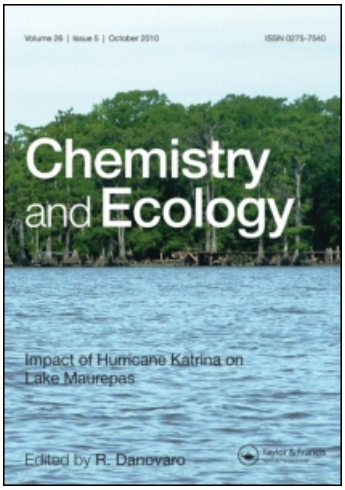

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DO MUCILAGE EVENTS INFLUENCE PICO- AND NANOPLANKTON SIZE AND STRUCTURE IN THE ADRIATIC SEA?

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Temporal changes in abundance and biomass of picophytoplankton, heterotrophic pico-eukaryotes, and nanoplankton assemblages were investigated along a transect crossing the Adriatic Sea, from the Italian to the Croatian coast. This 15-months (June 1999–August 2000) investigation allowed comparing microbial parameters during summer 1999 (year without mucilage) and summer 2000 when a major mucilage event occurred. Pico- and nanoplankton assemblages displayed significant differences between the 2 summer periods. The main differences can be summarized as: (i) presence of cyanobacterial blooms (up to 10^8 cells l^{-1}) in summer 2000, absent in summer 1999; (ii) an increasing fraction of heterotrophic pico-eukaryotes (up to 5.0×10^6 cells l^{-1}) and heterotrophic nanoplankton (size 2–5 μm) during mucilage event; (iii) a reduced abundance of small-sized (2–3 μm) phototrophic nanoplankton in summer 2000. Changes in community structure were signals of changes in trophic condition of the system, which resulted in a competitive advantage for small sized pico- and nanoheterotrophs. Data presented here indicated that mucilage events are associated with changes in microbial community structure and functioning in ambient water and induced the amplification of 3-step microbial food chain. The potential use of the heterotrophic pico-eukaryotes for describing alterations of the trophic pathways during mucilage events is discussed.

Keywords: Mucilage; Nanoplankton; Microbial food web; Heterotrophic pico-eukaryotes

1 INTRODUCTION

Episodes of marine mucilage (the so called "mare sporco"; Stachowitsch *et al.*, 1990) of various extension have been reported in the Adriatic sea since the 18th century (Fonda Umani *et al.*, 1989), but in the last 20 years the northern Adriatic has been increasingly plagued by exceptional events of large floating amounts of mucilage (Herndl, 1988; Herndl and Peduzzi, 1988; Cataletto *et al.*, 1996), which attracted public attention and concern. In the last years, acute dystrophic crises associated to mucilage have been reported in 1988, 1989, 1991, 1997 and again in 2000. *In situ* observations reported that gelling substances (*i.e.*, the starters of the "mare sporco" event) generally first appear in the upper part of the water column of the eastern part of the Adriatic Sea (*i.e.*, its oligotrophic region) and then gradually move towards

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the western coast, settling on the pycnocline (*i.e.*, the eutrophic one; Degobbis *et al.*, 1995; Degobbis *et al.*, 1997). These aggregates might last up to few months (3 months, Degobbis *et al.*, 1999), being generally confined in the northern Adriatic Sea by the circular counterclockwise current, coupled with the summer water column stratification (Orlic, 1987).

Several studies have been devoted to the identification of the possible causes and the triggering mechanisms of this phenomenon, but the reasons for the massive formation of mucilage are still substantially unknown. Among the several hypotheses proposed to explain the origin of mucilage phenomena in the northern Adriatic (Fogg, 1995; Miklestad, 1995; Herndl *et al.*, 1995; Degobbis *et al.*, 1999), Azam *et al.* (1999) suggested that they are the result of polysaccharide production, accumulation and flocculation due to the interactions between phytoplankton, microbial loop and organic matter pools. Once mucilage aggregates are produced, bacterial enzymatic activities hydrolyze their polysaccharides and proteoglycans, releasing DOM into seawater (a process defined "uncoupled solubilization"; Smith *et al.*, 1992). Mucilage hydrolysis is largely due to exo-enzymatic activities released by the microbial components in and/or out side the mucilage, but in turn DOM released during mucilage degradation is likely to provide an important source of *C* fuelling the microbial loop. Therefore, the knowledge of the trophic dynamics and size structure of pico- and nanoplankton assemblages is likely to provide important insights on the interactions between microbes and mucilage formation and its role on microbial loop functioning.

Also mechanisms that set and maintain the ambient levels of microbial abundance are not perfectly understood. Two types of controlling mechanisms have been proposed: a bottom-up control (*i.e.*, substrate limitation) and a top-down control (*i.e.*, predator-prey interactions; Gasol and Vaqué, 1993; Gasol, 1994), but relationships between bacteria and nanoflagellates are quite controversial (Sanders *et al.*, 1992; Gasol and Vaqué, 1993). A strong coupling between bacteria and heterotrophic flagellates is typically found in extremely oligotrophic conditions (Sanders *et al.*, 1992; Christaki *et al.*, 2001), whereas, in eutrophic systems, bacterial and heterotrophic nanoflagellate abundance are generally uncoupled (Gasol and Vaqué, 1993; Gasol, 1994; Weinbaurer and Peduzzi, 1995). Possible reasons for such decoupling are: (i) heterotrophic flagellates might have sources of nutrition other than bacteria (such as DOM, viruses, picophytoplankton, nanophytoplankton; Campbell and Carpenter, 1986; Sherr, 1988; Sherr and Sherr, 1988; Sherr and Sherr, 1991; González and Suttle, 1993; Marchant and Murphy, 1994; Verity *et al.*, 1996; González *et al.*, 1998; Safi *et al.*, 2002); (ii) bacteria are grazed by ciliates and mixotrophic flagellates (Bird and Kalff, 1986; Sherr and Sherr, 1987; Lovejoy *et al.*, 2000); (iii) bacteria are controlled by viral infection (Furhman, 1999; Wommack and Colwell, 2000).

Despite large information on microbial loop structure is available for the northern Adriatic Sea (Kaltenböck and Herndl, 1992; Vanucci *et al.*, 1994; Revelante and Gilmartin, 1995; Turk *et al.*, 1992; Weinbaurer and Peduzzi, 1995), the interactions between mucilage appearance and free living pico- and nanoplankton dynamics (*i.e.*, outside the mucilage) have been almost completely neglected (Degobbis *et al.*, 1995; Cataletto *et al.*, 1996).

In this paper we provide information on the potential role of aggregates in influencing free-living microbial assemblages. To do this we investigated temporal changes in abundance and biomass of picophytoplankton, heterotrophic picoeukaryotes, and nanoplankton assemblages along a transect crossing the Adriatic Sea from Italian to Croatian coasts. This investigation lasted 15-months and allowed comparing microbial parameters during summer 1999 (year without mucilage) and 2000, when a major event of mucilage was recorded. The aim of this investigation is to identifying microbiological descriptors of ecosystem trophic pathways alteration during mucilage events.

2 MATERIAL AND METHODS

2.1 Study Area and Sampling

The northern Adriatic Sea is a shallow continental-shelf basin, which deepens along the axis towards the southeast. The boundary between northern and middle Adriatic is assumed to be the Senigallia-Suzak transect (Hopkins *et al.*, 1999), whose maximum depth is ~70 m. The western coast of the northern Adriatic Sea is eutrophic and characterized by a large runoff and nutrient input. Conversely, the eastern coast, influenced by a mixture of Ionian Surface Waters and Levantine Intermediate Waters, is rather oligotrophic (Hopkins *et al.*, 1999). Three stations (C4, C7, C12) were selected along this transect (Fig. 1). Stations C4 (western coast) C7 (central), C12 (eastern coast) are at 57, 70 and 60-m depth, respectively. Sampling was carried out on monthly basis from mid June 1999 to the end of August 2000. Water samples were taken with Niskin bottles at 0, 10 m and at 2 m above the bottom at all stations. Hydrographic data were recorded by a SBE 9/11 Plus CTD. The appearance, shape and distribution of mucilage was recorded using a Remote Operative Vehicle (ROV) and mucilage were classified according to Stachowitsch *et al.* (1990).

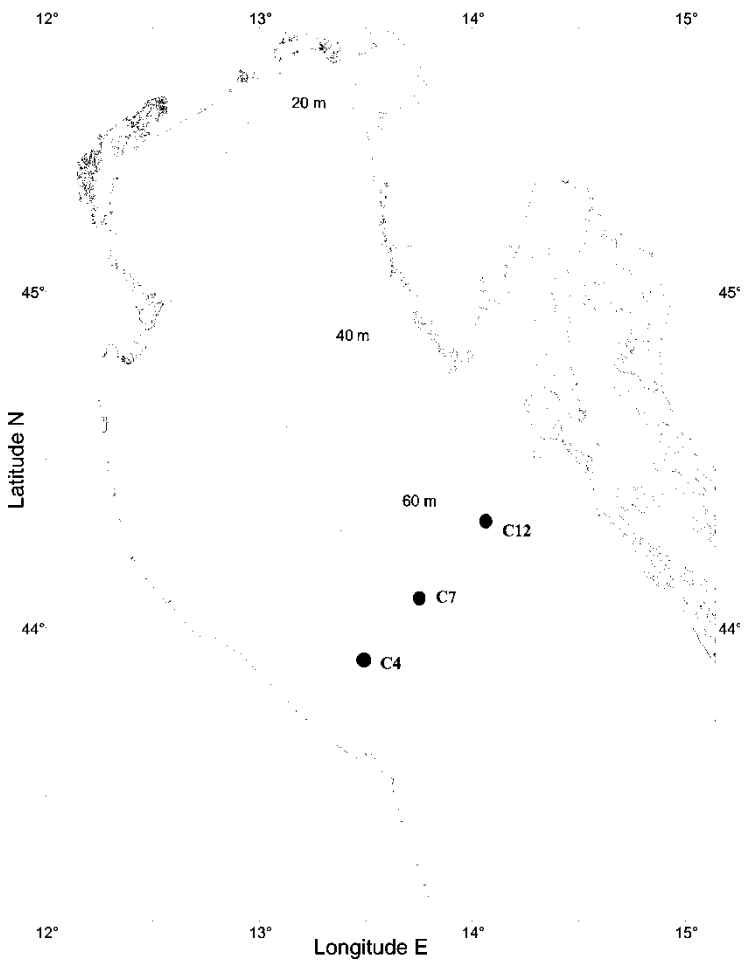

FIGURE 1 Sampling area and station location.

2.2 Microscopic Observations

After collection, samples (500 ml) for nanoplankton, picophytoplankton and heterotrophic picoeukaryotes analyses were preserved using glutaraldehyde (0.5% final sample concentration). Samples were stored in the dark at 4 °C until slides were prepared (*i.e.*, within few days from sampling).

Subsamples (35–40 ml, 10–30 ml for nanoplankton and picoplankton respectively) were stained with DAPI (4′,6-diamidino-2-phenylindole) and filtered at low pressure onto Nuclepore filters (<50 mm Hg; 25 mm, diameter 0.2 and 2.0 μm pore size for picoplankton and nanoplankton, respectively). Filters were then washed with 0.2 μm prefiltered seawater and mounted on glass slides (Sherr and Sherr, 1993). Duplicate slides were prepared for each sample. Slide were stored (−20 °C) in the dark until microscopic examination (within few days).

Slides for nanoplankton enumeration were examined at 1000 × magnification for both DAPI and chlorophyll fluorescence using a Zeiss Axioplan epifluorescence microscope (G365/FT395/LP420; BP450-490/FT510/LP520). A minimum of 100–150 cells per filter were counted and phototrophs (PNAN) were discriminated from heterotrophs (HNAN) by their chlorophyll autofluorescence.

All cells were measured using an ocular micrometer and classified into three size groups: 2–3, 3–5, and 5–20 μm. When one of the two main axis (*i.e.*, length and width) of the cell exceeded the upper limit of one size group the cell was considered as belonging to the next size group.

Slides for picophytoplankton (PPP) and heterotrophic pico-eukaryotes (HPE) were examined at 1000 × magnification for both DAPI and chlorophyll fluorescence. A minimum of 20–30 randomly chosen fields were observed. Picophytoplankton was discriminated from heterotrophic picoeukaryotes by their chlorophyll autofluorescence. Picophytoplankton cells were also classified as either cyanobacteria (prokaryotic PPP) or eukaryotic picophytoplankton (eukaryotic PPP) on the base of their autofluorescent spectrum (Waterbury *et al.*, 1986).

Cell volume was calculated by assigning simplified geometrical shapes to cells or, in some cases, a combination of more geometrical shapes, and applying or combining the standard formulae (Edler, 1979). For each size group of each sample a minimum of 20–30 cells were measured for cell volume calculation. Cell volumes (or plasma for diatoms) were converted to carbon content using a factor 0.11 pgC μm^{-3} for diatoms (Strathmann, 1967) and 0.22 pgC μm^{-3} for both phototrophic and heterotrophic nano- and picoflagellates (Børsheim and Bratbak, 1987) whereas, for cyanobacteria, a constant carbon content of 294 pgC $cell^{-1}$ was assumed (Cuhel and Waterbury, 1984).

3 RESULTS

3.1 Environmental Parameters

During the entire period of investigation (June 1999–August 2000) surface temperature ranged from 9.7 to 27.0 °C whereas salinity ranged from 34.8 to 38.6. Water column was stratified from May to October and vertically mixed from November to April. In several occasions (*i.e.*, in July 1999, May, July and August 2000) at station C4 a strong stratification of the upper 10 m of the water column was observed, likely due to the presence of less saline waters of riverine origin.

The hydrological conditions and the appearance and characteristics of mucilage aggregates during summer 1999 and 2000 are summarized in Table I. Large amorphous mucus aggregates were observed along the water column at all stations from the beginning of June 2000 till the end of August. Generally these aggregates had the consistence of flocs and macroflocs at surface and of large strings at the bottom of the water column.

TABLE I Comparison of Hydrological Characteristics and Mucilage Appearance in Summer 1999 and Summer 2000. Reported are: Temperature, Salinity, Mucilage Presence and Shape.

			Temperature (°C)		*Salinity*		*Pycnocline depth (m)*		*Type of mucilage*	
Month	*Station*	*Depth (m)*	*1999*	*2000*	*1999*	*2000*	*1999*	*2000*	*1999*	*2000*
June	C4	0	21.18	23.50	37.69	37.38	11	10	–	flocs
		10	21.12	21.84	37.72	38.23			–	macroflocs
		55	11.85	12.55	38.21	38.45			–	stringers
	C7	0	21.80	23.74	38.05	37.67	10	10	–	flocs and macroflocs
		10	21.49	23.66	38.04	37.70			–	stringers and clouds
		67	12.53	12.44	38.50	38.50			–	macroflocs and stringers
	C12	0	22.34	23.29	38.08	38.47	10	10	–	–
		10	22.27	23.13	38.05	38.48			–	flocs
		58	13.14	12.84	38.32	38.39			–	macroflocs
July	C4	0	24.95	25.04	35.20	37.11	8	10	–	macroflocs
		10	20.74	24.55	37.20	38.05			–	macroflocs and stringers
		55	12.59	12.78	38.19	38.41			–	flocs
	C7	0	24.05	25.39	38.05	38.00	10	10	–	macroflocs
		10	23.65	23.74	38.01	38.64			–	flocs
		67	12.74	12.31	38.49	38.48			–	stringers and flocs
	C12	0	24.28	24.74	37.92	38.45	10	10	–	stringers
		10	23.46	24.43	38.00	38.46			–	flocs
		58	13.82	13.36	38.44	38.45			–	stringers
August	C4	0	25.40	23.40	37.90	37.38	8	11	–	–
		10	22.38	23.01	38.09	38.02			–	flocs
		55	13.50	12.90	38.47	38.33			–	flocs
	C7	0	26.15	23.45	37.49	38.47	7	20	–	flocs
		10	18.29	23.22	38.38	38.47			–	flocs and macroflocs
		67	12.89	13.05	38.49	38.51			–	flocs
	C12	0	25.60	23.11	37.99	38.44	8	20	–	flocs and macroflocs
		10	23.42	23.06	37.84	38.44			–	stringers
		58	14.30	13.88	38.54	38.49			–	stringers and macroflocs
September	C4	0	23.41	27.08	37.65	36.88	10	11	–	flocs
		10	23.19	25.40	37.82	38.38			–	macroflocs and stringers
		55	14.21	12.99	38.49	38.29			–	stringers
	C7	0	22.37	27.06	38.08	38.44	10	15	–	–
		10	21.17	25.27	38.15	38.58			–	flocs
		67	12.65	13.56	38.43	38.54			–	flocs
	C12	0	22.87	26.55	38.39	37.33	10	11	–	flocs
		10	22.47	24.23	38.39	38.08			–	flocs
		58	13.82	14.51	38.50	38.59			–	macroflocs and stringers
Mean			19.61	20.53	38.03	38.19				

3.2 Picoplankton Assemblages

During the study period along the transect, picophytoplankton (PPP, *i.e.*, prokaryotic picophytoplankton plus eukaryotic picophytoplankton) abundance ranged from 0.19 to 10.60×10^7 cells l^{-1} with a mean value of 2.23×10^7 cells l^{-1}. Picophytoplankton biomass ranged from 0.76 to 31.32 μgC l^{-1} with a mean value of 6.69 μgC l^{-1} (data not shown). At all stations, prokaryotic picophytoplankton (*i.e.*, chroococcoid cyanobacteria of the *Synechococcus*-type; mean values: 2.52, 1.87 and 2.1 $\times$ 10^7 cells l^{-1} at station C4, C7 and C12, respectively) outnumbered eukaryotic picophytoplankton (mean values: 0.94, 0.71 and 0.70×10^6 cells l^{-1} at station C4, C7 and C12, respectively, data not shown) by more than one order of magnitude. The ratio of eukaryotic picophytoplankton to prokaryotic picophytoplankton was, on average, double at stations C7 and C12 (eukaryotic PPP: prokaryotic PPP = 0.9) compared to station C4 (eukaryotic PPP: prokaryotic PPP = 0.5). During the entire sampling period, prokaryotic picophytoplankton and eukaryotic picophytoplankton abundance and the eukaryotic picophytoplankton to prokaryotic picophytoplankton ratio did not show significant differences among stations (ANOVA, ns).

Temporal and spatial changes of prokaryotic picophytoplankton abundance are reported in Figure 2. Prokaryotic picophytoplankton showed similar temporal and vertical patterns at all stations, with lower values in winter and higher values in summer 2000 (especially between June and July). Highest prokaryotic picophytoplankton densities have been observed at beginning of July 2000, in the deeper layers of the water column (*i.e.*, under the pycnocline, 11.0, 7.3 and 9.0×10^7 cells l^{-1} at Stations C4, C7 and C12, respectively). Conversely, during summer 1999 highest values have been observed in the surface layer, even under stratified conditions.

Heterotrophic pico-eukaryotes (HPE) displayed low densities at all sampling periods (range: 0.32–474×10^4 cells l^{-1}; mean value: 49.6×10^4 cells l^{-1}) except in July and August 2000, when HPE abundance reached values up to 10^6 cells l^{-1} (Fig. 3). Heterotrophic picoeukaryotes abundance did not show significant differences among stations (ANOVA, ns).

3.3 Nanoplankton Assemblages

Total nanoplankton (as phototrophic plus heterotrophic nanoplankton) ranged from 2.0 to 49.7×10^5 cells l^{-1}, with a mean value of 15.2×10^5 cells l^{-1}. Nanoplankton carbon biomass ranged from 3.4 to 107.4 μgC l^{-1}, with a mean value of 14.8 μgC l^{-1}. On average, phototrophic nanoplankton accounted for 91% of total nanoplankton density and 80% of its biomass. The ratio of phototrophic to heterotrophic nanoplankton abundance (PNAN:HNAN) ranged from 2.1 to 218.5 (at Station C7, 67 m, April 2000 and at Station C4, 0 m, June 1999, respectively; data not shown) with a mean value of 21.5.

3.4 Phototrophic Nanoplankton

Phototrophic nanoplankton ranged from 1.8 to 45.7×10^5 cells l^{-1} (station C7, 10 m, August 2000 and station C4, May 2000 respectively) with a mean value of 14.0×10^5 cells l^{-1}. The mean PNAN abundance was similar at all stations (Tab. II). All autotrophic nanoplankton size classes considered here (*i.e.*, 2–3 μm, 3–5 μm, 5–20 μm) were, on average, more abundant on the western Adriatic side (Station C4) than on the Center and Eastern Coast (Stations C7 and C12), but their relative importance was quite similar at all stations. PNAN 2–3 μm in size was dominated by prasinophytes (on average, 57–61% of PNAN abundance). Cells 3–5 μm in size were dominated by primnesiophyte (26–29%) and cells 5–20 μm in size

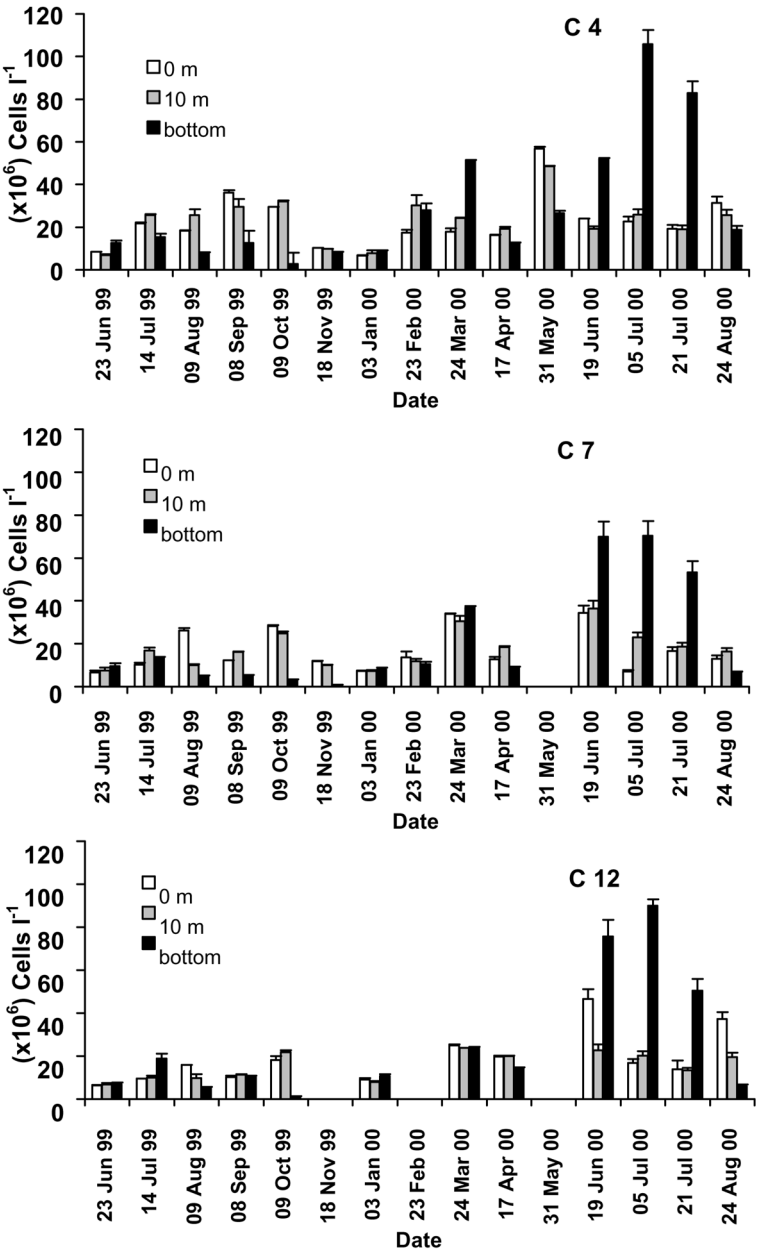

FIGURE 2 Temporal and spatial changes in prokaryotic picophytoplankton (cyanobacteria) abundance at the three sampling stations. Bars indicate standard deviation.

were largely accounted by cryptophytes and small diatoms (11–15%; Tab. II). During the period of investigation no significant differences were found among stations (ANOVA, ns).

Temporal and spatial changes in abundance and size structure of nanophytoplankton are reported in Figures 4 and 5, respectively. Temporal changes in PNAN abundance were evident at station C4, which displayed highest densities at the end of May 2000 in correspondence to a sharp halocline (10 m) due to the presence of low saline waters (35) and lowest

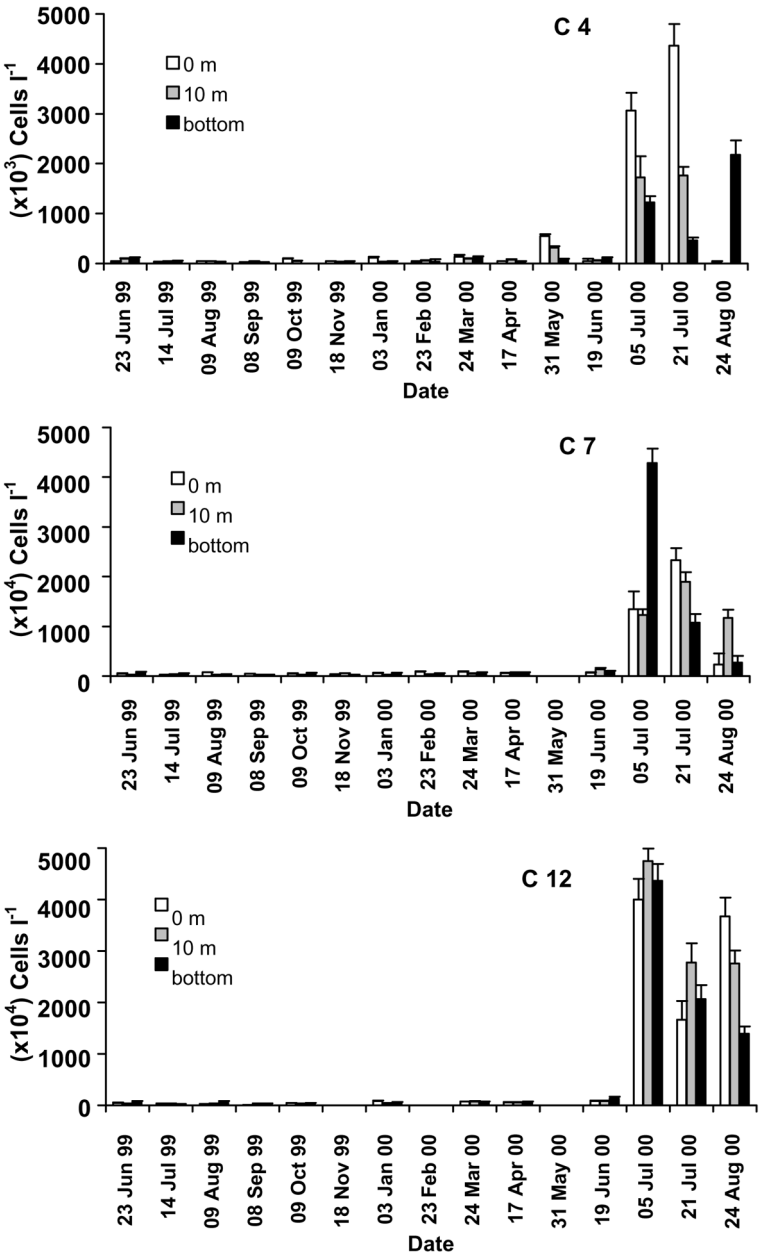

FIGURE 3 Temporal and spatial changes in heterotrophic pico-eukaryotes abundance at the three sampling stations. Bars indicate standard deviation.

values in November 99 and August 2000 after mucilage event. Conversely temporal changes were much less evident at Station C7 and C12.

The analysis of the vertical distribution of PNAN revealed, in summer 1999, highest densities in deeper layers of the water column (when no mucilage appeared), whereas, in spring and summer 2000 (when mucilage appeared), highest values were observed in the surface water layer (0–10 m). Comparing the two summer periods, evident changes were also

TABLE II Range and Average Abundance and Biomass of Phototrophic Nanoplankton at the Three Sampling Stations During the Investigation Period.

	PNAN (10^5 l^{-1})		*2–3 μm PNAN (10^5 l^{-1})*		*3–5 μm PNAN (10^5 l^{-1})*		*5–20 μm PNAN (10^5 l^{-1})*		*2–3 μm PNAN (%)*		*3–5 μm PNAN (%)*		*5–20 μm PNAN (%)*	
Stations	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*
C4	3.9–45.7	16.1	0.8–27.4	9.2	1.3–14.2	4.5	0.4–20.6	2.4	20.0–86.0	57.1	11.9–60.9	28.5	2.2–53.8	14.4
C7	1.8–23.5	12.9	0.4–17.1	7.8	0.3–7.1	3.4	0.3–4.6	1.7	20.5–87.2	57.7	5.4–59.1	28.3	3.6–30.1	14.0
C12	3.0–22.1	12.6	1.1–17.1	7.5	0.7–8.4	3.7	0.2–3.8	1.4	25.3–80.8	58.7	11.6–56.2	29.4	1.7–29.3	11.8
Entire data set	1.8–45.7	14.0	0.4–27.4	8.2	0.3–14.2	3.9	0.2–20.6	1.9	20.0–87.2	57.8	5.4–60.9	28.7	1.7–53.8	13.5
	PNAN (μg C l^{-1})		*2–3 μm PNAN (μg C l^{-1})*		*3–5 μm PNAN (μg C l^{-1})*		*5–20 μm PNAN (μg C l^{-1})*		*2–3 μm PNAN (%)*		*3–5 μm PNAN (%)*		*5–20 μm PNAN (%)*	
Stations	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*
C4	2.8–104.0	14.2	0.1–6.1	1.5	0.6–11.2	2.7	0.9–98.3	9.9	1.1–45.4	15.9	4.4–48.6	25.3	20.1–94.5	58.8
C7	1.2–38.4	10.3	0.0–2.4	1.1	0.0–4.6	1.9	0.4–36.5	7.4	1.7–51.3	14.3	0.0–44.8	21.1	24.9–95.1	64.6
C12	2.4–30.7	11.3	0.2–2.0	1.1	0.3–4.7	2.0	0.6–26.9	8.2	2.0–44.6	13.7	1.7–47.6	22.1	15.6–95.0	64.1
Entire data set	1.2–104.0	12.0	0.0–6.1	1.2	0.0–11.2	2.2	0.4–98.3	8.5	1.1–51.3	14.7	0.0–48.6	22.9	15.6–95.1	62.4

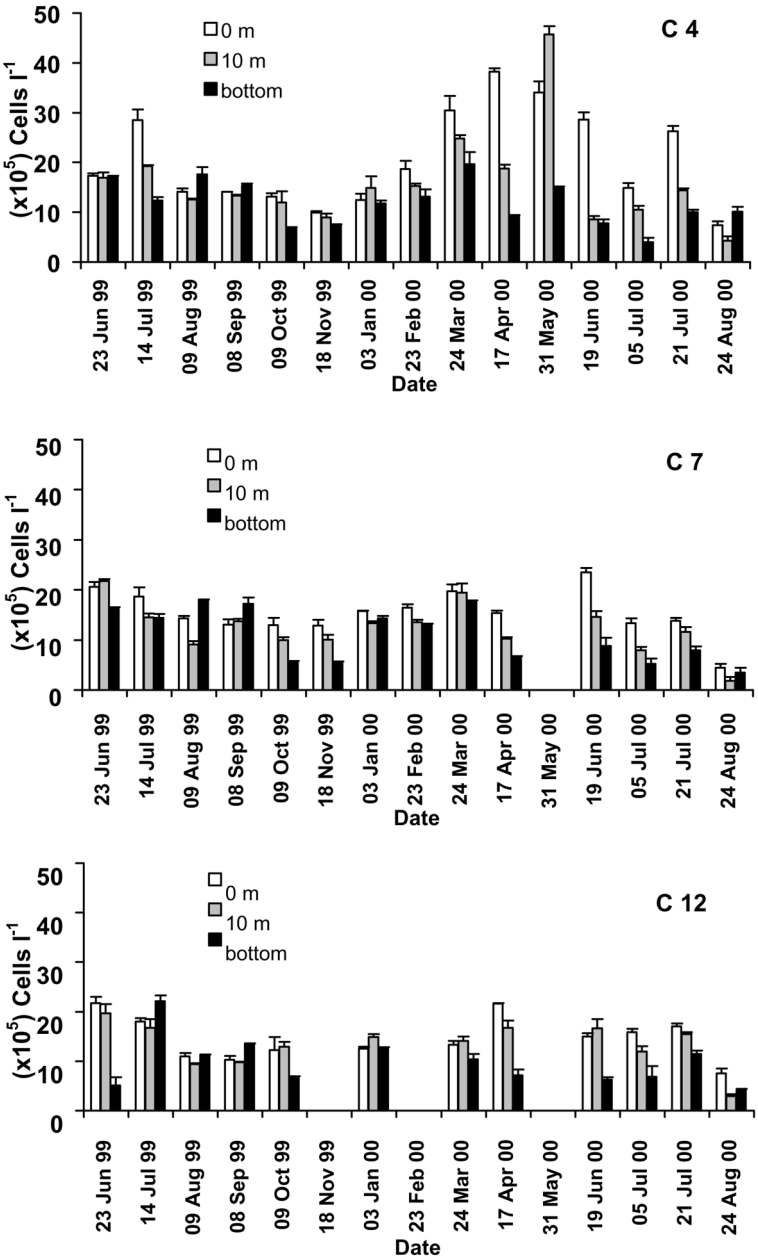

FIGURE 4 Temporal and spatial changes in phototrophic nanoplankton abundance at the three sampling stations. Bars indicate standard deviation.

observed in terms of abundance and PNAN structure (Fig. 5). In July 2000, during the mucilage event, the abundance of 2–3 μm sized PNAN decreased significantly compared to July 1999. Moreover, in summer 2000, PNAN was dominated primnesiophyte (3–5 μm in size accounting for 50–60% of total PNAN abundance), whereas, in summer 1999, PNAN was dominated by prasinophyte (2–3 μm in size). Such shift in size was associated also with the presence of thin diatoms, such as *Cylindrotheca closterium*.

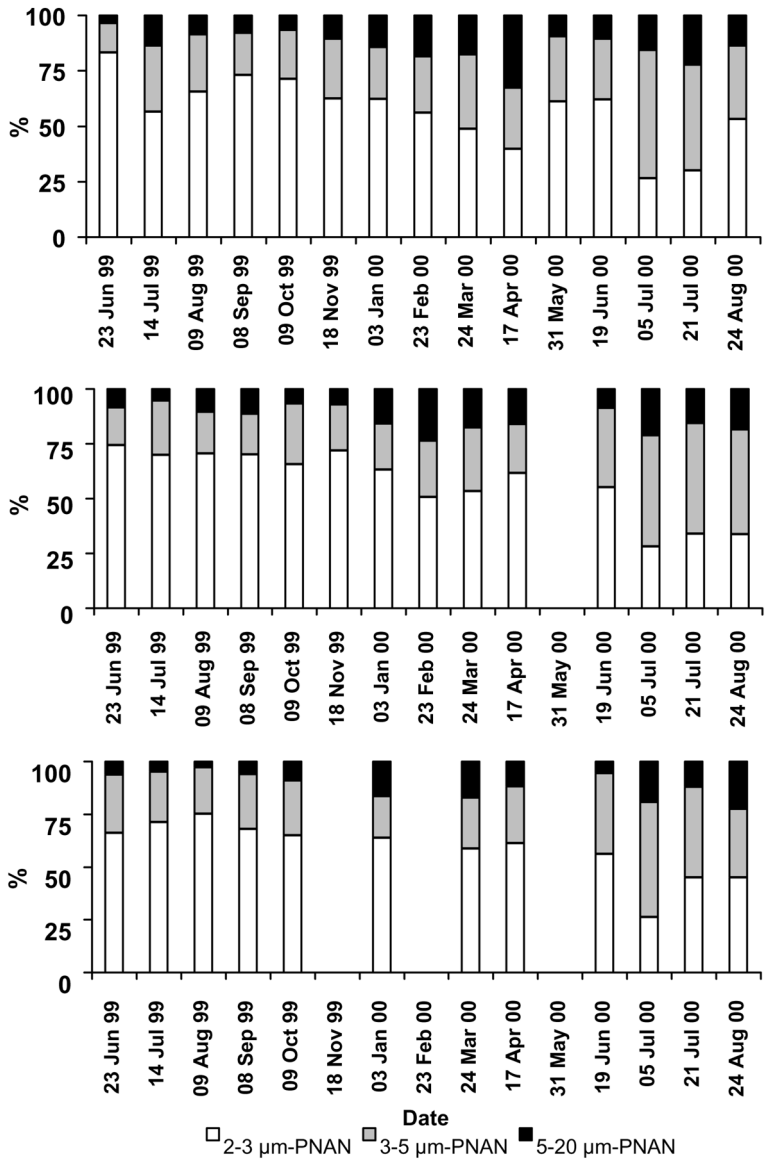

FIGURE 5 Temporal and spatial changes in phototrophic nanoplankton assemblage structure at the three sampling stations (integrated values of the three sampling depths).

PNAN biomass ranged from 1.2 to 104.0 $\mu gC\,l^{-1}$ (Station C12, August 2000 and Station C4, March 1999) with a mean value of 12.0 $\mu gC\,l^{-1}$ (Tab. II). Highest biomass values were largely closely related with the distribution of the 5–20 µm PNAN.

3.5 Heterotrophic Nanoplankton

Heterotrophic nanoplankton (HNAN) abundance ranged from 0.08 to 4.05×10^5 cells l^{-1} (Station C4 0 m, June 1999 and May 2000 respectively) with a mean value of 1.17×10^5 cells l^{-1}. HNAN abundance and the size structure were similar at all stations (Tab. III).

TABLE III Range and Average Abundance and Biomass of Heterotrophic Nanoplankton at the Three Sampling Stations During the Investigation Period.

	HNAN (10^5 l^{-1})		*2–3 μm HNAN (10^5 l^{-1})*		*3–5 μm HNAN (10^5 l^{-1})*		*5–20 μm HNAN (10^5 l^{-1})*		*3–5 μm HNAN (%)*		*5–20 μm HNAN (%)*		*5–20 μm (%)*	
Stations	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*
C4	0.08–4.05	1.23	0.00–1.13	0.34	0.00–1.16	0.45	0.08–2.52	0.44	1.20–52.94	24.89	5.26–67.33	36.06	15.27–76.85	39.05
C7	0.16–3.20	1.16	0.00–1.84	0.40	0.02–0.96	0.40	0.13–1.47	0.36	0.06–57.27	29.40	10.00–58.95	33.28	12.85–87.70	37.32
C12	0.17–2.99	1.09	0.01–1.46	0.36	0.00–1.17	0.37	0.07–0.98	0.35	2.66–56.50	29.70	13.79–57.50	35.13	11.91–75.44	35.17
Entire data set	0.08–4.05	1.17	0.00–1.84	0.37	0.00–1.17	0.41	0.07–2.52	0.39	0.06–57.27	27.74	5.26–67.33	34.88	11.91–87.70	37.38
	HNAN (μg C l^{-1})		*2–3 μm HNAN (μg C l^{-1})*		*3–5 μm HNAN (μg C l^{-1})*		*5–20 μm HNAN (μg C l^{-1})*		*3–5 μm HNAN (%)*		*5–20 μm HNAN (%)*		*5–20 μm (%)*	
Stations	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*
C4	0.48–12.35	2.90	0.00–0.21	0.07	0.00–0.82	0.31	0.39–11.64	2.54	0.11–10.74	2.43	1.30–32.91	12.15	65.69–97.64	85.41
C7	0.90–10.72	2.84	0.00–0.33	0.08	0.02–0.58	0.28	0.87–10.12	2.51	0.18–11.24	3.05	1.85–30.76	11.33	66.48–97.56	85.62
C12	0.42–5.72	2.52	0.00–0.28	0.08	0.00–0.75	0.25	0.36–4.94	2.21	0.23–11.58	3.45	3.40–28.43	12.66	60.89–95.31	83.89
Entire data set	0.42–12.35	2.77	0.00–0.33	0.08	0.00–0.82	0.28	0.36–11.64	2.43	0.11–11.58	2.92	1.30–32.91	12.03	60.89–97.64	85.06

HNAN 5–20 µm in size was dominated (43–44%) by heterotrophic cryptophytes (genus *Leukocryptos*) and unarmoured dinoflagellates, followed by HNAN 3–5 µm in size (31–33%), dominated by unidentified flagellates and choanoflagellates. Finally HNAN 2–3 µm in size accounted for 23–25% and were dominated by unidentified flagellates. HNAN abundance and assemblage structure of HNAN did not display significant differences among stations (ANOVA, ns). The ratio of HNAN to PNAN abundance (size 5–20 µm)

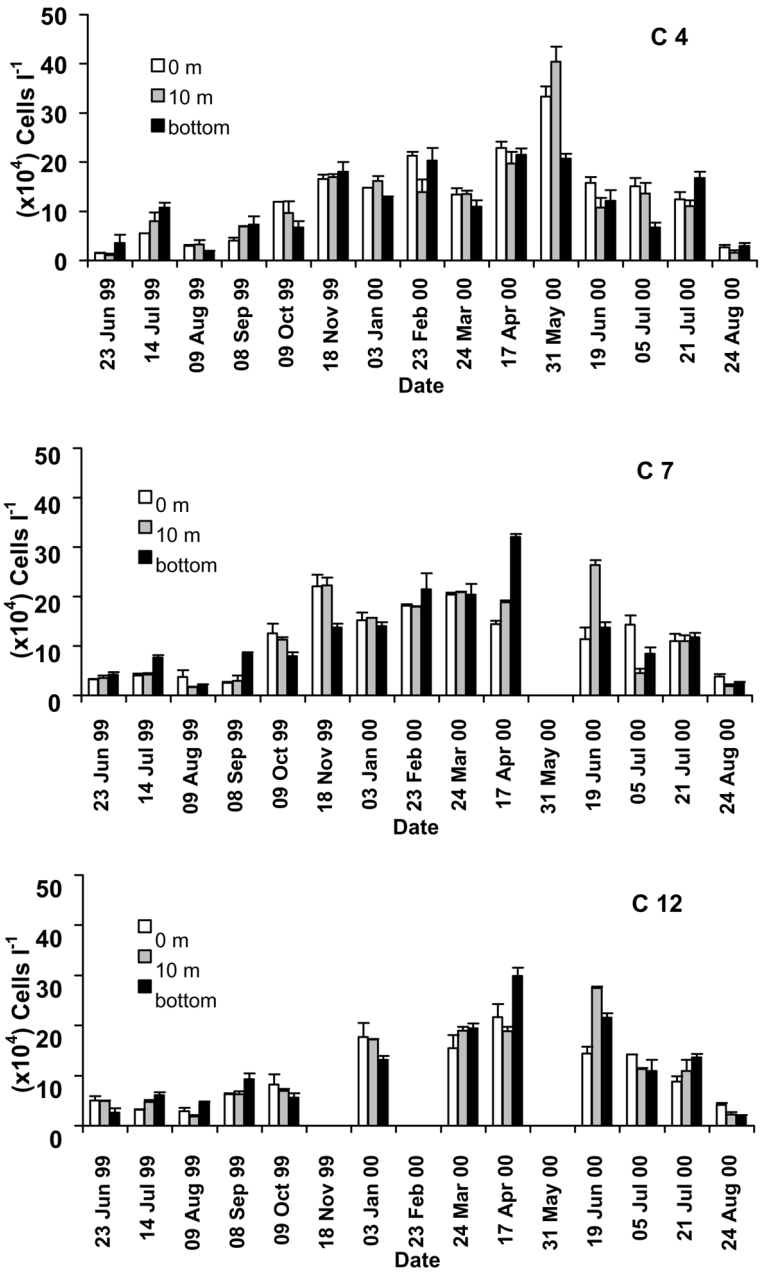

FIGURE 6 Temporal and spatial changes in heterotrophic nanoplankton assemblage abundance at the three sampling stations. Bars indicate standard deviation.

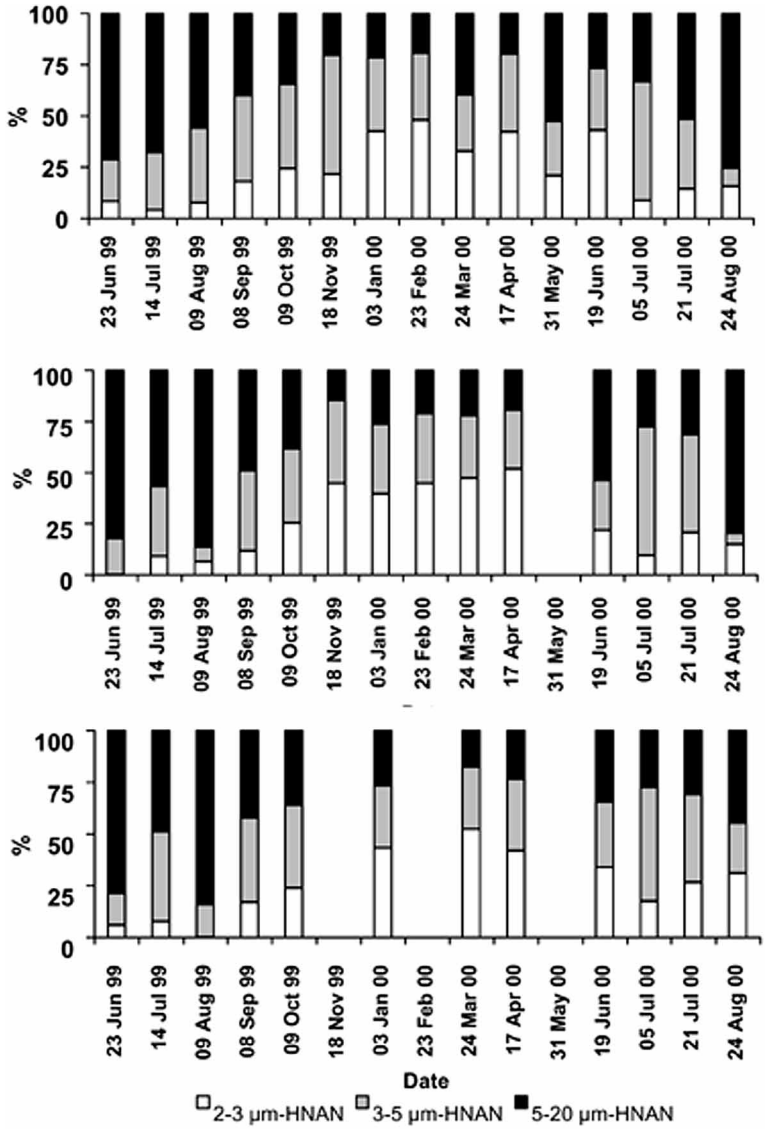

FIGURE 7 Temporal and spatial changes in heterotrophic nanoplankton assemblage structure at the three sampling stations (integrated values of the three sampling depths).

increased eastward and was significantly higher at station C12 than at station C4 (0.26 and 0.18, respectively; ANOVA, $p < 0.05$). No significant differences were found for all other ratios dealing with the different nanoflagellate components (ANOVA, ns).

Temporal and spatial changes of HNAN abundance and their size structure are reported in Figures 6 and 7 respectively. Generally, HNAN showed an irregular vertical distribution especially during summer 2000. The lowest HNAN abundance was observed in June 1999 (at Station C4) and in late August 2000 (at Stations C7 and C12), whereas the highest values were observed in spring 2000 at all stations. A high HNAN abundance was also observed in mid June 2000 at stations C7 and C12.

TABLE IV Results of Statistical Analyses for Testing Differences Among Size Classes of Pico- and Nano-Size Classes at the Three Sampling Stations: Comparison Between Summer 1999 and Summer 2000.

	C4		*C7*		*C12*	
Variable	*t-test*	*p*	*t-test*	*p*	*t-test*	*p*
Prokaryotic picophytoplankton (prokaryotic PPP)	−2.16	0.0416	−2.76	0.0114	−3.14	0.0048
Heterotrophic pico-eukaryotes (HPE)	−2.99	0.0068	−3.26	0.0036	−4.73	0.0001
2–3 μm Phototrophic nanoplankton (2–3 μm PNAN)	3.45	0.0023	4.53	0.0002	3.36	0.0028
3–5 μm Phototrophic nanoplankton (3–5 μm PNAN)	−1.19	0.2460	−1.34	0.1939	−1.25	0.2252
5–20 μm Phototrophic nanoplankton (5–20 μm PNAN)	−1.21	0.2400	0.29	0.7773	−2.91	0.0081
2–3 μm Heterotrophic nanoplankton (2–3 μm HNAN)	−3.23	0.0039	−3.40	0.0025	−3.21	0.0040
3–5 μm Heterotrophic nanoplankton (3–5 μm HNAN)	−2.36	0.0276	−3.20	0.0041	−3.20	0.0040
5–20 μm Heterotrophic nanoplankton (5–20 μm HNAN)	−1.54	0.1387	−1.44	0.1649	−1.59	0.1261
Total phototrophic nanoplankton (PNAN)	1.67	0.1093	3.10	0.0052	1.44	0.1633
Total heterotrophic nanoplankton (HNAN)	−3.07	0.0056	−2.97	0.0070	−3.10	0.0051
Total nanoplankton (PNAN plus HNAN)	1.42	0.1704	2.65	0.0146	1.07	0.2948
PNAN/HNAN	2.62	0.0155	6.16	0.0000	4.59	0.0001
(%) 2–3 μm PNAN/PNAN	4.79	0.0001	6.35	0.0000	4.92	0.0001
(%) 3–5 μm PNAN/PNAN	−4.30	0.0003	−6.38	0.0000	−3.48	0.0021
(%) 5–20 μm PNAN/PNAN	−2.97	0.0070	−2.95	0.0075	−4.46	0.0002
(%) 2–3 μm HNAN/HNAN	−3.34	0.0030	−3.77	0.0010	−3.47	0.0022
(%) 3–5 μm HNAN/HNAN	−0.47	0.6446	−1.72	0.0996	−1.32	0.2007
(%) 5–20 μm HNAN/HNAN	1.97	0.0617	3.15	0.0047	3.18	0.0044
Heterotrophic pico-eukayotes to 5–20 μm HNAN ratio	−2.81	0.0102	−2.92	0.0079	−3.43	0.0024

At all stations, the relative importance of the three size classes changed with time. Generally HNAN 5–20 μm in size dominated from June to early September 1999, whereas HNAN 2–3 μm in size dominated from November 1999 to April 2000. HNAN 3–5 μm in size dominated in summer 2000 and, in this size class, choanoflagellates dominated at all stations (on average 53–60%). HNAN biomass ranged from 0.42 to 12.35 $\mu gC\,l^{-1}$, with a mean value of 2.8 $\mu gC\,l^{-1}$ (Tab. III) and spatial patterns of HNAN biomass were dependent upon the distribution of the 5–20 μm size fraction.

3.6 Comparison Between Summer 1999 and Summer 2000

The analysis of changes occurred between summer 1999 and summer 2000 was performed comparing two sampling periods: (i) from June 23, 1999 to September 8, 1999 and (ii) from June 19, 2000 to August 24, 2000. At all stations, prokaryotic picophytoplankton abundance in summer 2000 was significant higher (on average 2–3 times) than in summer 1999 (Tab. IV). At all stations, in summer 2000, heterotrophic pico-eukaryotes abundance was significantly (two orders of magnitude) higher than in summer 1999 (Tab. IV). Conversely, the abundance of phototrophic nanoplankton 2–3 μm in size in summer 2000 was significantly lower than in summer 1999. No significant differences were observed comparing the 3–5 μm fraction of the two sampling periods. Finally at station C12 phototrophic

TABLE V Comparison of Nanoplankton Abundance from Different Locations.

Area	*Phototrophic nanoplankton ($10^5 \times$ Cells l^{-1})*	*Heterotrophic nanoplankton ($10^5 \times$ Cells l^{-1})*	*Mixotrophic nanoplankton ($10^5 \times$ Cells l^{-1})*	*Source*
Antarctica (Scotia-Weddell Sea)	<10.0–80.0	na	na	Bak *et al.*, (1992)
Southern Ocean (Atlantic Sector)	1.6–24.9	na	na	Buma *et al.*, (1990)
WN Pacific Ocean	na	<5–56.0	na	Lee *et al.*, (2001)
Tropical Pacific Ocean	na	7.0 (mean value)	5.0 (mean value)	Chavez *et al.*, (1996)
NE Pacific Coast	na	0.3–67.0	na	Tanaka *et al.*, (1997)
Sargasso Sea	na	1.1–6.2	0–2.9	Arenovski *et al.*, (1995)
Sargasso Sea	1.0–10.0	1.0–10.0	na	Caron *et al.*, (1999)
NW Atlantic Ocean	0.2–12.3	0.3–3.3	0.2–8.1	Lovejoy *et al.*, (2000)
Atlantic Ocean (Cape Hatteras)	10.0–70.0	10.0–30.0	na	Verity *et al.*, (1996)
Arabian Sea	na	0.15–8.5	na	Stelfox *et al.*, (1999)
Arabian Sea	1.9–8.3	1.67–4.10	na	Dennet *et al.*, (1999)
NW Mediterranean Sea	9.0–55.0	10.0–40.0	na	Klein *et al.*, (1997)
W Mediterranean Coast	na	8.2 (mean value)	na	Del Giorgio *et al.*, (1996)
E Mediterranean Sea	5.3–12.8	2.5–14.6	0.3–1.3	Christaki *et al.*, (1999)
Northern Adriatic Sea	>0.0–19.8	na	na	Fonda Umani *et al.*, (1999)
Adriatic Sea	1.8–45.7	0.1–4.0	na	This work

Note: na = data not available.

nanoplankton 5–20 μm in size in summer 2000 was significantly higher than in summer 1999 (Tab. IV).

In summer 2000, at all stations, the relevance of the small sized (2–3 μm) phototrophic nanoplankton was significantly lower than in summer 1999 (40–44 *vs.* 68–71%, respectively), whereas the importance of the fraction 3–5 μm was significantly higher (40–45 *vs.* 20–26%; Tab. IV). Finally the contribution of the abundance of 5–20 μm phototrophic nanoplankton increased significantly between the two summer periods. Conversely, at all stations, both abundance and relative importance of the 2–3 and 3–5 μm size classes of HNAN assemblages increased significantly in summer 2000 compared to summer 1999 (Tab. IV), but the contribution of the 5–20 μm fraction decreased significantly.

Overall, the average size of phototrophic nanoplankton increased from summer 1999 to summer 2000, whereas the average size of heterotrophic nanoplankton decreased.

4 DISCUSSION

4.1 Characteristics of Pico- and Nanoplankton Assemblages in the Adriatic Sea

Phototrophic nanoplankton abundance and biomass observed in this study are close to values previously reported for the northern Adriatic, Atlantic Ocean (*i.e.*, Gulf of St. Lawrence and

offshore Nova Scotia) and in a highly productive sector of the Arabian Sea, but are 2–4 times higher than those reported in the highly oligotrophic Eastern Mediterranean Sea (Tab. V, Verity *et al.*, 1996; Caron *et al.*, 1999; Dennett *et al.*, 1999, Lovejoy *et al.*, 2000; Christaki *et al.*, 1999 for biomass values).

The structure of nanoplankton assemblages, as well as the ratio of phototrophic to heterotrophic nanoplankton, were completely different from those reported in previous studies carried out in the Mediterranean Sea (Klein *et al.*, 1997; Christaki *et al.*, 1999). We found that, during the entire sampling period, nanoplankton assemblages were largely dominated by phototrophs, and that the heterotrophic component never exceeded 33%. Conversely, both in the northwestern Mediterranean Sea and in the Aegean Sea phototrophs were dominant in spring, but heterotrophs dominated during summer, when a strong stratification of the water column was observed (reaching densities up to 10^6 cells l^{-1}, Klein *et al.*, 1997; Christaki *et al.*, 1999; Mihalatou and Moustaka-Gouni, 2002; Siokou-Frangou *et al.*, 2002).

Lovejoy *et al.* (2000) found that mixotrophic plus phototrophic nanoplankton exceeded heterotrophic nanoplankton in spring and summer. They used the term mixotroph in a broad sense that includes species ranging from those that are predominantly photosynthetic, but rely on osmotrophic uptake of organic compounds, to those that are predominantly phagotrophic, but capable of photosynthesis (*i.e.*, chrysophytes, euglenoids, primnesiophytes, dinoflagellates rhaphidophytes, non-scaly prasinophytes; Jones, 1994; Raven, 1997). According to this criterion, we found that mixotrophs (*sensu* Lovejoy *et al.*, 2000) accounted on average for 68% of total phototrophic nanoplankton abundance. In this regard, Lovejoy *et al.* (2000) defined a conceptual model including four nanoplankton domains in relation with environmental and bacterial variables. According to this model, the strong relevance of mixotrophs within the nanoplankton assemblage indicates the presence of low nutrient concentrations and high irradiance levels. These conditions, strengthened by the relevance of mixotrophs among phototrophic nanoplankton, have been reported in the Adriatic Sea during the period of investigation, and were coupled with very low primary production values ($\sim$56 gC m^{-2} y^{-1}; Hopkins, 1999). In these conditions, low densities of heterotrophic nanoplankton were found, and is consistent with the conceptual model proposed by Lovejoy *et al.* (2000).

Despite no information is available in the Adriatic Sea for comparison, the strong dominance of mixotrophs in this system is intriguing and could indicate an opportunist behavior of nanoplankton assemblages able to acquire major nutrients (nitrogen and phosphorus) by phagotrophy (Arenovski *et al.*, 1995).

4.2 Mucilage Aggregates and Free-Living Microbial Assemblages: A Comparison Between Summer 1999 and 2000

Pico- and nanoplankton assemblages displayed significant differences when summer 1999 and summer 2000 (*i.e.*, when mucilage were present) are compared. The main differences can be summarized as follows: (i) the presence of major cyanobacterial blooms in summer 2000 (absent in summer 1999); (ii) an increasing fraction of heterotrophic pico-eukaryotes and heterotrophic nanoplankton (size 2–5 μm) during mucilage event; (iii) a reduced abundance of small sized (2–3 μm) phototrophic nanoplankton in summer 2000 (Tab. IV).

Summer cyanobacterial blooms of the same order of magnitude of those reported in the northern Adriatic Sea have been previously observed both during mucilage events (Kaltenböck and Herndl, 1992; Fuks, 1995) and in years without mucilage (Vanucci *et al.*, 1994). Moreover, conversely to what observed in previous studies, in summer 2000 mucilage aggregates were present all along the water column, but cyanobacterial blooms were confined to the bottom layers of the water column (layer 55–67 m over a bottom depth of

70 m). At this depth ca 10% of incidence PAR was still available, and nutrient concentrations were high (Degobbis *et al.*, 2000), so that optimal light and nutrient conditions were present. This suggests that it is not possible to identify a direct link between mucilaginous aggregates and cyanobacterial blooms, although it has been hypothesized that free-living *Synechococcus* cyanobacteria, when present in high concentration in surrounding waters, become easily entrapped into the mucilage matrix. Once cyanobacteria start colonizing the mucilage they display enhanced growth rates and this contribute to DOC excretion (Stachowitch *et al.*, 1990; Kaltenböck and Herndl, 1992; Baldi *et al.*, 1997; Flander *et al.*, 1998).

The appearance of mucilage was associated with a change in the trophic structure of microbial assemblages as, during summer 2000, the ratio of heterotrophic to phototrophic nanoplankton abundance increased dramatically. The high abundance of heterotrophic pico-eukaryotes and of small sized heterotrophic nanoflagellates observed during mucilage event is another clear sign of a shift in the trophic structure of the microbial assemblage. Changes in community structure are signals of changes in the trophic conditions of the systems, and reflect modified trophic pathways, which are able to optimize the exploitation of the available nutrient sources (Legendre and Rassoulzadegan, 1995; Mousseau *et al.*, 1996).

Azam *et al.* (1999) reported extremely high phosphatase activities in mucilage aggregates in the northern Adriatic Sea, and suggested that these mucilage aggregates have to be considered hot-spots of *P* regeneration, able to further sustain carbon fixation, cyanobacterial and heterotrophic growth into the aggregates. Recently, Simon *et al.* (2002) reviewed on microbial ecology of organic aggregates in aquatic ecosystem and pointed out that not only the aggregates but also their surroundings are sites and hot-spots of microbial processes, with the plume of solutes leaking out of the aggregates. Therefore, it is likely that organic molecules are released also during mucilage decomposition, and successfully taken up by heterotrophic and mixotrophic flagellates, thus contributing to the growth of free-living microbial components. This would, at least partially, explain the reason for the increase of small hetero-

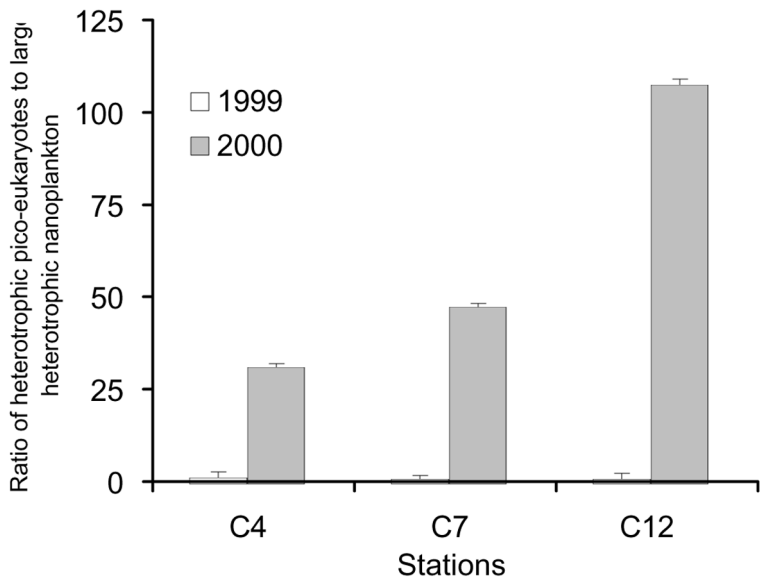

FIGURE 8 Comparison of heterotrophic pico-eukaryotes to 5–20 μm heterotrophic nanoplankton ratio in summer 1999 and summer 2000 at the three sampling stations (mean values and standard error are reported).

trophs and mixotrophs during summer 2000. In fact, during mucilage event in summer 2000, along the transect in ambient waters, β-glucosidase activity, and bacterial *C* production were higher than in summer 1999 (mean values: ~9.9 *vs.* 1.3 nmol l^{-1} h^{-1} and 0.23 *vs.* 0.12 µg C l^{-1} h^{-1} for β-glucosidase activity and bacterial *C* production, respectively; Degobbis *et al.*, 2000). Moreover during summer 2000 mucilage aging was responsible for the fuelling of DOM into the ambient water. This was evident in terms of dissolved protein and carbohydrate concentration (Degobbis *et al.*, 2000).

The size shift towards the smaller size classes displayed by the heterotrophic nanoplankton (*i.e.*, increasingly dominated by cells <5 µm) supports the hypothesis of a competitive advantage of small sized heterotrophs in utilizing the organic molecules released from mucilage and/or grazing bacteria growing on them. Moreover, significant relationships between heterotrophic nanoflagellates and β-glucosidase activity ($n = 36$, $p < 0.01$; data not shown) were observed. Although simple correlation analysis does not allow identifying cause-effect relationships, data presented here support the hypothesis that mucilage appearance could be responsible not only for the formation of specific assemblages into the aggregates, but also for a change in the structure of the microbial loop in ambient water.

Information on heterotrophic pico-eukaryotes is generally scant and data for the Adriatic Sea are lacking. During mucilage event all small sized heterotrophic flagellates (2–3 and 3–5 µm) increased their densities, but the increase displayed by heterotrophic pico-eukaryotes was much more evident. The strong increase of the pico-eukaryote component appears a specific feature of the structure of flagellate assemblages during mucilage event (Fig. 8). Heterotrophic pico-eukaryotes feed directly upon bacterioplankton (which during mucilage degradation notably increased; Degobbis *et al.*, 2000) and are characterized by extremely high growth rates and respond rapidly to any environmental change.

Recently, it has been reported for subtropical waters (Calbet *et al.*, 2001) densities of heterotrophic pico-eukaryotes close to those reported in this study during summer 2000. Calbet *et al.* (2001) showed that heterotrophic flagellates <2 µm (*i.e.*, pico-eukaryotes), heterotrophs 2–3 µm and heterotrophs 3–5 µm represent a well established 3-step predatory chain that significantly influence bacteria growth dynamics. In our study, heterotrophic pico-eukaryotes indicate that the presence of mucilage induced the amplification of a 3-steps microbial food chain (pico-eukaryotes-nanoflagellates 2–3 µm–nanoflagellates 3–5 µm). The only component that apparently did not "profit" of the mucilage event was composed by nanoflagellates 5–20 µm, probably due to their link to the autotrophic component (Sherr and Sherr, 1991; Verity *et al.*, 1996).

Further studies are needed to better understand factors responsible for the increase of heterotrophic pico-eukaryotes in ambient waters, but results reported in this study suggest that the ratio of pico-eukaryote to nanoflagellates 5–20 µm abundance should be taken into account in the future for both detecting changes in microbial food web pathways and for monitoring trophic changes occurring in the ambient water during the presence of mucilage aggregates.

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