledge (Dortch and Whitledge, 1992) and Justić *et al.* (Justić *et al.*, 1995), the following criteria were applied: (i) there was a Si limitation if Si/P <10 and Si/TIN <1; (ii) an N limitation if Si/P >10 and Si/TIN >1; (iii) a P limitation if Si/P >20 and TIN/P >20.

The program Statistica, version 8.0 (Statsoft, Tulsa, OK, USA) was used for statistical procedures. A logarithmic transformation ($\log_{10}(x + 1)$) was used on the data prior to statistical analyses.

RESULTS

Description of the species by light and electron microscopy

Chaetoceros vixvisibilis Schiller *in* Hustedt (Figs 2 and 3).
Reference: Hustedt, 1930, p. 727, figs 417 a-d.

Cells are arranged in straight chains of variable length, usually 4–45 cells per chain (Fig. 2A). The cells are joined together by the sibling setae, which are fused (Fig. 2A). One large, plate-like chloroplast is present per cell (Fig. 2A). In girdle view, frustules are rectangular to square in shape, most commonly with the pervalvar axis longer and valves slightly concave (Fig. 2A, B and D). Apertures between sibling valves are lanceolate and very narrow (Fig. 2A). The valves are elliptical to circular in valve view (Fig. 2C). The setae are long and delicate, all about the same direction: almost perpendicular to the chain axis or in a certain angle (about 45°) of the chain axis (Fig. 2A).

Valves are thinly silicified with a very conspicuous pattern: a slightly eccentric annulus from which a series of costae, sometimes bifurcated, run to the margins (Figs 2B–D). The valve mantle is very low and is divided from the valve face by a rim (Fig. 2B). Terminal valves show the same basic features, but additionally, the presence of a tiny, slit-like, rimoportula only on terminal valves was detected (Fig. 2D). Various open bands of the cingulum were found (Fig. 2D). All setae rise from the apices of the valves and are circular in

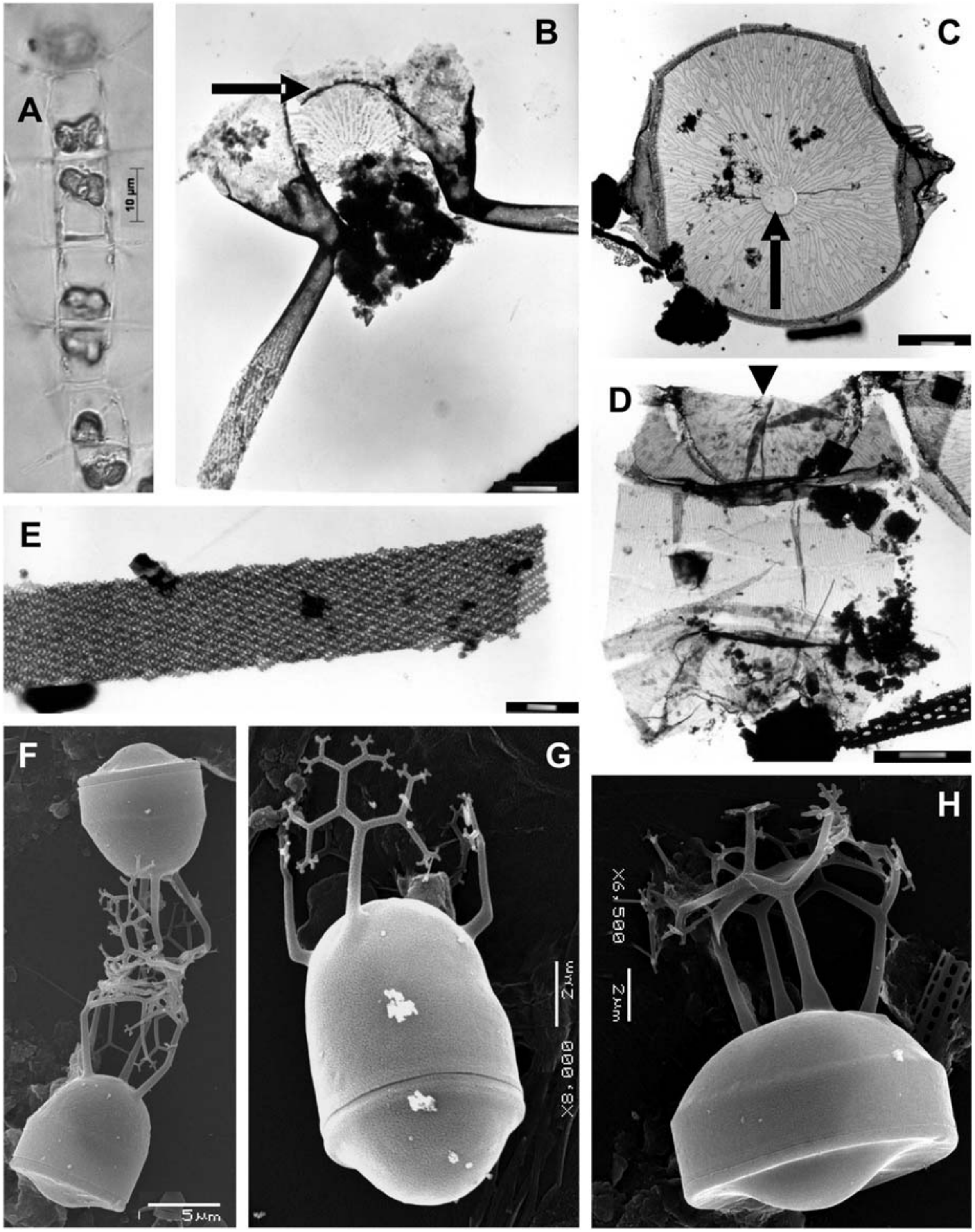

Fig. 2. *Chaetoceros vixvisibilis*, LM, TEM and SEM. (**A**) A chain with seven cells, all of them with a single chloroplast, LM. (**B**) Terminal valve showing valve face, valve mantle and terminal setae (a rim separating valve face from mantle is arrowed), TEM. (**C**) Intercalary valve in valve view, with broken setae and costae radiating from the annulus (arrow), TEM. (**D**) A terminal frustule, showing bands of the cingulum and rimoportula at the terminal valve (arrowhead), TEM. (**E**) Detail of a seta, TEM. (**F**) Resting spores with long, branched spines, SEM. (**G**) A single resting spore with three spines, SEM. (**H**) Another resting spore with four spines, SEM. Scale bars = 10 µm (A), = 2 µm (B–D), = 1 µm (E).

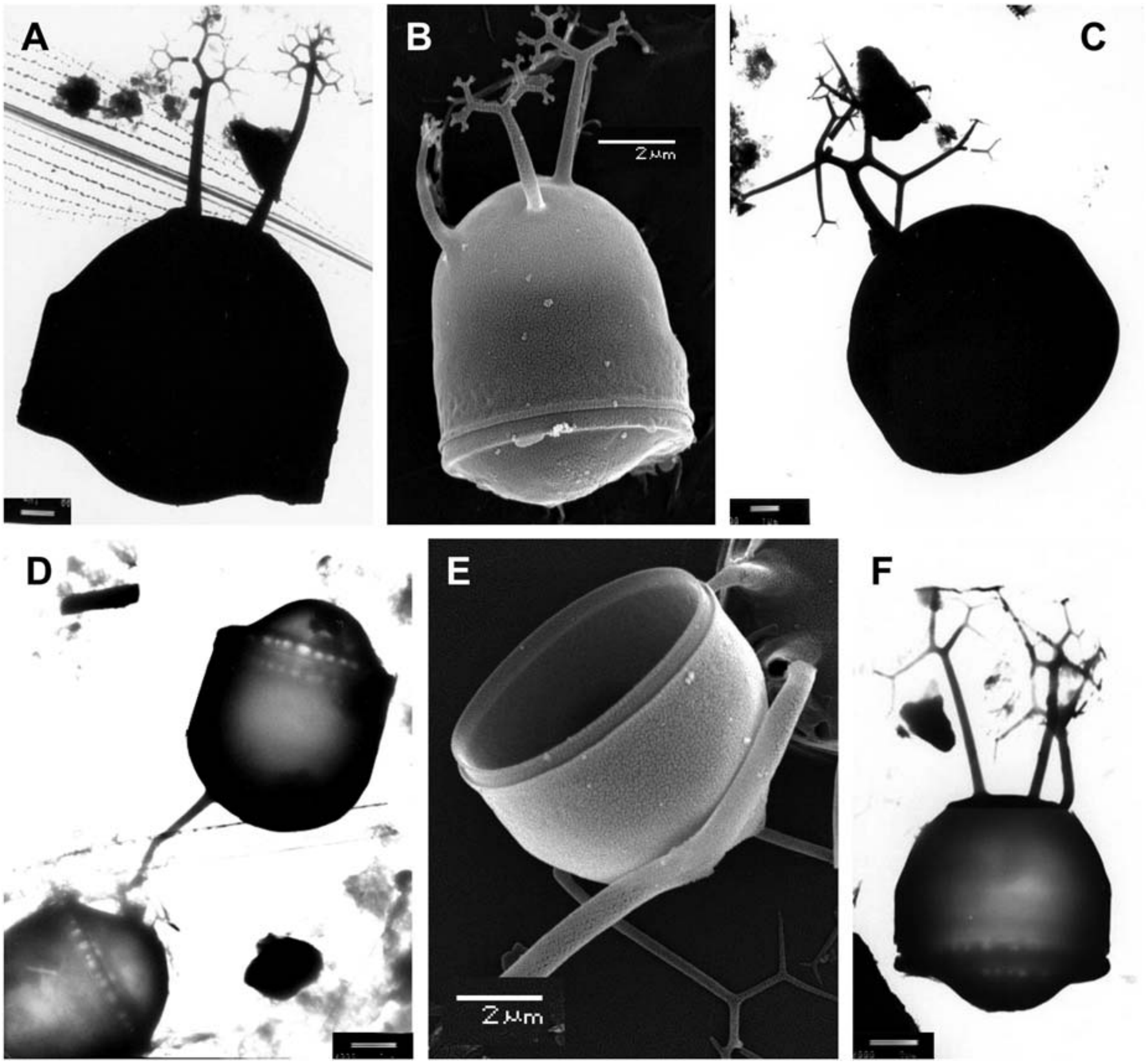

Fig. 3. *Chaetoceros vixvisibilis*, TEM and SEM. (**A**) Resting spore with two spines, TEM. (**B**) Another resting spore with four spines, SEM. (**C**) A resting spore with only one strong and branched spine, TEM. (**D**) A couple of resting spores, the upper one showing a single spine, TEM. (**E**) Primary valve with two spines, SEM. (**F**) Single resting spore with three branched spines, TEM. Scale bars = 2 µm (D, F), = 1 µm (A, C).

cross section throughout (Fig. 2A, B and E). They do not show spines, but have their walls perforated by spiral rows of tiny poroids (Fig. 2E) (measurements: apical axis, 11–28 µm).

Resting spores displayed a wide range of morphological variation in shape and size (Figs 2F–H and 3A–F). They were usually found to be solitary, but some couples were also seen (Figs 2F and 3D). Both valves are convex, domed or more rounded, depending on size (Figs 2F–H and 3A–D and F). The primary valve is smooth, with a variable number (1–4) of strong and sometimes long spines (Figs 2F–H and 3A–D and F); these spines may be very simple, with no branches, to very complicated, with many dichotomic branches (Figs 2F–H and 3A–D and F). The valve mantle may be imperceptible to relatively high and may have shallow pores (Figs 2G and H and 3B and E). Notches between primary and secondary valves are evident (Figs 2G and 3B, D and E). Secondary valves are more convex to domed with no major structures, but small granules in some specimens (Figs 2F–H and 3B, D and F).

Seasonality and distribution of phytoplankton in the NE Adriatic trophic gradient

Chaetoceros vixvisibilis was found in abundances up to 1.5×10^6 cells L^{-1}, in 22% of taken samples.

Seasonality of *C. vixvisibilis* is characterized by its appearance mostly in the warmer part of the year, probably greatly regulated by Po River discharge. The Po River discharge, followed a typical seasonality in 2002 and 2004, with distinct spring (snow melting period) and autumnal (rainfall period) maxima. The mean discharge in the period January–May reached 1600 m^3 s^{-1}. In 2003 and in 2005–2007, there were weak spring maxima, while autumn maxima were the highest annual discharges (see below).

Nutrient concentrations were low and showed biannual seasonality with summer minima (Fig. 4). Mean orthophosphate concentrations were mostly less than 0.04 μmol L^{-1}, TIN less than 1.8, SiO_4 <2 μmol L^{-1}. Nutrient concentrations and phytoplankton abundances decreased from northern and western stations (Zi 012, Sj 107) to southern (Sj 209, Zi 052) and eastern stations (Vv 034, Ri 011, Ri 022, Cr 001) of the investigated profile. There was an approximately 6-fold decrease in the average

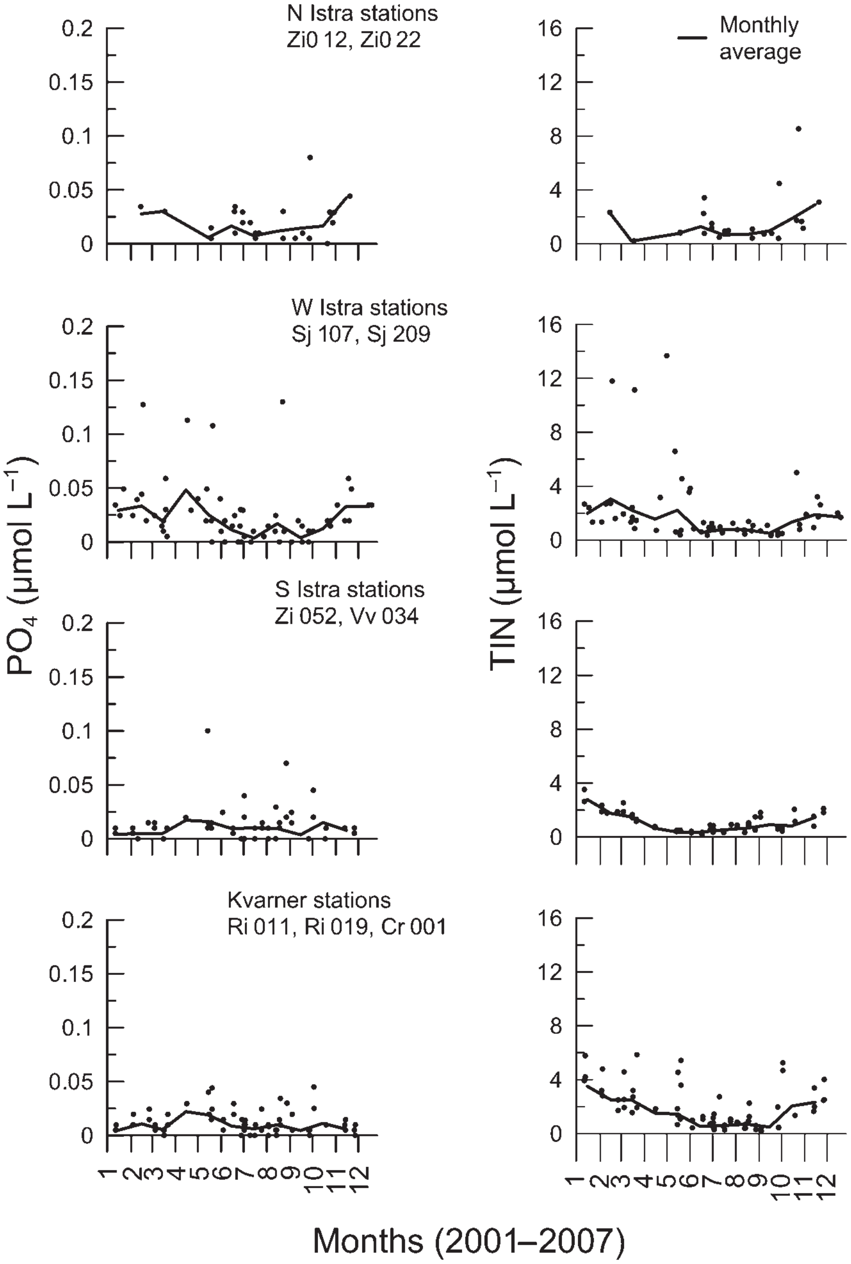

Fig. 4. Seasonal variations of orthophosphate (PO4) and total inorganic nitrogen (TIN) concentrations along the Istria profile and in the Kvarner region, in the period 2001–2007. Data are maximum concentrations in the 0–10 m layer (325 data). For station locations see Fig. 1.

abundance of diatoms from western stations (1.9×10^5 cells L^{-1}) to southern and Kvarner stations (3.1×10^4 cells L^{-1}). The abundance of *Ch. vixvisibilis* along the profile reached 1.5×10^3 cells L^{-1} at western stations and decreased eastward. Among other phytoplankton, average diatom abundance at all stations (1.4×10^5 cells L^{-1}) was about three times greater than the abundance of dinoflagellates (4.5×10^4 cells L^{-1}) and about five times greater than the abundance of coccolithophorids (2.9×10^4 cells L^{-1}).

The mean January–May Po River discharge in the period 2005–2007 (780 $m^3 s^{-1}$) declined by nearly half in comparison to 2002 and 2004 (1480 $m^3 s^{-1}$), with two orders of magnitude lower average abundance of total diatoms (Fig. 5). Reduced Po River discharge and the prevalence of oligotrophic conditions in 2005–2007 generally resulted in decreased abundances of *Chaetoceros vixvisibilis* (Figs 6 and 7). The annual maximum abundance of *Chaetoceros vixvisibilis* mostly appeared in the period May–July. Such seasonality was confirmed by

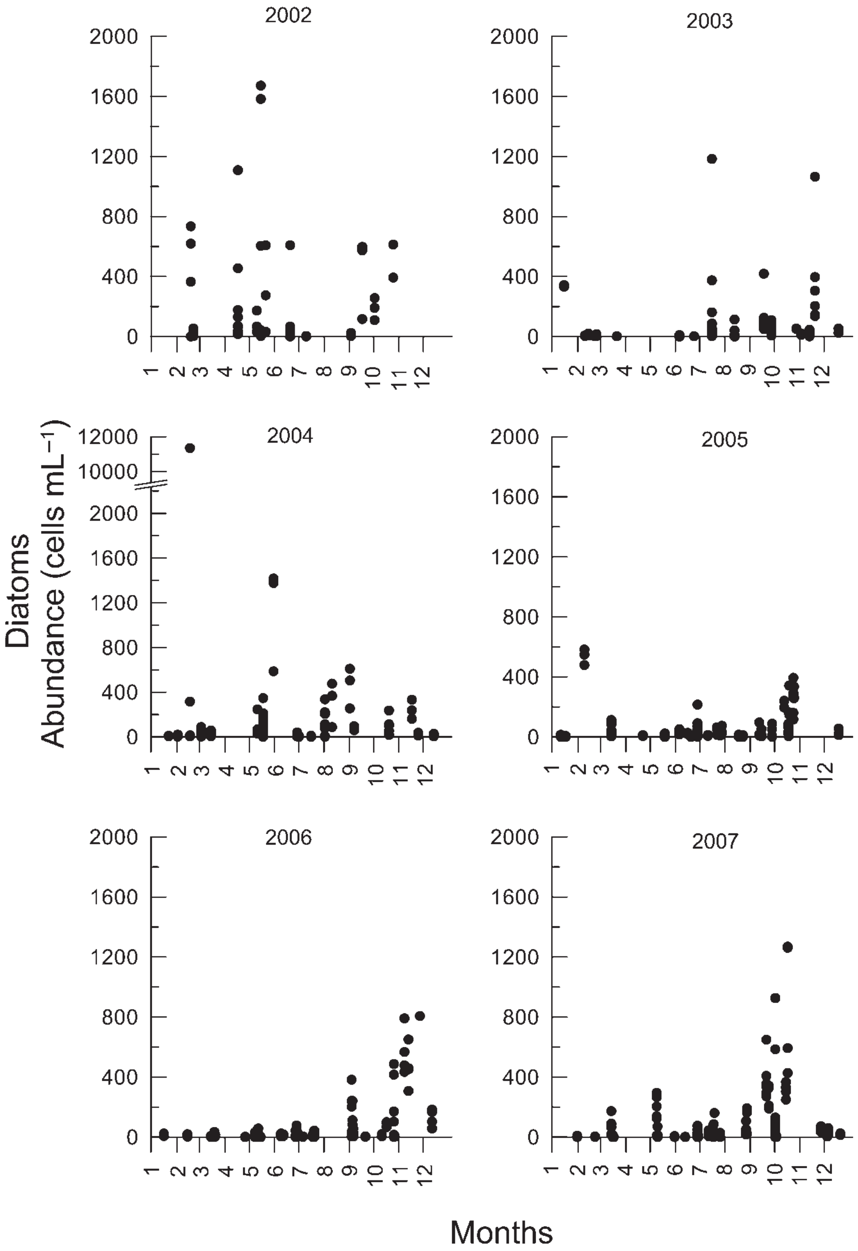

Fig. 5. Seasonal abundances of diatoms along the Istrian profile and in the Kvarner region, in the period 2002–2007. Data are maximum abundance in the 0–10 m layer (325 data). For station locations see Fig. 1.

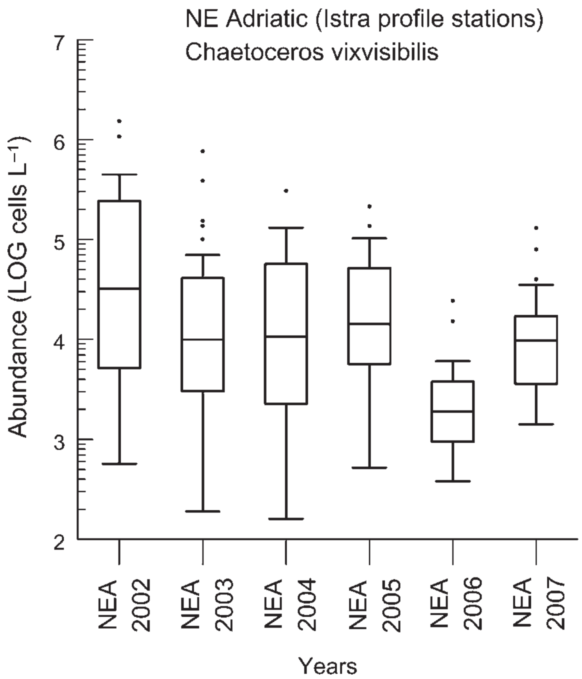

Fig. 6. Box diagrams showing variations of abundances in *Chaetoceros vixvisibilis* in the period 2002–2007.

significant positive correlations (Pearson, $P < 0.01$) with abundances of total diatoms, dinoflagellates and phosphorus, but negatively correlated with temperature and salinity (Table I). The analysis performed with data taken at all stations indicated significant positive correlation (Sperman, $P < 0.5$) between maximum annual *Chaetoceros vixvisibilis* abundance and average annual Po River inflow (Fig. 8).

The summer time appearance of the *C. vixvisibilis* was confirmed by T–S plankton diagram, where *C. vixvisibilis* preferred a temperature range between 12 and 16°C, and salinity between 33 and 38 (Fig. 9). Total inorganic nitrogen (TIN) plankton and total phosphorus (TP) plankton diagrams (Fig. 10) suggest that this species reaches maximum development if TP is higher than 0.25 μmol L^{-1}, and TIN is higher than 0.3 μmol L^{-1}. The same abundances were found if orthophosphate concentrations were as low as 0.02 μmol L^{-1}.

The Si:P ratios were mostly higher than the Redfield molar ratio, and the N:P ratios were much higher than 16:1, providing indirect evidence for strong stoichiometric P limitation of diatom growth rates (Fig. 11). All orthophosphate concentrations were less than 0.3 μmol L^{-1}. The Si:TIN and Si:P ratios clearly demonstrate that potential stoichiometric Si limitation of diatoms is rare at the western station Sj 107 and no Si limitation was indicated at the eastern Station Cr 001. At these two stations 46–55% of Si values were <2 μmol L^{-1}. Although nitrogen concentrations <1 μmol L^{-1} were detected in 46–51% of samples, nitrogen was only occasionally shown to be limiting.

DISCUSSION

Morphology

The general morphology of *Chaetoceros vixvisibilis* is similar to those species currently allocated in the subgenus Hyalochaete: the cells and chains (which are straight, of variable length, usually 4–8 cells per chain, but can be up to 45 per chain) have a delicate appearance, with the valves thinly silicified, bearing a slightly eccentric annulus and costae radiating from it, a single rimoportula present only at terminal valves, long, thin and delicate setae perforated by tiny poroids with no spines, and one plate-like chloroplast per cell. The absence of spines on the setae can be considered a remarkable morphological feature: most species belonging to the genus *Chaetoceros* and the subgenus Hyalochaete, which have been studied by EM so far, show the presence of, at least, minute spines. One notable exception is the species *Chaetoceros socialis* Lauder, which has a longer seta (than the others in sibling valves), with no spines (Hernández-Becerril, 1996).

The most characteristic feature of this commonly found species is, however, the resting spore. Showing morphological variability, solitary spores have both valves convex to domed with a smooth surface or a surface with small granules, and one to four strong spines often branching dichotomically. These resting spores appear unique among other members of the subgenus Hyalochaete, only superficially comparable to those occurring in *Chaetoceros lorenzianus* Grunow: they have two large arms or branches with various smaller branches, although the number of large arms is always two and the vegetative cells of *C. lorenzianus* are quite different.

Ecology

Chaetoceros vixvisibilis is one of the most remarkable, warm oceanic species and is an abundant diatom in the northern (Viličić *et al.*, 2009) and southern Adriatic (Viličić *et al.*, 1995), mostly contributing to southern Adriatic cyclonic gyre offshore phytoplankton blooms in spring (Viličić *et al.*, 1989), having specific environmental preferences (Table II). The taxonomic composition of phytoplankton in the eastern Mediterranean basin and the south-eastern Adriatic Sea has been defined by dominating diatoms such as: *Chaetoceros, Pseudo-nitzschia, Proboscia, Rhizosolenia, Bacteriastrum, Cerataulina, Leptocylindrus* and *Thalassionema* (Kimor *et al.*, 1987; Viličić *et al.*, 1995).

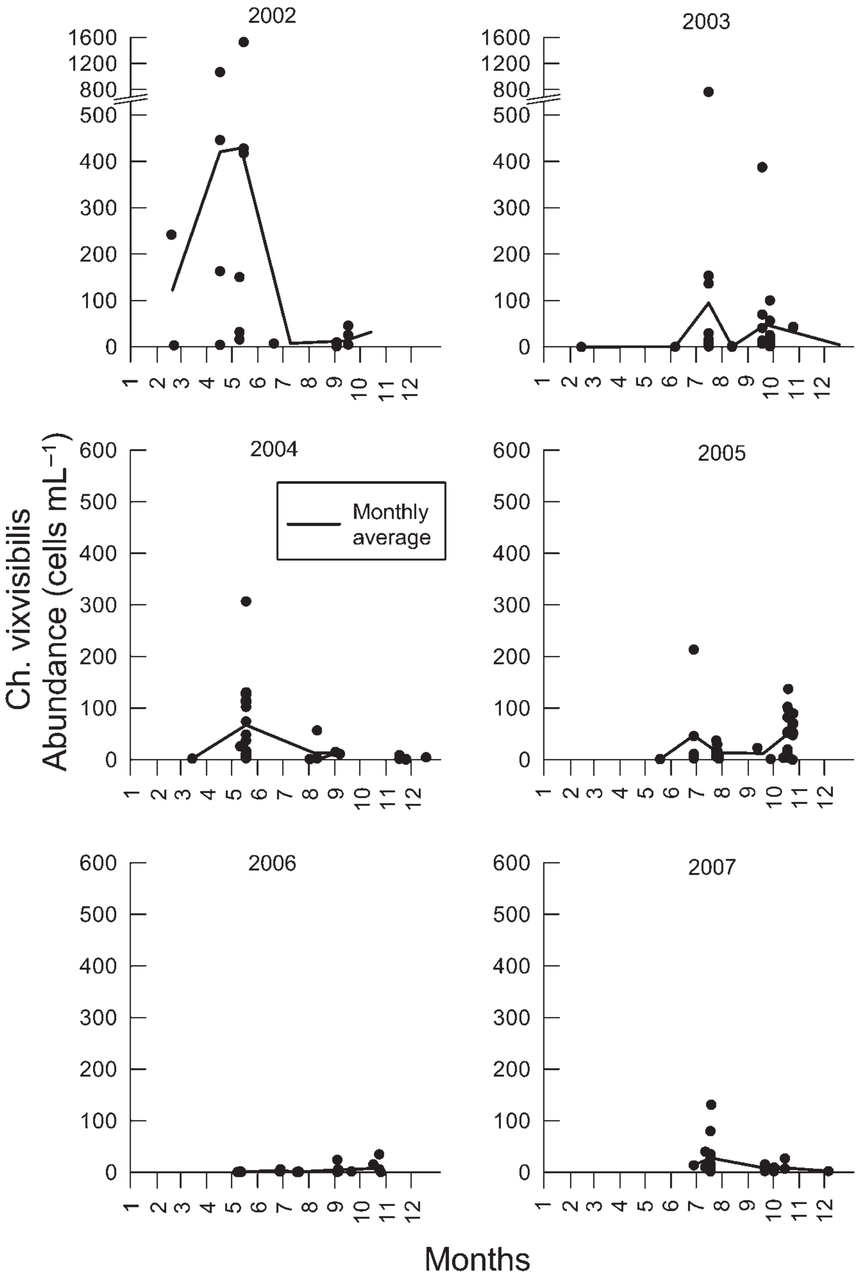

Fig. 7. Seasonal abundances of *Chaetoceros vixvisibilis* along the Istrian profile and in the Kvarner region, in the period 2002–2007. Data are maximum abundance in the 0–10 m layer (325 data). For station locations see Fig. 1.

Table I: Pearson correlation matrix calculated for abundances of Chaetoceros vixvisibilis (Ch. vix), total diatoms (DIA), dinoflagellates (DINO), total nanoplankton (NANO), as well as temperature (T), salinity (S), total phosphorus (TP), total inorganic nitrogen (TIN) and orthosilicates (SiO_4)

	DIA	DINO	NANO	T	S	TP	TIN	SiO_4
Ch vix	0.76**	0.55*	0.39	−0.49*	−0.58*	0.72**	−0.34	−0.15

Eighteen surface samples (Log + 1 transformed) were taken at station Sj 107, during 2002–2007. Significance level at $*P < 0.05$, $**P < 0.01$.

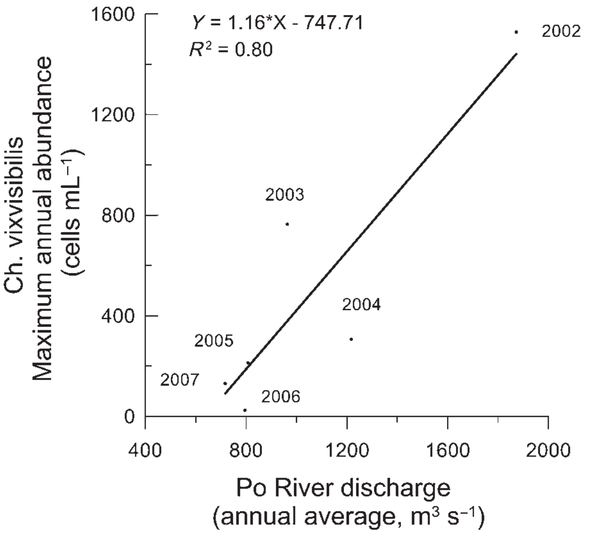

Fig. 8. Correlation between annual average Po River inflow and annual maximum abundance of *Chaetoceros vixvisibilis*, along the Istrian profile and in the Kvarner region, in the period 2002–2007.

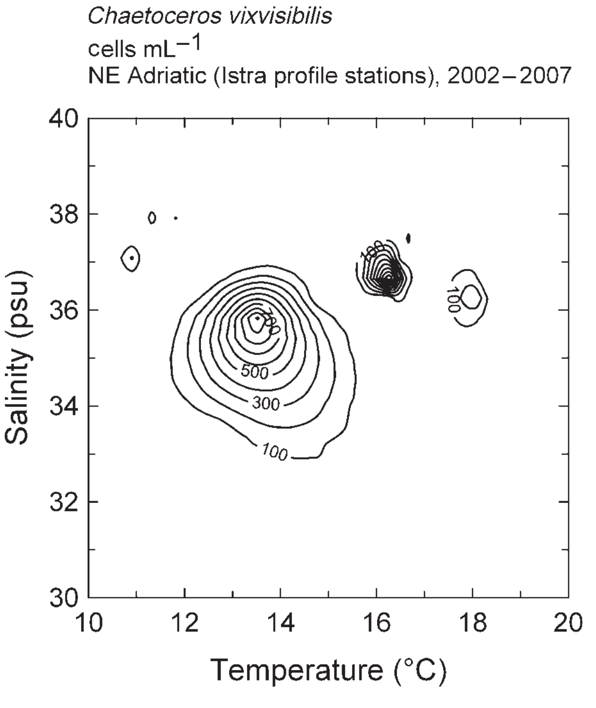

Fig. 9. Salinity–temperature *Chaetoceros vixvisibilis* abundance diagram, along the northeastern Adriatic profile for the period 2002–2007.

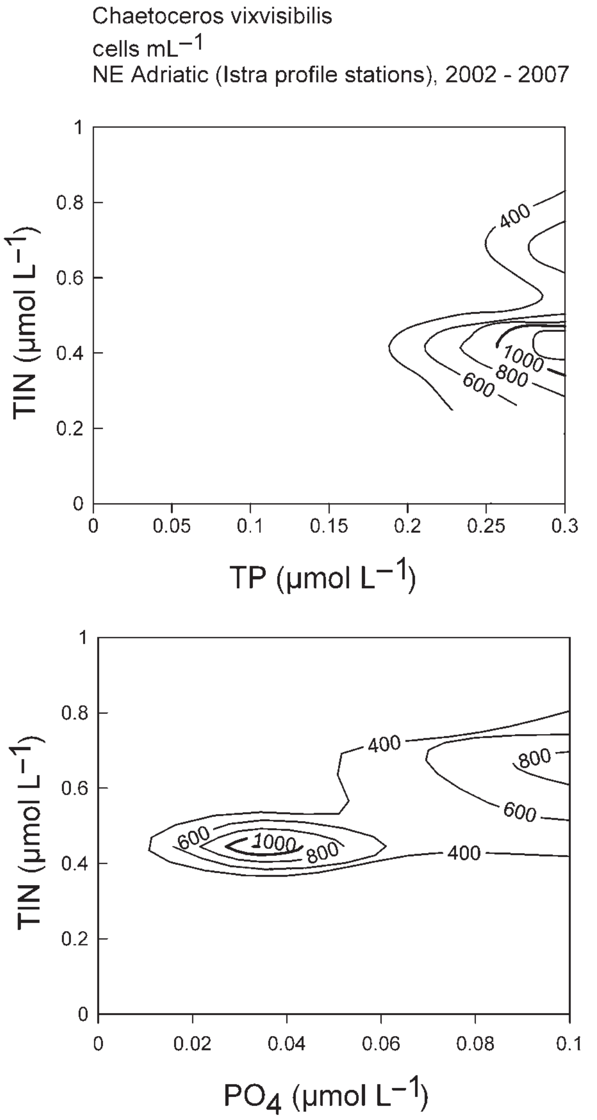

Fig. 10. Total phosphorus (TP), total inorganic nitrogen (TIN), *Chaetoceros vixvisibilis* diagram, along the northeastern Adriatic profile for the period 2002–2007.

Nutrient stoichiometries from this study indicate that phosphorus strongly limited phytoplankton growth in the northeastern Adriatic Sea; occasionally nitrogen and silica. Lack of silica limitation resulted in the domination of diatoms in the phytoplankton community. The north Adriatic is enriched with nutrients mostly due to the Po River discharge, which regulates diatom abundances seasonally. Accordingly, the westernmost Istrian station Sj 107 and the northernmost station Zi 012 differed mostly from other stations throughout the year (Viličić *et al.*, 2009). Po River outflows and meteorological factors are the main components triggering the alternation of stratification and mixing of the water column that strongly affect the trophic state of the northern basin (Socal *et al*, 2008). There is a close coupling of physical and biological processes over a range of space and time scales in the northern Adriatic (Spillman *et al.*, 2007). Episodes of strong north winds (bora) may provoke a double-gyre system (mostly in winter): a cyclonic gyre in the Gulf of Trieste, an anticyclonic gyre off western Istria (Kuzmić

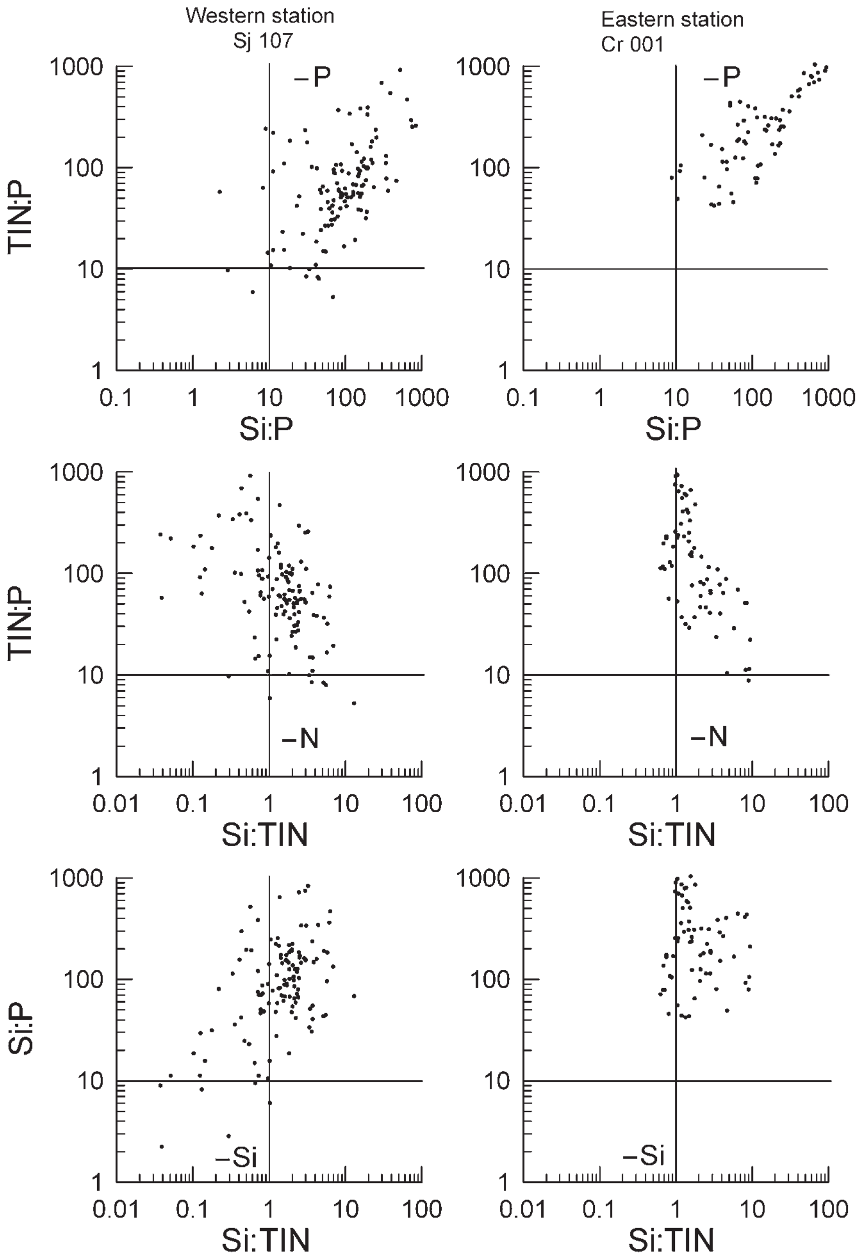

Fig. 11. Molar ratios of the nutrients (N:P:Si) at western and eastern stations.

et al., 2006; Lyons *et al.*, 2007; Pullen *et al.*, 2007) as well as the formation of the Istrian Coastal Counter Current which transfers water enriched with nutrients from the Po River close to the Istrian coast (Supić *et al*, 2006), resulting in higher productivity (at stations Zi 012, Zi 022 and occasionally at Sj 209). The eastern basin is mostly influenced by scarce sources of nutrients such as karstic rivers and the EAC (Viličić *et al*, 2008).

Chaetoceros vixvisibilis showed the greatest abundances at the western station Sj 107 in May 2002, which probably coincided with relatively sufficient concentrations of nutrients and increase in daylight (Fig. 6). Among other abundant diatoms at station Sj 107, we recorded *Skeletonema marinoi* (February 2004), *Chaetoceros socialis* (November 2003) and *Pseudo-nitzschia* sp. (May 2002, October 2007). Abundance of *C. vixvisibilis* was significantly correlated with phosphorus ($r^2 = 0.7$, $P < 0.01$, Table I), which is the main limiting nutrient in this region (Maestrini *et al.*, 1997; Vukić Lušić *et al.*, 2008). *Chaetoceros vixvisibilis* seasonality was directly proportional to the annual regime of the Po River influx. In the period 2002–2004, the high winter-spring Po River

Table II: Chaetoceros vixvisibilis—main morphometric characteristics and environmental preferences

Parameter	Min–max	Average	References
Valve diameter (μm)	11–28	11.3	This paper
Cell length (μm)	6.8–10.2	8.2	This paper
Volume per cell (μm^3)	170–4570		Viličić *et al.* (1989)
Cells per chain	4–45	18	This paper
Abundance (cells mL^{-1})	1500		This paper
	107		Viličić *et al.* (1989)
Optimum temperature range (°C)	12–17		This paper
	14–15.8		Viličić *et al.* (1989)
Optimum salinity range (psu)	33.00–37.50		This paper
	38.82–38.86		Viličić *et al.* (1989)
Optimum total phosphorus range (μmol L^{-1})	0.25–0.3		This paper
Optimum ortophosphate range (μmol L^{-1})	0.02–0.05		This paper
Optimum inorganic nitrogen range (μmol L^{-1})	0.03–0.5		This paper

freshwater inputs were accompanied by spring *C. vixvisibilis* maxima. However, in the period 2006–2007, annual abundance maxima of *C. vixvisibilis* decreased drastically. In the same period, phytoplankton maxima moved from spring to autumn. The trend of autumnal highest Po discharge and highest chlorophyll *a* biomass has been recorded since 1993 (Supić *et al*, 2006).

Minimum *Chaetoceros vixvisibilis* abundances appeared at oligotrophic southern stations (Vv 034 and Zi 052) which are mostly influenced by the EAC. The diatoms are compensated by a greater contribution of nanoplankton to total primary biomass (Radić *et al*, 2009). The north and western stations (Zi 012, Sj 107) are relatively isolated, due to the greater Po River influence (Viličić *et al.*, 2009). On the other hand, the southwestern station Zi 209 is isolated likely due to its position in the transition area where the influences of the EAC and north Adriatic less saline water frequently change.

Recent correlations among the thermophyle diatom *Chaetoceros vixvisibilis* abundance, temperature, salinity, phosphorus and nitrogen, provide information for the evaluation of possible ecosystem changes in the future. Possible further physical changes (warming, circulation) could influence seawater chemistry, phytoplankton physiology and composition.

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