water

MDPI

Article

Tracking the spatio-temporal distribution of organic particles to predict macroaggregation in the northern Adriatic Sea

Romina Kraus [1,*] †, and Nadica Ivošević DeNardis [2,*] †

[1] Center for Marine Research, Ruđer Bošković Institute, Giordano Paliaga 5, 52210 Rovinj, Croatia; kraus@cim.irb.hr

[2] Division for Marine and Environmental Research, Ruđer Bošković Institute, Bijenička 54, 10000 Zagreb, Croatia; ivosevic@irb.hr

* Correspondence: kraus@cim.irb.hr, ivosevic@irb.hr

Abstract: In the last two decades, the phenomenon of macroaggregation known as "mucilage events" has become increasingly common in the Mediterranean Sea and beyond, causing severe damage to the marine ecosystem and its associated economy. Interestingly, there have been no mucilage events in the northern Adriatic, where this phenomenon has been recurrent since the 18th century. The aim of this study was to present a unique data set on the spatial and temporal distribution of surface-active organic particles at selected sampling stations in the northern Adriatic Sea, as well as phytoplankton, chlorophyll data, and physical properties of seawater, to study the role of surface-active particles in the mucilage event. We used an electrochemical approach for direct and high throughput characterization of fragile organic matter in terms of its dissolved and particulate state in seawater samples over a three-year period. Results show an increase in surfactant activity and concentration of surface-active particles in subsurface layer and stratified column prior to mucilage event that could be related to very abundant *Skeletonema marinoi*, a diatom characteristic of the winter bloom in the northern Adriatic. This study could contribute to the prediction of macroaggregation under certain environmental and oceanographic conditions, which then leads to the appearance of mucilage in marine systems.

Keywords: Adriatic Sea; adhesion; adsorption; chronoamperometry; diatoms; macroaggregation; organic matter; *Skeletonema marinoi;* surface-active particles

1. Introduction

We are witnessing profound changes in aquatic ecosystems due to multiple and long-lasting human activities that have caused global climate change. Global climate change is altering photosynthetic activities, diversity, and algal community composition, thereby affecting the functionality of aquatic ecosystems [1]. Aquatic semi-enclosed basins such as the Mediterranean Sea, which is warming faster than any other marine region in the world and where more than 600 non-native species have been observed, are particularly at risk [2,3,4].

The Adriatic Sea in the northernmost part of the Mediterranean, a semi-enclosed sea, is severely affected by freshwater runoff from the Po River and overpopulation. Nowadays it also faces the spread of tropical and subtropical non-indigenous species that threaten the marine ecosystem and fisheries [5].

Another serious consequence of human-induced activities and global climate change is the event of mucilage formation, which has become more frequent in the last two decades, spreading over the Mediterranean, i.e., the Tyrrhenian Sea, the Gulf of Thessaloniki in the Aegean Sea, the Bosporus, and the Dardanelles Strait [6,7,8,9,10,11] and beyond, i.e., in the Sea of Marmara and the Bay of Biscay [12,13]. The mucilage formation event causes anoxia and death of benthic species and many marine invertebrates, but also severe

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Lastname

Received: date
Revised: date
Accepted: date
Published: date

economic damage to fisheries and tourism [14]. Interestingly, there have been no mucilage events in the northern Adriatic (NA) in the last 15 years. However, records show that events occurred from 1729 to 2008, with an increased frequency from 1988 to 2008 [12,15,16,17]. Several comprehensive long-term studies have been conducted during that period in the NA to explain the mechanisms and evaluate the processes observed in the field, as well as to assess possible consequences and impacts on marine organisms [18-24]. Diatoms, for example, are sensitive to climate changes, which they cannot easily escape, so they have to deal with environmental changes by altering their cell metabolism. Our recent studies have shown that the adaptive response of algae to stress is species-specific and stressor-dependent. It seems that diatoms are more resistant to changes in temperature and salinity, with the cell wall having an important protective function in contrast to naked flagellates [25,26]. The adaptive response of microalgae exposed to drop in salinity or in temperature is manifested by an increase in cell stiffness, a change in hydrophobicity due to changes at the molecular level. Additionally, *Cylindrotheca* species are known to produce large amounts of extracellular material under nutrient stress in the marine system, which has been associated with the large-scale phenomenon of mucilage formation in the northern Adriatic Sea [27]. Moreover, the organic matter released by this diatom had the same chemical composition of polysaccharides as mucilage, suggesting that the exopolymeric substance of diatoms is the most likely origin of mucilage in the northern Adriatic [27].

For over two decades, we employ direct electrochemical approach to characterize dispersed micrometer-sized surface-active particles (organic droplets, living cells, vesicles, natural particles), flexible gel-like particles, and dissolved surface-active matter in the frame of mesocosm experiments and field studies [24,28-30].

The purpose of this study is to present the unknown data from the period 1998-2000, when a mucilage event occurred in NA region. We electrochemically characterized the organic matter in the form of microparticles and dissolved fractions and determined the phytoplankton community, chlorophyll content, and physical properties of seawater samples at six sampling stations along the Po-Rovinj transect in NA. The results obtained in this case study could contribute to the prediction of macroaggregation under certain environmental and oceanographic conditions, which then leads to the appearance of mucilage in marine ecosystems.

2. Materials and Methods

2.1. Seawater sampling

Sampling was conducted during 32 oceanographic cruises along the Po River Delta (Italy) and Rovinj (Croatia) transect in NA at six sampling stations (SJ108, SJ101, SJ103, SJ105, SJ107, and RV001, Figure 1). The cruises were conducted monthly to bi-monthly from February 1998 to December 2000. Samples for electrochemical and salinity measurements were collected in 5-liter Niskin bottles at 5-6 depths (0 m, 5 m, 10 m, 20 m, 30 m, and 2 m above bottom). Samples for phytoplankton and chlorophyll-*a* measurements were collected in 5-liter Van Dorn bottles at 3-5 depths (0 m, 5 m, 10 m, 20 m, and 2 m above the bottom).

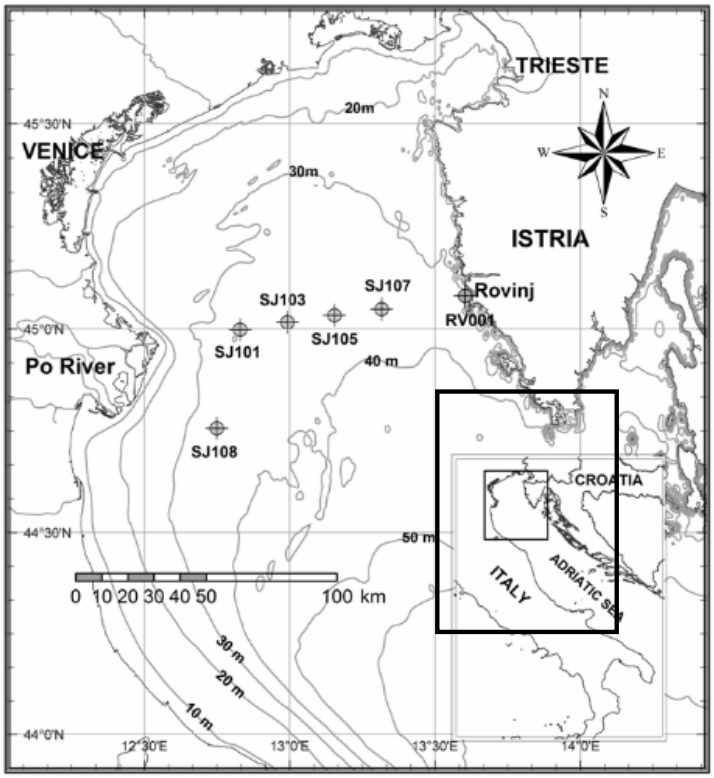

Figure 1. Map of the study area with sampling stations along the Po River Delta-Rovinj transect in the northern Adriatic [31].

2.2. Oceanographic measurements

Temperature was measured with protected reversible thermometers (Richter and Wiese, Berlin, Germany, accuracy ± 0.1°C) attached to 5-liter Niskin bottles. Salinity was measured with high-precision laboratory salinometers (±0.01) in 250 ml seawater subsamples.

Samples for determination of phytoplankton species and chlorophyll-*a* as an indicator of phytoplankton biomass were prefiltered through 300 mm Nybolt nets. Chlorophyll-*a* samples were further filtered through Whatman GF/C filters (1 mm pore size) immediately after collection, and the filters were stored at -20°C until analysis. The content of chlorophyll-*a* in seawater samples was determined by the fluorometric method [32] using a Farrand F-4 fluorometer. The accuracy of the method is 78% and the detection limit is 0.02 mg l^{-1}.

Phytoplankton subsamples of 200 ml were preserved with Lugol's solution (2% final concentration) buffered with sodium acetate. Abundance and composition of phytoplankton were determined at 200× magnification in 100 random fields of view (50, 200, or 400, depending on sample density) after 40 h of sedimentation of a 50 ml subsample with a Zeiss inverted microscope using the Utermöhl-Settling technique [33]. Microphyto-plankton (phytoplankton in the 20-200 μm size range) were determined to the species or genus level, whereas for the smaller (<10 μm) and larger nanophytoplankton (10-20 μm), only abundance was determined.

2.3. Model particles

Laboratory cultures of the marine nanoflagellate *Dunaliella tertiolecta* Butcher (strain CCMP 1320) from the Provascoli-Guillard Center for Culture of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences, were used as model particles. Cells were grown

in natural seawater filtered through a pore size of 0.22 μm and then enriched with the F-2 growth medium [34]. Cells were cultured in a water bath under controlled conditions (constant shaking 20 rpm), 12 hours of light: 12 hours of darkness at an irradiance of 31 μmol photons m^{-2} s^{-1}). The average cell number in triplicate samples was determined using a Fuchs-Rosenthal haemocytometer (Fein-Optik Jena, Germany, depth 0.2 mm) and a light microscope (Olympus BX51, Olympus Corporation, Japan). Cells were harvested at stationary phase (15 days) by centrifugation (2000 g, 3 min), and the washed pellets were resuspended twice with seawater. The final pellet was resuspended in 2 ml of filtered seawater and served as the stock cell suspension. To generate the calibration curves, different aliquots of the stock cell suspension were added to seawater with the selected surfactant activity.

2.4. Electrochemical method

In this study, the electrochemical methods of polarography and chronoamperometry of oxygen reduction at the dropping mercury electrode were used. These electrochemical methods allow direct characterization of organic matter by distinguishing between the adhesion of individual organic particles and the collective adsorption of biopolymers at the charged mercury/aqueous electrolyte interface [23,24]. In short, the adhesion of organic particles to the mercury electrode is registered as a well-defined amperometric signals, while the adsorption of biopolymers is registered as a gradual decrease in current proportional to the biopolymer concentration in the sample [23,35-38].

2.5. Electrochemical measurements

Electrochemical measurements were performed in a standard Methrom vessel (thermostatted to 20 ± 1 °C) containing 15 ml of seawater and three electrode systems. A mercury electrode with the following characteristics served as the working electrode: lifetime 2.0 s, flow rate 6.03 mg s^{-1}, and maximum surface area 4.57 mm^2. An Ag/AgCl electrode served as the reference electrode and a Pt wire served as the counter electrode. The reference electrode was separated from the measured suspension by a ceramic frit, and its potential in 0.1 m NaCl was +2 mV with respect to the calomel electrode (1 M KCl). Electrochemical measurements were performed using a polarographic analyzer PAR 174A (Princeton Applied Research) and a Nicolet 3091 digital oscilloscope connected to PC. The seawater sample was characterized in terms of organic particle concentration and surfactant activity as a measure of dissolved organic fraction. The concentration of organic particles was determined by recording of current-time curves across 50 mercury drops at a constant potential of -400 mV (time resolution 50 s). Signal frequency was expressed as the number of amperometric signals of cells over a 100 s period. The surfactant activity of the sample was measured by adding 0.5 mL of 0.1 M $HgCl_2$ to the seawater before the measurement and then recording the polarograms of Hg (II) reduction (current-potential curves). The surfactant activity of the seawater is expressed in mg l^{-1}, which corresponds to the amount of the nonionic synthetic surfactant Triton-X-100, M=600.

3. Results

The determination of surface-active particles (SAP) in the seawater sample could be hampered by the dissolved organic matter content, as their adsorption is faster from particle adhesion on the interface. Therefore, we constructed two calibration curves showing the dependence of the amperometric signal frequency by varying the cell density of *D. tertiolecta* in seawater samples with surfactant activity (SA) of 1.2 mg l^{-1} or 2. 0 mg l^{-1} expressed in equivalents of Triton-X-100 (Figure 2). These calibration curves were used to determine the concentration of surface-active particles in seawater samples.

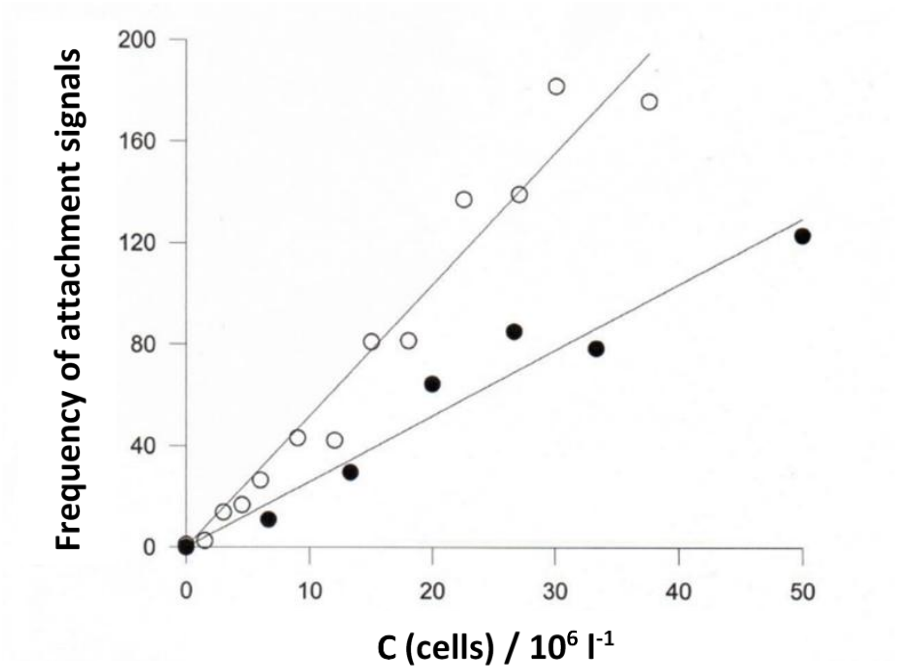

Figure 2. Calibration curves: dependence of the frequency of attachment signals on the cell abundance in seawater with a surfactant activity of 1.2 (○) and 2.0 mg l^{-1} (●).

Surface-active particles are organic micrometer-sized particles (3-500 μm) produced by phytoplankton activity, exudation and organic matter transformation that tend to accumulate in the interfaces in water column (thermocline, halocline, pycnocline). Figure 3. shows the spatio-temporal variation of the concentration of SAP in seawater over a three-year period measured at six selected stations and at 5-6 depths. In general, the average concentration of SAP is twice as high up to 10 m and decreases towards the bottom. On average, the concentration of SAP varied more in the upper part of the water column than in the lower part of the water column. Minimum and maximum SAP concentrations are 0.2 to 20.5x10^6 l^{-1}, respectively. Moreover, the concentrations of SAP were higher at the western sampling stations (SJ108, SJ101 and SJ103) than at the eastern sampling stations (SJ107, RV001), probably related to the inflow from the Po River Delta. The distribution of the concentration of SAP on western sampling stations was usually higher in spring/summer and early fall, probably because of increased abundance of phytoplankton and favorable growing temperature. Figure 4 provides an overview of phytoplankton abundance, chlorophyll-*a* concentration, temperature, and salinity at the selected sampling stations during the three-year period. Chlorophyll-*a* and phytoplankton followed the seasonal pattern in each year and at all stations. The pattern was most pronounced at western stations SJ108 and SJ101. Large peaks were observed at these stations in spring, spring-summer, and fall. Chlorophyll-*a* and phytoplankton maxima occurred predominantly in the surface layer and decreased from the western stations (SJ108 and SJ101), known to be eutrophic, to the easternmost, RV001, known to be oligotrophic. Interestingly, the maximum and highest average values of chlorophyll-*a* and phytoplankton were recorded at stations SJ108 and SJ101 in the spring of 1998 and 1999, respectively.

Temperature followed the same seasonal pattern at all stations, varying from 6.45°C (SJ108, 5 Jan 2000, 0 m) to 29.15°C (SJ101, 25 Aug 2000, 0 m). At the western stations (SJ108, SJ101, and SJ103), a sharp drop in salinity was occasionally observed in spring (May-June) and fall (October), probably due to the intense freshwater outflow periods of the Po River. In the upper part of the water column, the extreme salinity minimum of 8.17‰ was observed at the surface of SJ108 on 25.10.2000. and the maximum of 38.42‰ in 0-10 m layer

at SJ107 on 18.12.1999. Moreover, the variations of salinity in the eastern region were not pronounced during the three-year period.

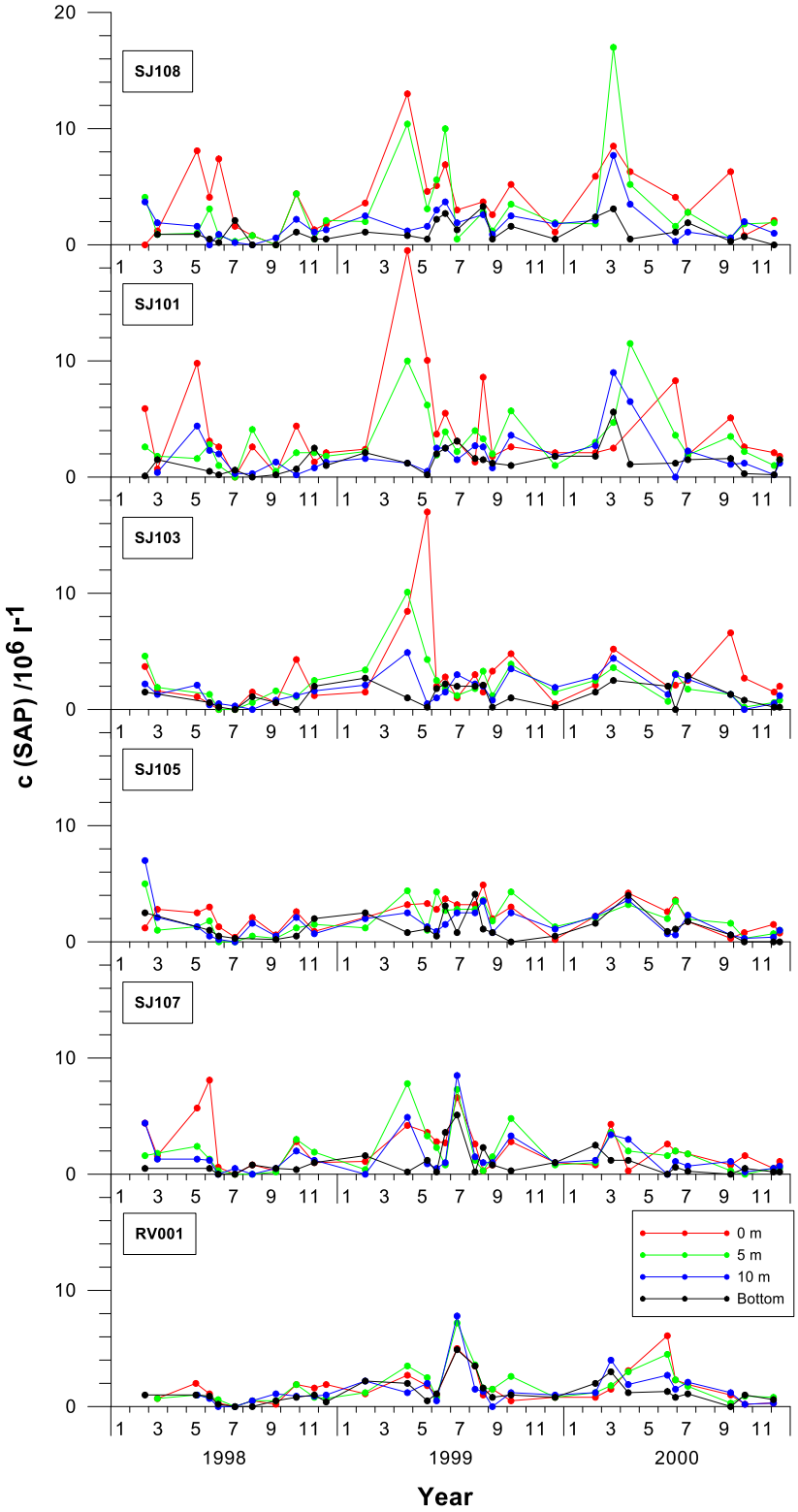

Figure 3. Spatio-temporal distribution of SAP concentration (10^6 l^{-1}) at the six stations of the Po River delta-Rovinj transect at 0 m, 5 m, 10 m and at the bottom in the period from 1998 to 2000.

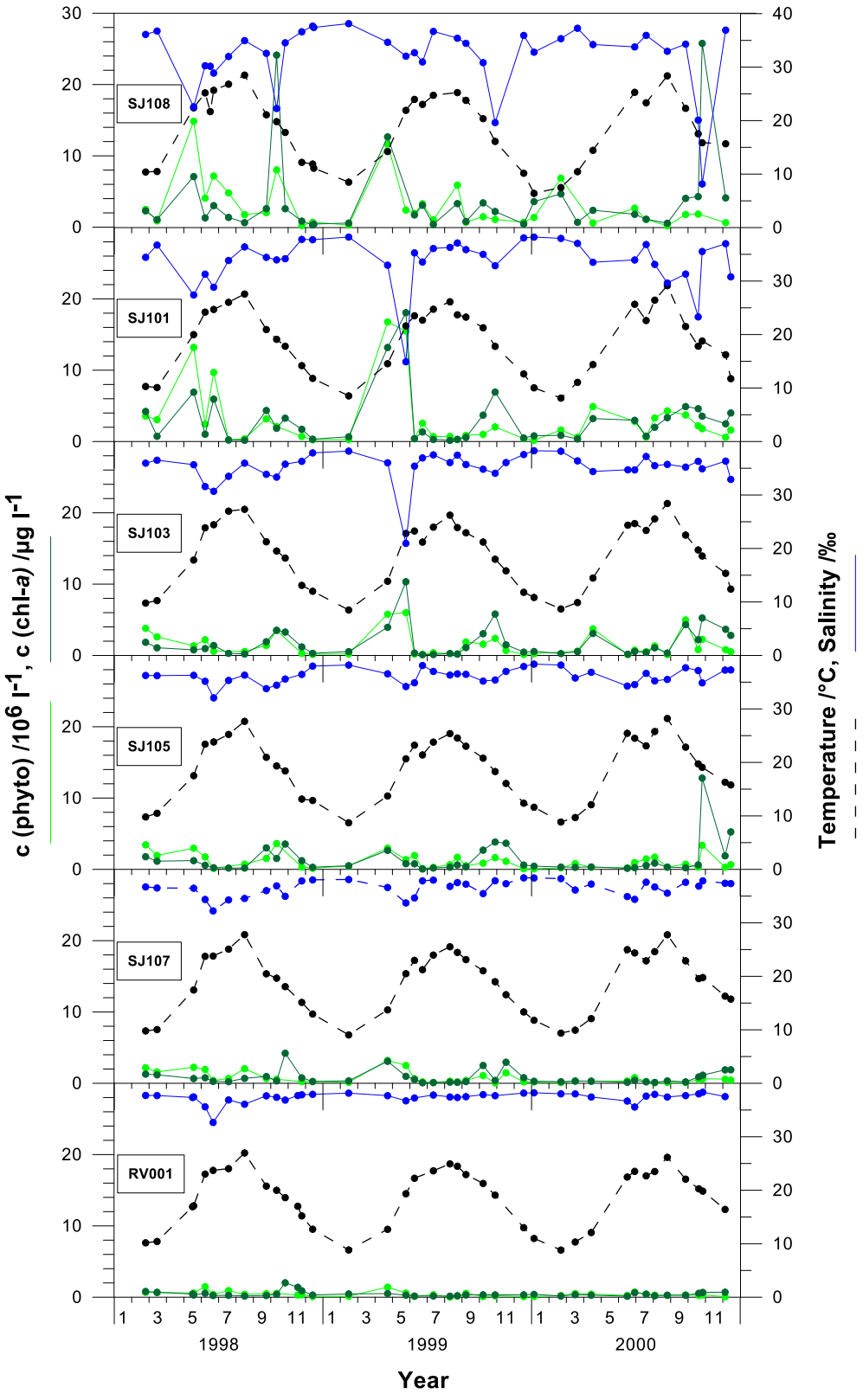

Figure 4. Spatio-temporal distribution of phytoplankton (10^6 l^{-1}), chlorophyll-*a* (µg l^{-1}), temperature (°C) and salinity (‰) at the six stations of the Po River delta-Rovinj transect at 0 m, 5 m, 10 m and at the bottom in the period from 1998 to 2000.

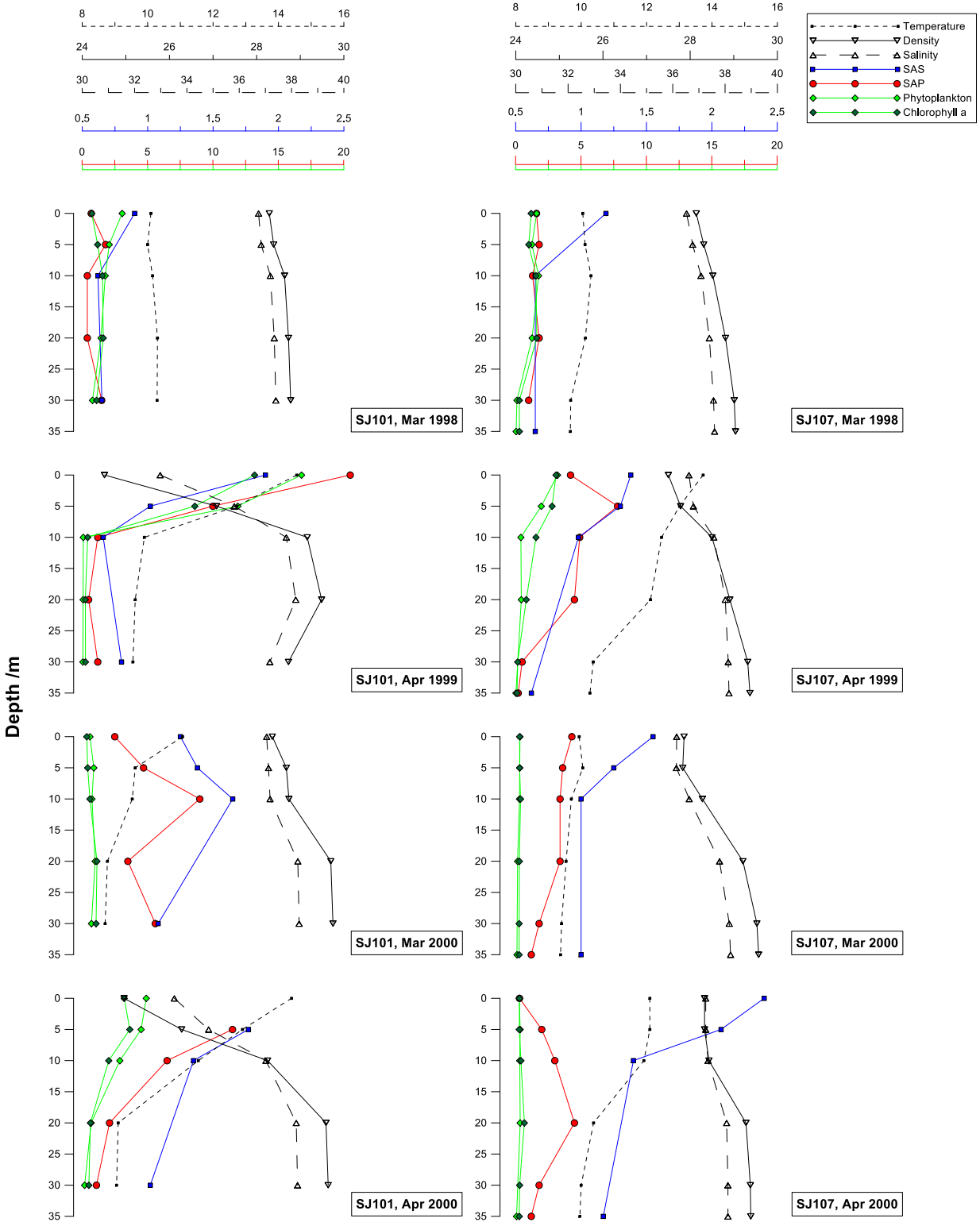

Figure 5. Vertical distribution of SAP (10^6 l^{-1}), SA (mg l^{-1}), phytoplankton (10^6 l^{-1}), chlorophyll-*a* (μg l^{-1}), temperature (°C), density (kg m^{-3}), and salinity (‰) at stations SJ101 and SJ107 in March 1998, April 1999, March 2000, and April 2000.

Next, we compare the determined parameters in the years without the event (1998, 1999) and with the event (2000) using two selected stations, in the western part SJ101 and in the eastern part SJ107 of the northern Adriatic (Figure 5).

In March 1998, the water column at SJ101 and SJ107 was mostly mixed; temperature was about 9 °C, and chlorophyll-*a* and phytoplankton were rather low (Figure 5). SAP and SA generally showed the same distribution as the biotic factors, and only SA at SJ107, 0m was 1.5 mg l^{-1} and from 5 m to the bottom was 0.5 mg l^{-1}.

In April 1999, very high levels of phytoplankton, chlorophyll-*a*, SAP, and SA were observed at SJ101 at 0 m and 5 m at the sharp thermocline, halocline, and pycnocline. At the surface, phytoplankton abundance reached 1.68x10^7 cells l^{-1}, chlorophyll-*a* 13.19 μg l^{-1}, SAP 2.1x10^7 l^{-1}, and SA 1.9 mg l^{-1}, while the values from 10 m to the bottom were much lower (1x10^5 cells l^{-1}, 0.4 μg l^{-1}, 1.2x10^6 l^{-1}, and 0.8 mg l^{-1}, respectively). Temperature was 16°C at the surface and about 10°C at 10 m depth. Salinity and density were 32 ‰ and 25 kg m^{-3}, respectively, in the surface layer and 39‰ and 29 kg m^{-3}, respectively, from 10 m to the bottom. For SJ107, those gradients were less pronounced.

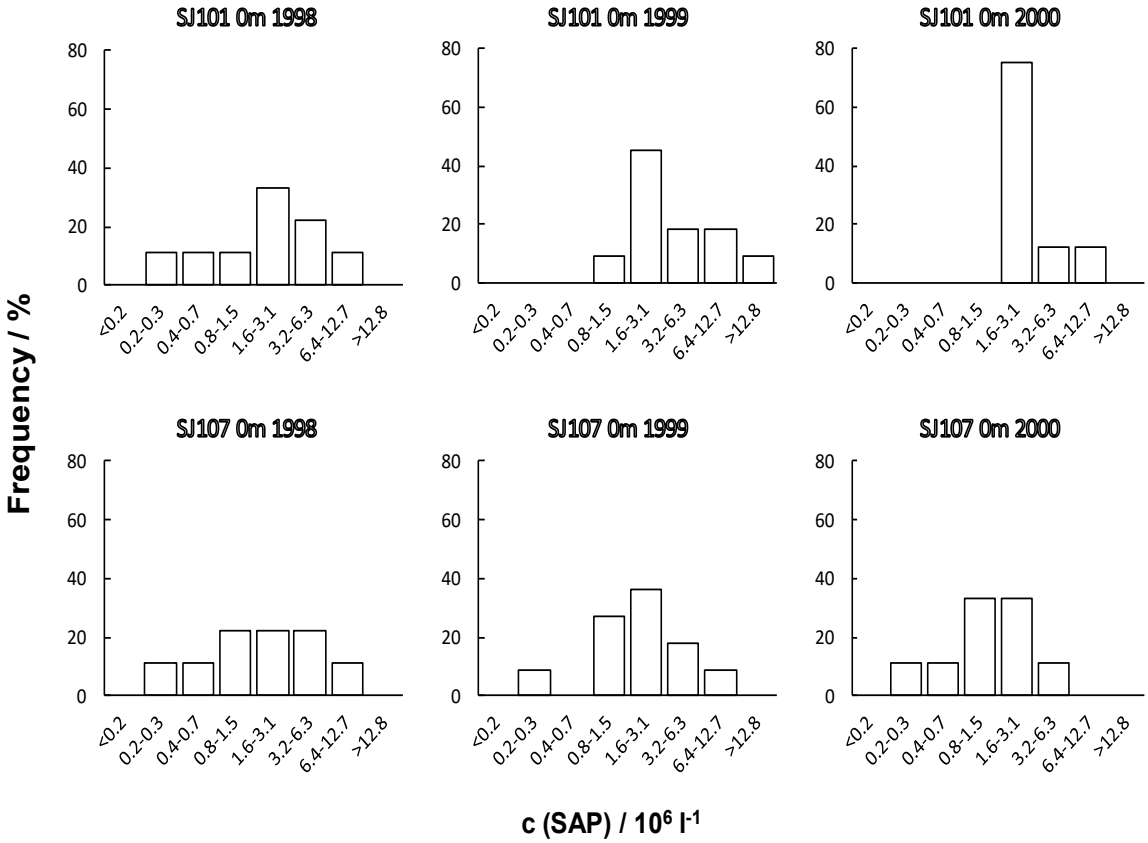

Figure 6. Frequency distribution (%) of concentration of SAP (10^6 l^{-1}) at 0 m depth from SJ101 and SJ107 in 1998, 1999, and 2000.

In March 2000, we note that high concentrations of SAP and SA at 10 m depth at SJ101 (9x10^6 l^{-1} and 1.7 mg l^{-1}, respectively) were accompanied by rather low phytoplank-ton and chlorophyll-*a* (7.5x10^5 l^{-1} and 0.6 μg l^{-1}, respectively). Significantly less pronounced thermo-, halo-, and pycnoclines were formed in March than in the following month of

April. In April 2000, phytoplankton, chlorophyll-*a*, SAP, and SA levels in the 0-10 m layer of SJ101 were high but lower than in April 1999, as follows: $5x10^6$ l^{-1}, 3.7 μg l^{-1}, $6.5x10^6$ l^{-1}, and 1.8 mg l^{-1}, respectively. Thus, in 1999 the concentration of SAP was high only in the surface layer, while in 2000 the concentrations of SAP were high in the 5-10 m layer.

At SJ107 in April 2000 the halo- and pycnocline were less pronounced than in March 2000. Temperature decreased from 12°C at the surface to 10°C at 20 m depth and chlorophyll-*a* and phytoplankton were rather low. Under these conditions, an increase in SAP from 0.3 $x10^6$ l^{-1} at the surface to 4.5 $x10^6$ l^{-1} at 20 m depth and a decrease to 1.2 $x10^6$ l^{-1} at the bottom was observed. SA was highest at 2.4 mg l^{-1} at the surface and decreased to 1.2 mg l^{-1} at 10 m depth.

We determined frequency distributions of SAP concentration in the years without the event (1998, 1999) and with the event (2000) using the same stations in the western part SJ101 and in the eastern part SJ107 (Figure 6).

At SJ101, the concentrations of SAP from 1998 to 2000 were mainly 1.6-3.1 x 10^6 l^{-1} with a perspective increasing abundance from 33 to 45 and 75%. The low SAP concentrations (<1.5 10^6 l^{-1}) on SJ 101 were not detected. In contrast, a wide variety of concentrations were detected on SJ107. For SJ107, the distribution was 22% evenly represented in three concentration classes, 0.8-1.5, 1.6-3.1, and 3.2-6.3 x 10^6 l^{-1} in 1998, 27% and 36% in 1999 and 33% in 2000, respectively.

We identified phytoplankton species in the upper layer from the surface to 10 m depth of SJ101 and SJ107, and summarized the abundance of cells during the study period in Tables 1 and 2. In February 1998, diatom *Skeletonema marinoi* (*S. marinoi)* peaked in SJ101 ($1.5x10^5$ cells l^{-1}), while in March 1998 in SJ101 and in February and March in SJ107, diatom *Nitzschia delicatissima* complex (*N. delicatissima* complex*)* was the dominant diatom ($5.9x10^4$, $9.3x10^4$, and $1.2x10^5$ cells l^{-1}, respectively).

In February 1999, *S. marinoi* was dominant in SJ101 ($8.5x10^4$ cells l^{-1}), while the phytoplankton community in SJ107 had no particular dominant species and total abundance was low ($3.7x10^3$ cells l^{-1}). In April 1999, an exceptionally intense diatom bloom was observed in SJ101, where the *N. delicatissima* complex and *S. marinoi* ($5.2x10^6$ and $6.9x10^6$ cells l^{-1}, respectively) were jointly dominant. In comparison, the abundance of the diatom genus *Chaetoceros* was much lower in SJ107, but also significant ($3.6x10^5$ cells l^{-1}).

High abundance of *S. marinoi* was observed at SJ101 in February 2000 ($1.3x10^6$ cells l^{-1}), with low total abundance at this station in March 2000 and April 2000, while at SJ107 total abundance was low in February 2000 and April 2000, with *S. marinoi* reaching 7-$8x10^4$ cells l^{-1} in March 2000.

Table 1. Phytoplankton community in February and March 1998, February and April 1999, and February to April 2000 at 0 m, 5 m and 10 m at SJ101. Sample and species abundance and contribution to sample abundance, with cumulative contribution cut-off at least of 80 %.

Station	Date	Depth	Species	Species abundance	Total abundance	Species contribution	Cumulative (%)
SJ101	25.2.1998	0	*Skeletonema marinoi*	748,140	1,146,260	65.3	65.3
			Nitzschia delicatissima complex	290,820		25.4	90.6
		5	*Nitzschia delicatissima* complex	928,700	1,735,300	53.5	53.5
			Skeletonema marinoi	715,580		41.2	94.8
		10	*Nitzschia delicatissima* complex	583,120	768,120	75.9	75.9
			Skeletonema marinoi	170,200		22.2	98.1
SJ101	17.3.1998	0	*Nitzschia delicatissima* complex	59,570	61,050	97.6	97.6
		5	*Nitzschia delicatissima* complex	39,590	41,810	94.7	94.7
		10	*Nitzschia delicatissima* complex	38,110	39,590	96.3	96.3
SJ101	15.2.1999	0	*Skeletonema marinoi*	85,470	125,800	67.9	67.9
			Nitzschia delicatissima complex	28,120		22.4	90.3
		5	*Skeletonema marinoi*	87,690	128,020	68.5	68.5
			Nitzschia delicatissima complex	29,970		23.4	91.9
		10	*Skeletonema marinoi*	67,340	105,450	63.9	63.9
			Nitzschia delicatissima complex	29,600		28.1	91.9
SJ101	24.4.1999	0	*Skeletonema marinoi*	6,926,400	15,258,800	45.4	45.4
			Nitzschia delicatissima complex	5,220,700		34.2	79.6
			Chaetoceros sp.	1,713,100		11.2	90.8
		5	*Nitzschia delicatissima* complex	657,860	990,120	66.4	66.4
			Skeletonema marinoi	227,920		23.0	89.5
		10	*Skeletonema marinoi*	14,430	31,080	46.4	46.4
			Nitzschia delicatissima complex	13,690		44.0	90.5
SJ101	21.2.2000	0	*Skeletonema marinoi*	1,266,880	1,381,580	91.7	91.7
		10	*Skeletonema marinoi*	201,280	248,640	81.0	81.0
SJ101	21.3.2000	0	*Chaetoceros* sp.	10,730	21,460	50.0	50.0
			Skeletonema marinoi	4,440		20.7	70.7
		5	*Chaetoceros* sp.	14,060	38,110	36.9	36.9
			Skeletonema marinoi	8,510		22.3	59.2
			Nitzschia delicatissima complex	7,400		19.4	78.6
			Chaetoceros curvisetus	6,660		17.5	96.1
		10	*Nitzschia delicatissima* complex	29,230	48,840	59.8	59.8
			Skeletonema marinoi	10,360		21.2	81.1
			Chaetoceros curvisetus	5,180		10.6	91.7
SJ101	17.4.2000	0	*Nitzschia delicatissima* complex	102,490	113,960	89.9	89.9
		5	*Nitzschia delicatissima* complex	156,140	163,540	95.5	95.5
		10	*Nitzschia delicatissima* complex	33,670	39,220	85.8	85.8

Table 2. Phytoplankton community in February and March 1998, February and April 1999, and February to April 2000 at 0 m, 5 m and 10 m at SJ107. Sample and species abundance and contribution to sample abundance, with cumulative contribution cut-off at least of 80 %.

Station	Date	Depth	Species	Species abundance	Sample abundance	Species contribution	Cumulative (%)
SJ107	25.2.1998	0	*Nitzschia delicatissima* complex	93,240	124,320	75.0	75.0
			Rhizosolenia fragilissima	13,320		10.7	85.7
		5	*Nitzschia delicatissima* complex	386,280	481,000	80.3	80.3
			Rhizosolenia fragilissima	86,580		18.0	98.3
		10	*Nitzschia delicatissima* complex	123,210	140,970	87.4	87.4
SJ107	17.3.1998	0	*Nitzschia delicatissima* complex	123,580	127,280	97.1	97.1
		5	*Nitzschia delicatissima* complex	66,970	71,780	93.3	93.3
		10	*Nitzschia delicatissima* complex	94,720	99,160	95.5	95.5
SJ107	15.2.1999	0	*Skeletonema marinoi*	3,700	12,210	30.3	30.3
			Chaetoceros affinis	1,480		12.1	42.4
			Chaetoceros curvisetus	1,480		12.1	54.5
			Nitzschia tenuirostris	1,480		12.1	66.7
		5	*Skeletonema marinoi*	8,880	13,320	66.7	66.7
			Nitzschia tenuirostris	2,220		16.7	83.3
		10	*Skeletonema marinoi*	14,060	17,390	80.9	80.9
SJ107	24.4.1999	0	*Chaetoceros* sp.	364,450	836,200	43.6	43.6
			Nitzschia delicatissima complex	223,850		26.8	70.4
		5	*Nitzschia delicatissima* complex	246,420	695,600	35.4	35.4
			Skeletonema marinoi	155,400		22.3	57.8
			Chaetoceros socialis	138,380		19.9	77.7
			Chaetoceros curvisetus	76,220		11.0	88.6
		10	*Nitzschia delicatissima* complex	109,520	204,240	53.6	53.6
			Skeletonema marinoi	47,360		23.2	76.8
SJ107	21.2.2000	0	*Chaetoceros curvisetus*	492	1,476	33.3	33.3
			Nitzschia delicatissima complex	246		16.7	50.0
			Nitzschia tenuirostris	246		16.7	66.7
			Prorocentrum micans	246		16.7	83.3
		5	*Nitzschia tenuirostris*	555	1,850	30.0	30.0
			Melosira dubia	370		20.0	50.0
			Nitzschia delicatissima complex	370		20.0	70.0
			Achnanthes sp.	185		10.0	80.0
		10	*Nitzschia tenuirostris*	246	861	28.6	28.6
			Achnanthes sp.	123		14.3	42.9
			Mastogloia sp.	123		14.3	57.1
			Navicula sp.	123		14.3	71.4
			Nitzschia delicatissima complex	123		14.3	85.7
SJ107	17.3.2000	0	*Skeletonema marinoi*	75,110	105,080	71.5	71.5
			Chaetoceros sp.	17,760		16.9	88.4
		5	*Skeletonema marinoi*	81,770	92,500	88.4	88.4
		10	*Skeletonema marinoi*	36,260	45,140	80.3	80.3
SJ107	14.4.2000	0	*Nitzschia delicatissima* complex	555	1,110	50.0	50.0
			Prorocentrum micans	370		33.3	83.3
		5	*Nitzschia delicatissima* complex	1,353	1,353	100.0	100.0
		10	*Nitzschia delicatissima* complex	1,110	1,665	66.7	66.7
			Glenodinium sp.	185		11.1	77.8
			Gymnodinium sp.	185		11.1	88.9

4. Discussion

The NA is characterized by pronounced seasonal and long-term variations in oceanographic and biological conditions caused primarily by atmospheric influences, freshwater discharges, variable intrusions of high salinity water, and highly variable and complex circulation [e.g., 39-41 and references therein]. This area is considered the most productive part of the generally oligotrophic Mediterranean [42-44]. In NA, a horizontal trophic gradient is usually formed, reflecting eutrophic waters in the western part and oligotrophic waters in the eastern part, as can be seen in the gradients of chlorophyll-*a* [45,46], phytoplankton [47], zooplankton [48], dissolved organic carbon (DOC) [49,50] or surfactant activity [51]. Our results showed that the SAP spatio-temporal distribution followed the same pattern, i.e. the concentration was higher in eutrophic areas than in oligotrophic areas. However, under certain meterological and circulatory conditions, this gradient disappears and a phytoplankton bloom occurs in large parts of the region [52-55], resulting in a particularly high bioproduction (anchovy catch) in the entire Adriatic [31,56].

One of the features of the NA was the "mucilage event", where the massive mucilaginous material appears in the form of macroaggregates up to several meters in size, forming surface, subsurface and benthic layers tens of kilometers long [20,21]. In general, the mucilage event in the NA begins in late spring/early summer, when the stratification of the water column strengthens and the exchange of water masses between the northern and central sub-basins slows down [15]. The months leading up to the event (March-May) are thought to be an "incubation period" characterized by the advection of freshwater from the Po Delta to the east [57], and the mucilage event appears to begin in such low-salinity, oxygen-saturated, and P-limited waters [58,59]. Accumulation has been found to occur particularly efficiently in anticyclic eddies that occur under these stable conditions in NA and act as "hotspots" for macroaggregate formation [60].

Mucilage event features self-assembly of extracellular polymers, mainly polysaccharides from phytoplankton, into organic particle precursors at critical concentrations, which under certain conditions transform into a giant gel by phase transition [20,22]. There is a broad consensus that mucilage originate from diatoms [27,60-64] is formed during phosphorus-limited growth [e.g., 68 and references therein] and that the accumulation of released organic matter is maintained by water column stability [e.g., 18,19,58,65].

The northern Adriatic region was studied particularly extensively from June 1999 to July 2002, which provides important data for the interpretation of our results [21]. The authors reported that in the spring and summer of 1999, the intense western Adriatic current (WAC) and the cyclonic circulation transporting water from the NA were well developed and it seems that these factors prevented the development of the mucilage event. Contrary, in 2000, 2001 and 2002, the WAC was weak or receding and coupled with a strong Istrian coastal countercurrent (ICCC) [66]. These conditions allowed the Po River water to remain longer in the NA and were probably the crucial initial conditions for the accumulation of organic material and the development of the mucilage event. In the freshened upper part of the highly stratified water column, concentrations of particulate organic carbon were higher in the period before the 2000 and 2002 mucilage events, indicating the importance of the halocline and pycnocline, as well as the POC, for the accumulation of organic material [67]. Although the values of DOC remained low, the authors do not exclude that a smaller fraction of DOC, whose variations would not significantly affect the total content of DOC, plays an important role in the aggregation process and the mucilage event, such as SAP [68] and colloidal fractions. Indeed, colloids have been found to be a relevant fraction of DOC in the NA [69], and colloidal aggregation has been identified as an important mechanism in the transport of organic carbon to higher dimensions to form microaggregates that can coalesce into macroaggregates [20].

When the critical concentration of SAP (~ $2x10^7$ l^{-1}) is reached, a phase transition from dispersed microparticles to a gel state occurs when supported by meterological and

hydrodynamic conditions [22]. Our results showed that the critical concentration of SAP was reached in April 1999 and mucilage event did not occur (Fig. 5). In contrast, the concentration of SAP reached only 1.15×10^7 l^{-1} in April 2000, but mucilage event occurred. In March 1998, the concentration of SAP and the abundance of phytoplankton were relatively low, while in April 1999, a high concentration of SAP and an extremely intense diatom bloom were observed, indicating a relationship between SAP and diatoms. In contrast, moderate abundance of phytoplankton and high concentration of SAP were measured in March 2000 and April 2000, the period before the mucilage event that began in late May, suggesting that SAP is associated with diatoms but also with other fractions of organic matter.

However, the significant presence of diatoms in all types of macroaggregates was indicated by a significant proportion of highly refractory organic matter containing biogenic silica [70]. Diatoms in laboratory cultures were observed to excrete large amounts of deoxysugars, especially rhamnose and fucose [71-73]. On the other hand, fresher macroaggregates formed at the earlier stages of the mucilage event were also enriched in rhamnose and fucose, which was further confirmation of the hypothesis that diatoms contribute significantly to the formation of macroaggregates [70].

It should be noted that not only active phytoplankton contribute significantly to the DOM pool, but also past populations through their activity and decay [Marty with co-authors [74,75]. Our results indicate that the phytoplankton community during the winter months (February 2000) preceding the incubation period (presumably spring) may be important for the development of the mucilage event. Indeed, mucilage event may have begun in winter when very dense, cold water formed in the bottom layer and persisted into spring, enhancing water column stratification in the NA [57]. During this period, the bloom of *S. marinoi* was recorded on the studied transect in the western part of NA, but even further south [47]. *S. marinoi* was known to be a winter bloom, peaking in January [76], which is normally the annual maximum of phytoplankton abundance and biomass in the Adriatic [47,77]. However, since 2008, the bloom of *S. marinoi* has shifted to March and abundance has declined, both associated with ongoing climate change [78,79]. We hypothesize that this change in the timing of the seasonal cycle has contributed to the disappearance of the mucilage event at NA. Based on the reported evidence and our results, the mucilage event at NA could be anticipated by: i) specific oceanographic conditions that maintain stratification and limited water exchange in winter, ii) a winter diatom bloom that secretes polysaccharides, and iii) an order of magnitude higher concentration of SAP than typically observed in late winter/early spring. Depending on the composition of the diatom community and its response to environmental stress, the chemical composition of the mucilages may also differ accordingly, which could be important for understanding the stability and persistence of mucilages in the marine environment.

At the end, although the mucilage event has disappeared from the NA, the shallow and enclosed Sea of Marmara faces major challenges [80-82]. Due to global climate change and human-induced activities such as overfishing, phytoplankton biomass has increased, which contributes to the fact that the mucilage event continues throughout the year [6]. We believe that global climate change is having major impacts on semi-enclosed, closed, and shallow basins, triggering changes related to declines in freshwater inflow, nutrient availability, increases in temperature and salinity, changes in circulation patterns and water exchange, and the appearance of new species that could potentially disrupt existing trophic structure and ecosystem stability.

Author Contributions: Both authors contributed equally to the work.

Funding: This work was supported by the Croatian Ministry of Science, Education and Sports through the projects (i) Mechanism of long-term changes in the organic matter cycle of the northern

Adriatic ecosystem, Grant No. 00981303; (ii) National Monitoring Programme (Project Adriatic), Systematic Research of the Adriatic Sea as a Base for Sustainable Development of the Republic of Croatia and (iii) Surface Forces on Atomic Scale Applied in Marine Science, and Nanotechnology, Grant No. 0982934-2744, (iv) Croatian Science Foundation Project "From algal cell surface properties to stress markers for aquatic ecosystems" (IP-2018-01-5840), and (v) MAT Project (Mucilages in the Adriatic and Tyrrhenian Seas) supported by the Croatian Ministry of Science and Technology and Italian Ministry of Science and Technology.

Data Availability Statement: The datasets generated during the current study are available from the corresponding authors on request.

Acknowledgments: We thank all colleagues who participated in sampling during fieldwork at r/v Vila Velebita, data collection, processing, and creation of the CMR database, especially Dr. Robert Precali. Special thanks to Dr. Solveg Kovač for her contribution to the electrochemical measurements, to technician Anica Bakota for phytoplankton counting and identification, and to Romano Rabak for chlorophyll-a measurements. Special thanks to Dr. Vera Žutić and Dr. Vesna Svetličić for coordinating the study of organic microparticles within the Project Adriatic.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Templado, J. Future Trends of Mediterranean Biodiversity. In *The Mediterranean Sea*, 1st ed.; Goffredo, S., Dubinsky, Z., Eds.; Springer: Dordrecht, Netherlands, 2014; Volume 1, pp. 479–498. https://doi.org/10.1007/978-94-007-6704-1_28
2. Marbá, N.; Jordá, G.; Agustí, S.; Girard, C.; Duarte, C.M. Footprints of Climate Change on Mediterranean Sea Biota. *Front Mar Sci* **2015**, *2*, 56, 1-11. https://doi.org/10.3389/fmars.2015.00056
3. Schroeder, K.; Chiggiato, J.; Bryden, H.L.; Borghini, M.; Ben Ismail, S. Abrupt Climate Shift in the Western Mediterranean Sea. *Sci Rep* **2016**, *6*, 23009, 1-7. https://doi.org/10.1038/srep23009
4. Vargas-Yáñez, M.; García, M.J.; Salat, J.; García-Martínez, M.; Pascual, J.; Moya, F. Warming Trends and Decadal Variability in the Western Mediterranean Shelf. *Glob Planet Chang* **2008**, *63*, 177–184. https://doi.org/10.1016/j.gloplacha.2007.09.001
5. Budiša, A.; Paliaga, P.; Juretić, T.; Lučić, D.; Supić, N.; Pasarić, Z.; Đakovac, T.; Mladinić, M.; Dadić, V.; Tičina, V. Distribution, diet and relationships of the invasive ctenophore *Mnemiopsis leidyi* with anchovies and zooplankton, in the northeastern Adriatic Sea. *Medit Mar Sci* **2021**, *22*, 827-842. https://doi.org/10.12681/mms.23305
6. Savun-Hekimoğlu, B.; Gazioğlu, C. Mucilage Problem in the Semi-Enclosed Seas: Recent outburst in the Sea of Marmara. *Int J Environ Geoinformatics* **2021,** *8*, 402-413. https://doi.org/10.30897/ijegeo.955739
7. Aktan, Y.; Dede, A.; Ciftci, P.S. Mucilage Event Associated with Diatoms and Dinoflagellates in Sea of Marmara, Turkey. In: *Harmful Algae News*, The Intergovernmental Oceanographic Commission of UNESCO 2008, 36, 1–3.
8. Tüfekçi, V.; Balkıs, N.; Polat Beken, Ç.; Ediger, D.; Mantıkçı, M. Phytoplankton Composition and Environmental Conditions of a Mucilage Event in the Sea of Marmara. *Turkish J Biol* **2010**, *34*, 199–210. https://doi.org/10.3906/biy-0812-1
9. İşinibilir Okyar, M.; Üstün, F.; Orun, D.A. Changes in Abundance and Community Structure of the Zooplankton Population during the 2008 Mucilage Event in the Northeastern Marmara Sea. *Turkish J Zool* **2015**, *39*, 28–38. doi: 10.3906/zoo-1308-11
10. Özalp, H.B. First Massive Mucilage Event Observed in Deep Waters of Çanakkale Strait (Dardanelles), Turkey. *J Black Sea / Mediterr Environ* **2021**, *27*, 49–66.
11. Genitsaris, S.; Stefanidou, N.; Sommer, U.; Moustaka-Gouni, M. Phytoplankton blooms, red tides and mucilaginous aggregates in the urban Thessaloniki Bay, Eastern Mediterranean. *Diversity* **2019**, *11*, 136, 1-22. https://doi.org/10.3390/d11080136
12. Danovaro, R.; Fonda Umani, S.; Pusceddu, A.M. Climate Change and the Potential Spreading of Marine Mucilage and Microbial Pathogens in the Mediterranean Sea. *PLoS ONE* **2009**, *4*, 1-8. https://doi.org/10.1371/journal.pone.0007006
13. Rouaud, V.; Susperregui, N.; Fahy, A.; Guyoneaud, R.; Bichon, S.; Lienart, C.; Del Amo, Y.; Savoye, N.; Gaudin, P.; Duran, R.; Lauga, B. Dynamics of microbial communities across the three domains of life over an annual cycle with emphasis on marine mucilage in the Southern Bay of Biscay resolved by microbial fingerprinting. *Cont Shelf Res* **2019**, *186*, 127-137. https://doi: 10.1016/j.csr.2019.06.003
14. Karadurmuş, U.; Sari, M. Marine mucilage in the Sea of Marmara and Its Effects on the Marine Ecosystem: Mass Deaths. *Turkish J Zool* **2022**, *46*, 93-102. https://doi.org/10.3906/zoo-2108-14
15. Degobbis, D.; Malej, A.; Fonda-Umani, S. The mucilage phenomenon in the northern Adriatic: A critical review of the present scientific hypotheses. *Ann Ist Super Sanità* **1999**, *35*, 373-381.
16. Precali, R.; Giani, M.; Marini, M.; Grilli, F.; Ferrari, C.R.; Pecar, O., Paschini, E. Mucilaginous aggregates in the northern Adriatic in the period 1999-2002: typology and distribution. *Sci Total Environ* **2005**, 353, 10-23. https://doi: 10.1016/j.scitotenv.2005.09.066
17. Zingone, A.; Escalera, L.; Aligizaki, K.; Tejedor, M.F.; Ismael, A.; Montresor, M.; Mozetič, P.; Taş, S.; Totti, C. Toxic marine microalgae and noxious blooms in the Mediterranean Sea: A contribution to the Global HAB Status Report. *Harmful Algae* **2021** 102: 101843. https://doi: 10.1016/j.hal.2020.101843
18. Vollenweider, R.A., Rinaldi, A. Eds. Marine Mucilages. Special issue, *Sci Total Environ* **1995**, *165*, pp. 1-235.
19. Funari, E., Azam, F., Fonda Umani, S., Pagnotta, R. Eds. State of the art and new scientific hypotheses on the phenomenon of mucilages in the Adriatic Sea. Special issue, *Ann Ist Super Sanità* **1999**, *35*, pp. 353-476.
20. Žutić V.; Svetličić V. 2000. Interfacial processes. In *The Handbook of Environmental Chemistry*; Wangersky, P. Ed. Springer-Verlag: Berlin, Heidelberg, Germany; Volume 5 Part D. Marine Chemistry. pp. 149-165.
21. Giani, M., Degobbis, D., Rinaldi, A., Eds. Mucilages in the Adriatic and Tyrrhenian Seas. Special issue, *Sci Total Environ* **2005**, *353*, pp. 1-380.
22. Svetličić, V.; Žutić, V.; Zimmermann, A.H. Biophysical scenario of giant gel formation in the Northern Adriatic Sea. *Ann NY Acad Sci* **2005**, *1048*, 524–527.
23. Svetličić, V.; Balnois, E.; Žutić, V.; Chevalet, J.; Hozić Zimmermann, A.; Kovač, S.; Vdović, N. Electrochemical detection of gel microparticles in seawater. *Croat Chem Acta* **2006**, *79*, 107–113. https://hrcak.srce.hr/2619
24. Pletikapić, G.; Ivošević DeNardis, N. Application of surface analytical methods for hazardous situation in the Adriatic Sea: monitoring of organic matter dynamics and oil pollution. *Nat Hazards Earth Syst Sci* **2017**, *17*, 31-44. https://doi.org/10.5194/nhess-17-31-2017
25. Novosel, N.; Mišić Radić, T.; Zemla, J.; Lekka, M.; Čačković, A.; Kasum, D.; Legović, T.; Žutinić, P.; Gligora Udovič, M.; Ivošević DeNardis N. Temperature-induced response in algal cell surface properties and behaviour: an experimental approach. *J Appl Phycol* **2022a**, *34*, 243-259. https://doi.org/10.1007/s10811-021-02591-0
26. Novosel, N.; Mišić Radić, T.; Levak Zorinc, M.; Zemla, J.; Lekka, M.; Vrana, I.; Gašparović, B.; Horvat, L.; Kasum, D.; Legović, T.; Žutinić, P.; Gligora Udovič, M.; Ivošević DeNardis, N. Salinity induced chemical, mechanical and behavioral changes in marine microalgae. *J Appl Phycol* **2022b**, *34*, 1293–1309. https://doi.org/10.1007/s10811-022-02734-x

27. Pletikapić, G.; Mišić Radić, T.; Hozić Zimmermann, A.; Svetličić, V.; Pfannkuchen, M.; Marić, D.; Godrijan, J.; Žutić, V. AFM imaging of extracellular polymer release by marine diatom *Cylindrotheca closterium* (Ehrenberg) Reiman & J.C. Lewin. *J Mol Recognit* **2011**, *24*, 436-445. https://doi.org/10.1002/jmr.1114
28. Novosel, N.; Ivošević DeNardis, N. Structural Features of the Algal Cell Determine Adhesion Behavior at a Charged Interface. *Electroanal* 2 **2021**, *33*, 14336-1443. https://doi.org/10.1002/elan.202060580
29. Žutić, V.; Svetličić, V.; Ivošević, N.; Hozić, A.; Pečar, O. Northern Adriatic mesocosm experiment Rovinj 2003: dynamics of organic microparticles studied by the electrochemical technique. *Period Biol* **2004**, *106*, 67-74.
30. Ivošević DeNardis, N.; Šegota, S.; Svetličić, V.; Castelli, A.; Kljajić, Z. Characterization of marine organic matter in the bay of Boka Kotorska by electrochemical and atomic force microscopy imaging. *Stud Mar* **2013**, *26*, 5-21.
31. Kraus, R.; Supić, N.; Precali, R. Factors favoring phytoplankton blooms in the northern Adriatic: Towards the northern Adriatic empirical ecological model. *Ocean Sci* **2016**, *12*, 19–37. https://doi.org/10.5194/os-12-19-2016
32. Parsons, T.R.; Maita, Y.; Lalli, C.M. *A Manual of Chemical and Biological Methods for Seawater Analysis*, 2nd ed.; Pergamon Press New York, USA, 1985; 1-173.
33. Utermöhl, H. Zur Verfollkommnung der quantitativen Phytoplankton-Methodik. *Mitt Int Verein theor angew Limnol* **1958**, *17*, 47–71.
34. Guillard, R.R.L. Culture of Phytoplankton for Feeding Marine Invertebrates. In *Culture of Marine Invertebrates Animals*, Smith, M.L., Chanley, M.H., Eds.; Springer: Boston, MA, USA, 1975. pp. 29-60. http://dx.doi.org/10.1007/978-1-4615-8714-9_3
35. Ivošević, N.; Žutić, V. Polarography of marine particles: A model study. *Croat. Chem. Acta*, **1997**, *70*, 167-178.
36. Baldi, F.; Ivošević, N.; Minacci, A.; Pepi, M.; Fani, R.; Svetličić, V.; Žutić, V. Adhesion of *Acinetobacter venetianus* to diesel fuel roplets studied with In situ electrochemical and molecular probes. *Appl Environ Microbiol* **1999**, *65*, 2041-2048. https://doi.org/10.1128/AEM.65.5.2041-2048.1999
37. Žutić, V.; Ivošević, N.; Svetličić, V.; Long, R.A.; Azam, F. Film formation by marine bacteria at a model fluid interface. *Aquat Microb Ecol* **1999**, *17*, 231–238. https://doi.org/10.3354/ame017231
38. Kovač, S.; Kraus, R.; Geček, S.; Žutić, V. Cell suspension as a model system for electrochemical analysis. *Croat Chem Acta* **2000**, *73*, 279-291. https://hrcak.srce.hr/132095
39. Poulain, P.M.; Kourafalou, V.H.; Cushman-Roisin, B. Northern Adriatic Sea. In *Physical Oceanography of the Adriatic Sea*, Cushman-Roisin, B., Gačić, M., Poulain, P.M., Artegiani, A., Eds.; Springer: Dordrecht, Netherlands, 2001, pp. 143-165. https://doi.org/10.1007/978-94-015-9819-4_5
40. Supić, N.; Orlić, M.; Degobbis, D. Istrian Coastal Countercurrent and its Year-to-Year Variability. *Estuar Coast Shelf Sci* **2000**, *51*, 385–397. https://doi.org/10.1006/ecss.2000.0681
41. Supić, N.; Kraus, R.; Kuzmić, M.; Paschini, E.; Precali, R.; Russo, A.; Vilibić, I. Predictability of northern Adriatic winter conditions. *J Mar Syst* **2012**, *90*, 42–57. https://doi.org/10.1016/j.jmarsys.2011.08.008
42. Sournia, A. La production primaire planctonique en Mediterranée. Essai de mise a jour *Bull Etude Comm Méditerr* Num Spéc **1973**, *5*, pp. 1-128.
43. Fonda Umani, S.; Milani, L.; Borme, D.; de Olazabal, A.; Parlato, S.; Precali, R.; Kraus, R.; Lucić, D.; Njire, J.; Totti, C.; Romagnoli, T.; Pompei, M.; Cangini, M. Inter-annual variations of planktonic food webs in the northern Adriatic Sea. *Sci Total Environ* **2005**, *353*, 218-231. https://doi.org/10.1016/j.scitotenv.2005.09.016
44. Fonda Umani, S.; Del Negro, P.; Larato, C.; De Vittor, C.; Cabrini, M.; Celio, M.; Falconi, C.; Tamberlich, F.; Azam, F. Major inter-annual variations in microbial dynamics in the Gulf of Trieste (northern Adriatic Sea) and their ecosystem implications. *Aquat Microb Ecol* **2007**, *46*, 163–175. https://doi.org/10.3354/ame046163
45. Solidoro, C.; Bastianini, M.; Bandelj, V.; Codermatz, R.; Cossarini, G.; Canu, D.M.; Ravagnan, E.; Salon, S.; Trevisani, S. Current state, scales of variability, and trends of biogeochemical properties in the northern Adriatic Sea. *J Geophys Res* **2009**, *114*, 7-91. https://doi.org/10.1029/2008JC004838
46. Mozetič, P.; Solidoro, C.; Cossarini, G.; Socal, G.; Precali, R.; Francé, J.; Bianchi, F.; Vittor, C.D.; Smodlaka, N.; Fonda Umani, S. Recent Trends Towards Oligotrophication of the Northern Adriatic: Evidence from Chlorophyll *a* Time Series. *Estuaries Coast* **2010**, *33*, 362-375. http://dx.doi.org/10.1007/s12237-009-9191-7
47. Totti, C.; Cangini, M.; Ferrari, C.; Kraus, R.; Pompei, M.; Pugnetti, A.; Romagnoli, T.; Vanucci, S.; Socal, G. Phytoplankton size-distribution and community structure in relation to mucilage occurrence in the northern Adriatic Sea. *Sci Total Environ* **2005**, *353*, 204-17. https://doi.org/10.1016/j.scitotenv.2005.09.028
48. Varezić Bojanić, D.B.; Vidjak, O.; Kraus, R.; Precali, R. Regulating mechanisms of calanoid copepods variability in the northern Adriatic Sea: Testing the roles of west-east salinity and phytoplankton gradients. *Estuar Coast Shelf Sci* **2015**, 164, 288-300. http://dx.doi.org/10.1016/j.ecss.2015.07.026
49. Dautović, J.; Vojvodić, V.; Tepić, N.; Ćosović, B.; Ciglenečki, I. Dissolved organic carbon as potential indicator of global change: A long-term investigation in the northern Adriatic. *Sci Total Environ* **2017**, *587–588*, 185–195. https://doi.org/10.1016/j.scitotenv.2017.02.111
50. Ciglenečki, I.; Vilibić, I.; Dautović, J.; Vojvodić, V.; Ćosović, B.; Zemunik, P.; Dunić, N.; Mihanović, H. Dissolved organic carbon and surface active substances in the northern Adriatic Sea: Long-term trends, variability and drivers. *Sci Total Environ* **2020**, 730, 139104. https://doi.org/10.1016/j.scitotenv.2020.139104

51. Gašparović, B.; Ćosović, B. Surface-active properties of organic matter in the North Adriatic Sea. *Estuar Coast Shelf Sci* **2003**, *58*, 555-566. https://doi.org/10.1016/S0272-7714(03)00133-1
52. Revelante, N.; Gilmartin, M. Temporal succession of phytoplankton in the northern Adriatic. *Neth J Sea Res* **1976**, *10*, 377–396. https://doi.org/10.1016/0077-7579(76)90012-0
53. Revelante, N.; Gilmartin, M. The lateral advection of particulate organic matter from the Po Delta region during summer stratification, and its implications for the northern Adriatic. *Estuar Coast Shelf Sci* **1992**, *35*, 191-212. https://doi.org/10.1016/S0272-7714(05)80113-1
54. Kraus, R.; Supić, N. Impact of circulation on high phytoplankton blooms and fish catch in the northern Adriatic (1990–2004). *Estuar Coast Shelf Sci* **2011**, *91*, 198–210. https://doi.org/10.1016/j.ecss.2010.10.021
55. Marić, D.; Kraus, R.; Godrijan, J.; Supić, N.; Đakovac, T.; Precali, R. Phytoplankton response to climatic and anthropogenic influences in the north-eastern Adriatic during the last four decades. *Estuar Coast Shelf Sci* **2012**, *115*, 98-112. https://doi.org/10.1016/j.ecss.2012.02.003
56. Kraus, R.; Supić, N.; Lučić, D.; Njire, J. Impact of winter oceanographic conditions on zooplankton abundance in northern Adriatic with implications on Adriatic anchovy stock prognosis. *Estuar Coast Shelf Sci* **2015**, *167*, 56–66. https://doi.org/10.1016/j.ecss.2015.10.008
57. Russo, A:, Maccaferri, S.; Djakovac, T.; Precali, R.; Degobbis, D.; Deserti, M.; Paschini, E.; Lyons, D.M. Meteorological and oceanographic conditions in the northern Adriatic Sea during the period June 1999-July 2002: influence on the mucilage phenomenon. *Sci Total Environ* **2005,** 353, 24-38. https://doi.org/10.1016/j.scitotenv.2005.09.058
58. Degobbis, D.; Precali, R.; Ferrari, C.R.; Djakovac, T.; Rinaldi, A.; Ivančić, I.; Gismondi, M.; Smodlaka, N. Changes in nutrient concentrations and ratios during mucilage events in the period 1999-2002. *Sci Total Environ* **2005**, *353*, 103-114. https://doi.org/10.1016/j.scitotenv.2005.09.010
59. Volf, G.; Atanasova, N.; Kompare, B.; Precali, R.; Ožanić, N. Study of the impact of TIN/PO4 ratio on mucilage formation in the northern Adriatic using regression trees. *Acta Adriat* **2015**, *56*, 207-222. http://hrcak.srce.hr/152249
60. Kraus, R.; Supić, N. Sea dynamics impacts on the macroaggregates: A case study of the 1997 mucilage event in the northern Adriatic. *Prog Oceanogr* **2015**, *138*, 249–267. https://doi.org/10.1016/j.pocean.2015.06.005
61. Alldredge, A.L. The potential role of particulate diatom exudates in forming nuisance mucilaginous scums. *Ann Ist Super Sanità* **1999**, 35, 397–400.
62. Myklestad, S.M. Phytoplankton extracellular production and leakage with consideration on the polysaccharide accumulation. *Ann Ist Super Sanità* **1999**, *35*, 411-419.
63. Kraus, R.; Ivančić, I.; Đakovac, T.; Smodlaka, N. Northern Adriatic mesocosm experiment Rovinj 2003: changes in the microphytoplankton community. *Period Biol* **2004**, *106*, 31-37.
64. Najdek, M.; Blažina, M.; Đakovac, T.; Kraus, R. The role of the diatom *Cylindrotheca closterium* in a mucilage event in the northern Adriatic Sea: coupling with high salinity water intrusions. *J Plankton Res* **2005**, *27*, 851-862. https://doi.org/10.1093/plankt/fbi057
65. Degobbis, D.; Fonda-Umani, S.; Franco, P.; Malej, A.; Precali, R.; Smodlaka N. Changes in the northern Adriatic ecosystem and the hypertrophic appearance of gelatinous aggregates. *Sci Total Environ* **1995**, *165*, 43–58. https://doi.org/10.1016/0048-9697(95)04542-9
66. Grilli, F.; Paschini, E.; Precali, R.; Russo, A.; Supić N. Circulation and horizontal fluxes in the northern Adriatic Sea in the period June 1999-July 2002. Part I: geostrophic circulation and current measurement. *Sci Total Environ* **2005**, *353*, 57-67. https://doi.org/10.1016/j.scitotenv.2005.09.008
67. Giani, M.; Savelli, F.; Berto, D.; Zangrando, V.; Ćosović, B.; Vojvodić, V. Temporal dynamics of dissolved and particulate organic carbon in the northern Adriatic Sea in relation to the mucilage events. *Sci Total Environ* **2005**, *353*, 126-38. https://doi.org/10.1016/j.scitotenv.2005.09.062
68. Ćosović, B., Vojvodić, V. Mucilage events in the Northern Adriatic: search for tools for early warning. *Period Biol* **2000**, *102*, 255-259.
69. Pettine, M.; Capri, S.; Manganelli, M.; Patrolecco, L.; Puddu, A.; Zoppini, A. The dynamics of DOM in the northern Adriatic Sea. *Estuar Coast Shelf Sc*i **2001**, *52*, 471–489. https://doi.org/10.1006/ecss.2000.0752
70. Giani, M.; Berto, D.; Zangrando, V.; Castelli, S.; Sist, P.; Urbani, R. Chemical characterization of different typologies of mucilaginous aggregates in the Northern Adriatic Sea. *Sci Total Environ* **2005**, *353*, 232-46. https://doi.org/10.1016/j.scitotenv.2005.09.027
71. Allan, G.G.; Lewin, J.C.; Johnson, P. Marine Polymers. IV Diatom Polysaccharides. *Bot Mar*, **1972**, *15*, 102-108. https://doi.org/10.1515/botm.1972.15.2.102
72. Haug, A.; Myklestad, S.M. Polysaccharides of marine diatoms with special reference to *Chaetoceros* species. *Mar Biol* **1976**, *34*, 217-222. https://doi.org/10.1007/BF00388798
73. Urbani, R.; Magaletti, E.; Sist, P.; Cicero, A.M. Extracellular carbohydrates released by the marine diatoms *Cylindrotheca closterium, Thalassiosira pseudonana* and *Skeletonema costatum*: Effect of P-depletion and growth status. *Sci Total Environ* **2005**, *353*, 300–306. https://doi.org/10.1016/j.scitotenv.2005.09.026
74. Marty, J.C.; Žutić, V.; Precali, R.; Saliot, A.; Ćosović, B.; Smodlaka, N.; Cauwet, G. Organic matter characterization in the Northern Adriatic sea with special reference to the sea surface microlayer. *Mar Chem* **1988**, 25, 243-263. https://doi.org/10.1016/0304-4203(88)90053-9
75. Malfatti, F.; Turk, V.; Tinta, T.; Mozetič, P.; Manganelli, M.; Samoa, T.J.; Ugalde, J.A.; Kovač, N.; Stefanelli, M.; Antonioli, M.; Fonda-Umani, S.; Del Negro, P.; Cataletto, B.; Hozić, A.; Ivošević DeNardis, N.; Žutić, V.; Svetličić, V.; Mišić Radić, T.; Radić,

T.; Fuks, D.; Azam, F. Microbial mechanisms coupling carbon and phosphorus cycles in phosphorus-limited northern Adriatic Sea. *Sci Total Environ* **2014**, *470/471*, 1173-1183. https://doi.org/10.1016/j.scitotenv.2013.10.040
76. Totti, C.; Cucchiari, E.M.; Romagnoli, T. Intra and interannual variability of phytoplankton in coastal area of Senigallia (northern Adriatic Sea) from 1988 to 2000. *Biol Mar Mediterr* **2002**, *9*, 391-399.
77. Bernardi Aubry, F.; Berton, A.; Bastianini, M.; Socal, G.; Acri, F. Phytoplankton succession in a coastal area of the NW Adriatic, over a 10-year sampling period (1990-1999). *Cont Shelf Res* **2004**, *24*, 97-115. https://doi.org/10.1016/j.csr.2003.09.007
78. Cerino, F.; Fornasaro, D.; Kralj, M.; Giani, M.; Cabrini, M. Phytoplankton temporal dynamics in the coastal waters of the north-eastern Adriatic Sea (Mediterranean Sea) from 2010 to 2017. *Nat Conserv* **2019**, *34*, 343-372. https://doi.org/10.3897/natureconservation.34.30720
79. Totti, C.; Romagnoli, T.; Accoroni, S.; Coluccelli, A.; Pellegrini, M.O.; Campanelli, A.; Grilli, F.; Marini, M. Phytoplankton communities in the northwestern Adriatic Sea: Interdecadal variability over a 30-years period (1988–2016) and relationships with meteoclimatic drivers. *J Mar Syst* **2019**, *193*, 137-153. https://doi.org/10.1016/j.jmarsys.2019.01.007
80. Albay, M. MARMARA DENİZİ'NİN MÜSİLAJ SORUNU (The Mucilage Problem of the Marmara Sea, in Turkish), 1st ed.; Istanbul University Press: Istanbul, Turkey, 2023; pp. 1-405. Doi: 10.26650/B/LS32.2023.002.11
81. Yagci, A.L.; Colkesen, I.; Kavzoglu, T.; Sefercik, U.G. Daily monitoring of marine mucilage using the MODIS products: a case study of 2021 mucilage bloom in the Sea of Marmara, Turkey. *Environ Monit Assess* **2022**, *194*, 170. https://doi.org/10.1007/s10661-022-09831-x
82. Kavzoglu, T.; Goral, M. Google Earth Engine for Monitoring Marine Mucilage: Izmit Bay in Spring 2021. *Hydrology* **2022**, *9*, 135. https://doi.org/10.3390/hydrology9080135