

Article

Tracking the Spatio-Temporal Distribution of Organic Particles to Predict Macroaggregation in the Northern Adriatic Sea

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Abstract: In the last two decades, the phenomenon of macroaggregation has become more frequent in the Mediterranean Sea and beyond, but it has disappeared in the northern Adriatic. The aim of this study was to present, for the first time, the concentration of surface-active particles (SAP) at selected sampling stations in the northern Adriatic Sea, as well as phytoplankton and chlorophyll data and physical properties of seawater, in order to investigate the role of SAP in macroaggregation. We used an electrochemical method (polarography and amperometry) to directly and throughput characterize fragile organic material in terms of its dissolved and particulate state in seawater samples. The results show that the concentration of SAP is subject to seasonal variations and was generally higher in the western region. In addition, an increase in surfactant activity and concentration of SAP was detected in the subsurface layer and in the stratified column prior to the mucilage event, which may be related to the very high abundance of *Skeletonema marinoi*, a diatom characteristic of the winter bloom in the region. We noted an order of magnitude higher concentration of SAP than typically observed in late winter/early spring, which serves as a precursor to the macroaggregation that occurs under certain environmental and oceanographic conditions in marine systems.

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



Citation: Kraus, R.; Ivošević DeNardis, N. Tracking the Spatio-Temporal Distribution of Organic Particles to Predict Macroaggregation in the Northern Adriatic Sea. *Water* **2023**, *15*, 1665. <https://doi.org/10.3390/w15091665>

Academic Editors: Rolf D. Vogt and Chengyun Zhou

Received: 1 March 2023

Revised: 10 April 2023

Accepted: 21 April 2023

Published: 24 April 2023



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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 [2–4].

The Adriatic Sea, a semi-enclosed sea in the northernmost part of the Mediterranean, 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 Bosphorus, and the Dardanelles Strait [6–11], 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 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 [12,15–17]. 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 microalgae 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 microalgae 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 drops in salinity or in temperature is manifested by an increase in cell stiffness or 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].

Considering that organic matter is fragile and subject to transformation at different interfaces depending on environmental conditions, it is necessary to use an analytical method that maintains the integrity of organic matter. Thus, for over three decades, our research effort was to develop a 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 that was applied to mesocosm experiments and field studies [24,28–30].

The aim of this study is to present, for the first time, the concentration of surface-active particles (SAP) at selected sampling stations in the northern Adriatic Sea and to investigate the role of SAP in the phenomenon of macroaggregation known as “mucilage events”. We used an electrochemical method (polarography and amperometry) to directly and through-put characterize fragile organic material in terms of its dissolved and particulate state in seawater samples. The results obtained are related to the phytoplankton community, chlorophyll content, and physical properties of seawater samples at six sampling stations along the Po–Rovinj transect in the NA. The results obtained in this case study could contribute to the prediction of macroaggregation under specific environmental and oceanographic conditions, which could then lead to the occurrence 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 the 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-L 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-L Van Dorn bottles at 3–5 depths (0 m, 5 m, 10 m, 20 m, and 2 m above the bottom).

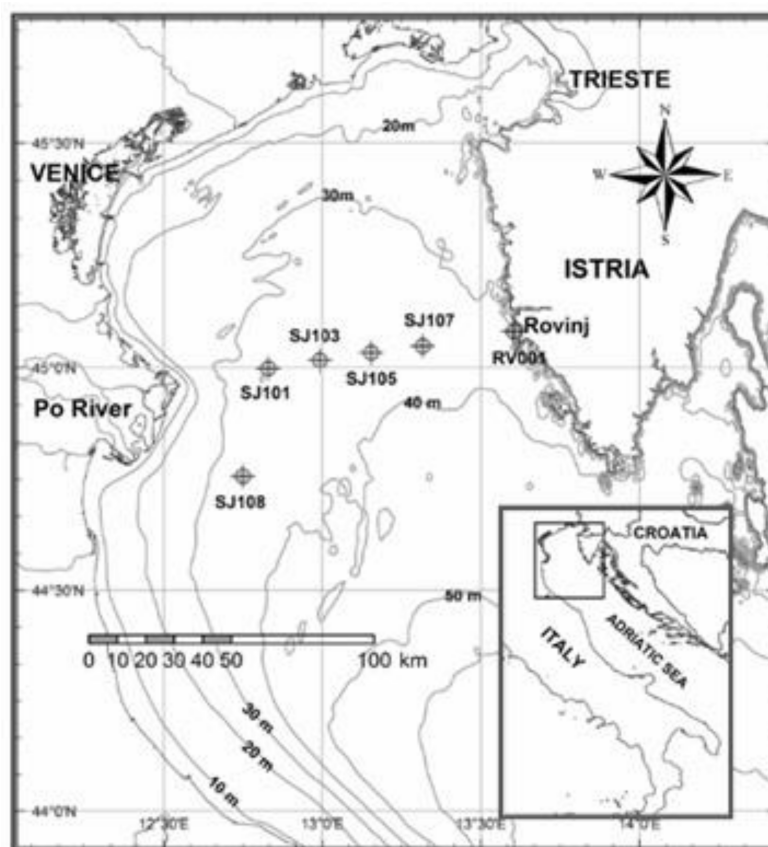


Figure 1. Map of the study area with sampling stations along the Po River Delta-Rovinj transect in the northern Adriatic, adopted from [31].

2.2. Oceanographic Measurements

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

Samples for the determination of phytoplankton species and chlorophyll-*a* as an indicator of phytoplankton biomass were prefiltered through 300 μm 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 was 78% and the detection limit was 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 $200\times$ 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]. Microphytoplankton (phytoplankton in the 20–200 μm size range) were determined at the species or genus level, whereas for the smaller ($<10 \mu\text{m}$) 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 $0.22 \mu\text{m}$ 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 h of light: 12 h of darkness at an irradiance of $31 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). The average cell number in triplicate samples was determined using a Fuchs-Rosenthal hemocytometer (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 ($2000 \times g$, 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 was registered as a well-defined amperometric signals, while the adsorption of biopolymers was 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 $20 \pm 1 \text{ }^\circ\text{C}$) 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 the dissolved organic fraction. The concentration of organic particles was determined by recording the 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.

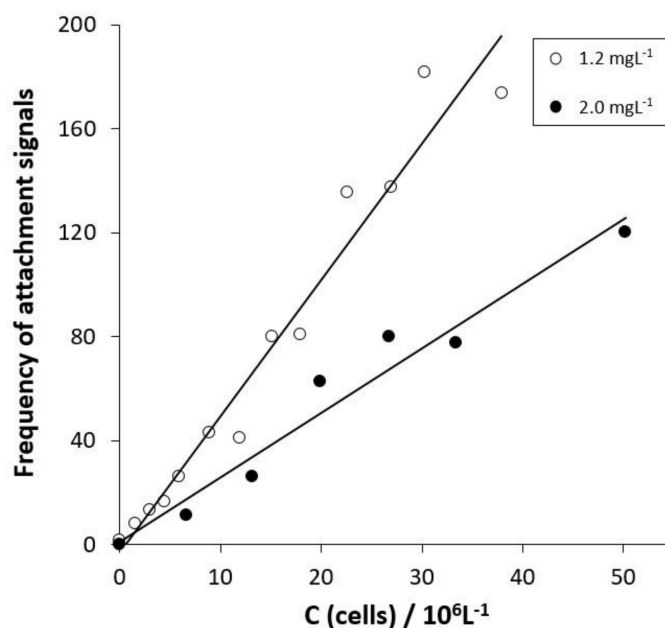


Figure 2. Calibration curves: dependence of the frequency of attachment signals on the cell abundance in seawater with a surfactant activity of 1.2 and 2.0 mg L⁻¹.

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 the 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 five or six 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 $20.5 \times 10^6 \text{ L}^{-1}$, 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 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.

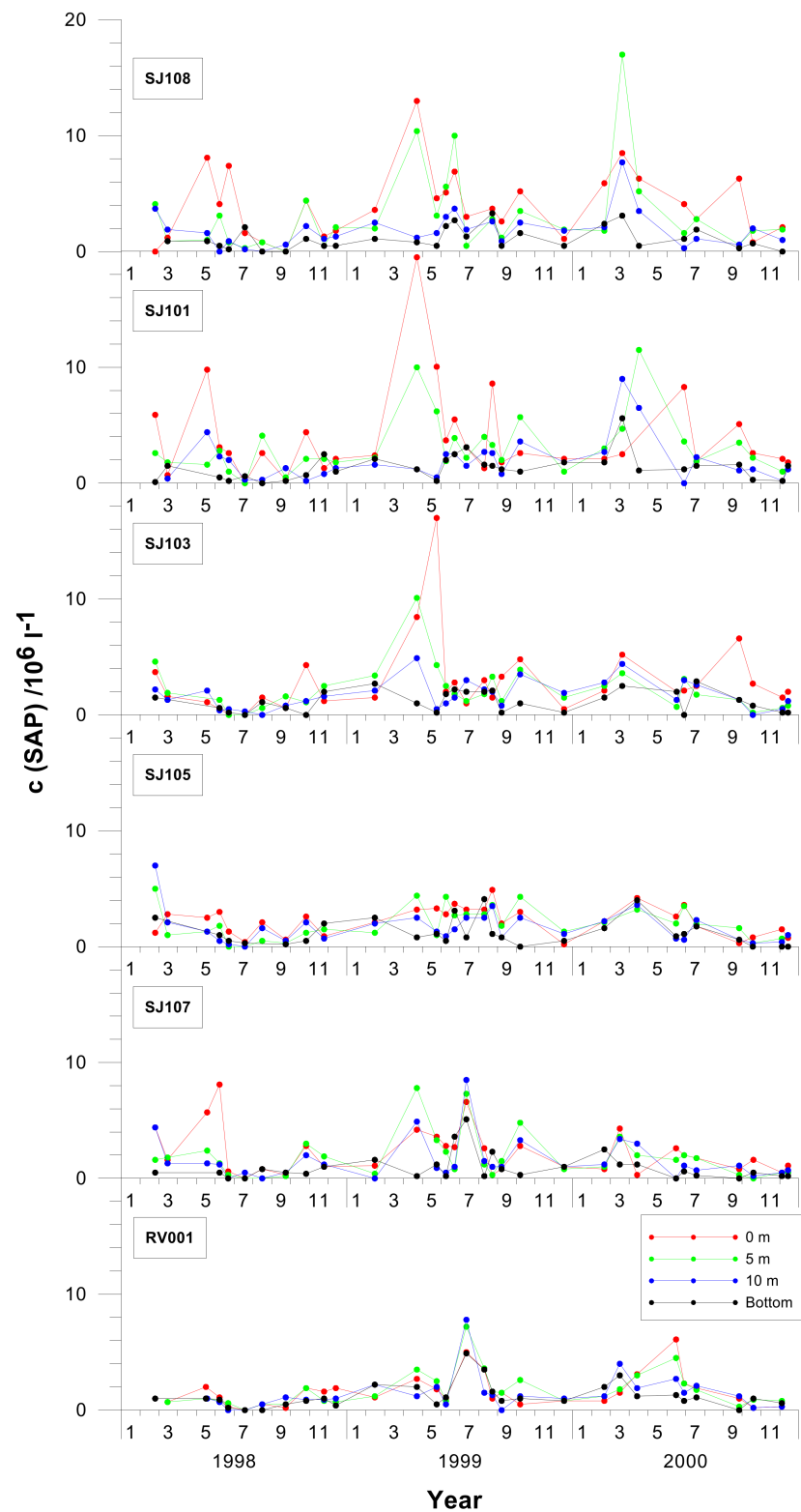


Figure 3. Spatio-temporal distribution of SAP concentration (10^6 L^{-1}) at the six stations of the Po River delta-Rovinj transect at 0 m, 5 m, and 10 m and at the bottom in the period from 1998 to 2000.

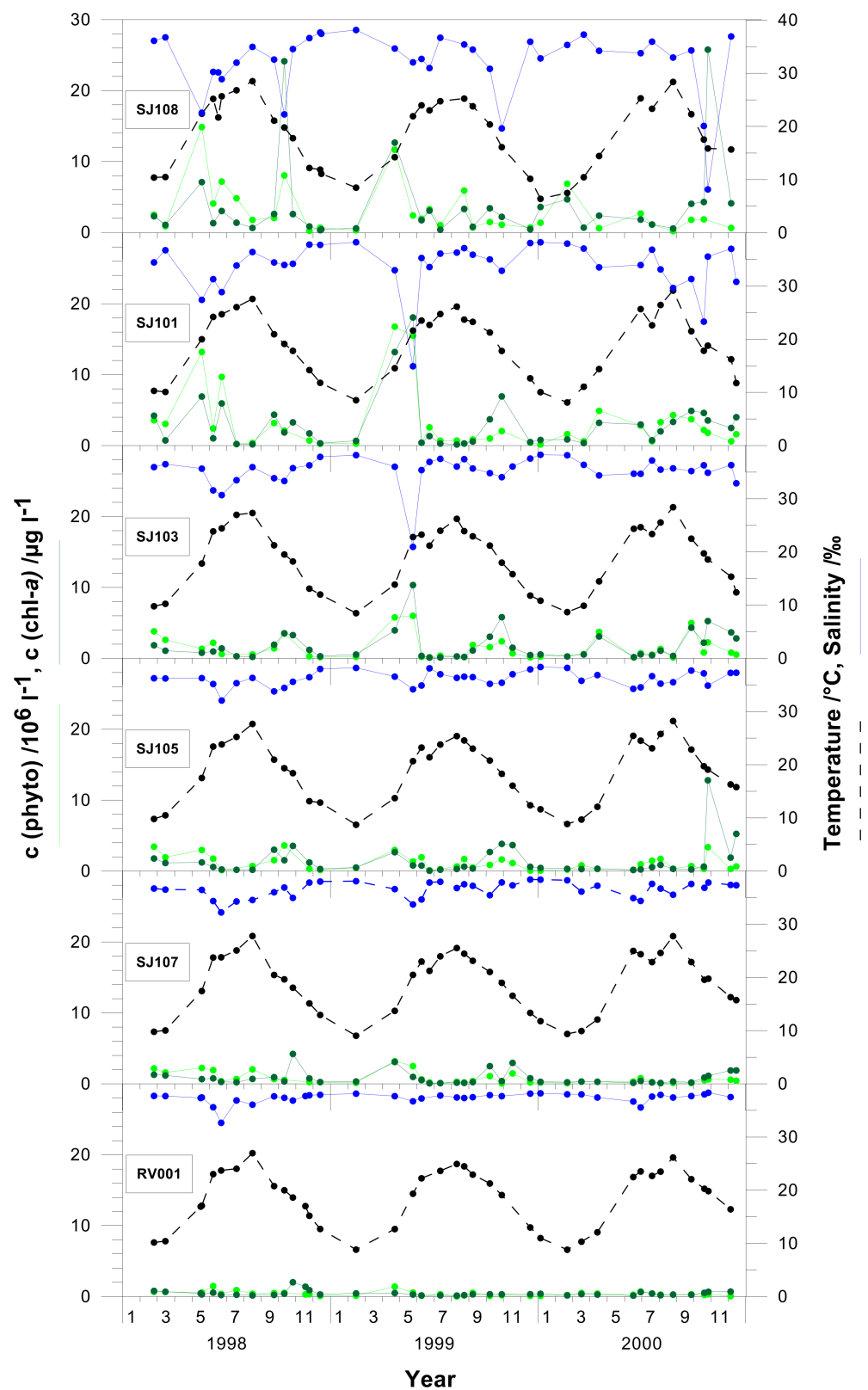


Figure 4. Spatio-temporal distribution of phytoplankton (10^6 L^{-1}), chlorophyll-*a* ($\mu\text{g L}^{-1}$), temperature ($^{\circ}\text{C}$), and salinity (‰) at the six stations of the Po River delta-Rovinj transect at 0 m, 5 m, and 10 m and at the bottom in the period from 1998 to 2000. Phytoplankton data-light green, chlorophyll-*a* data-dark green, temperature data-black, and salinity data-blue.

Temperature followed the same seasonal pattern at all stations, varying from 6.45 °C (SJ108, 5 January 2000, 0 m) to 29.15 °C (SJ101, 25 August 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, an extreme salinity minimum of 8.17‰ was observed at the surface of SJ108 on 25 October 2000, and a maximum of 38.42‰ in 0–10 m layer at SJ107 on 18 December 1999. Moreover, the variations of salinity in the eastern region were not pronounced during the three-year period.

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).

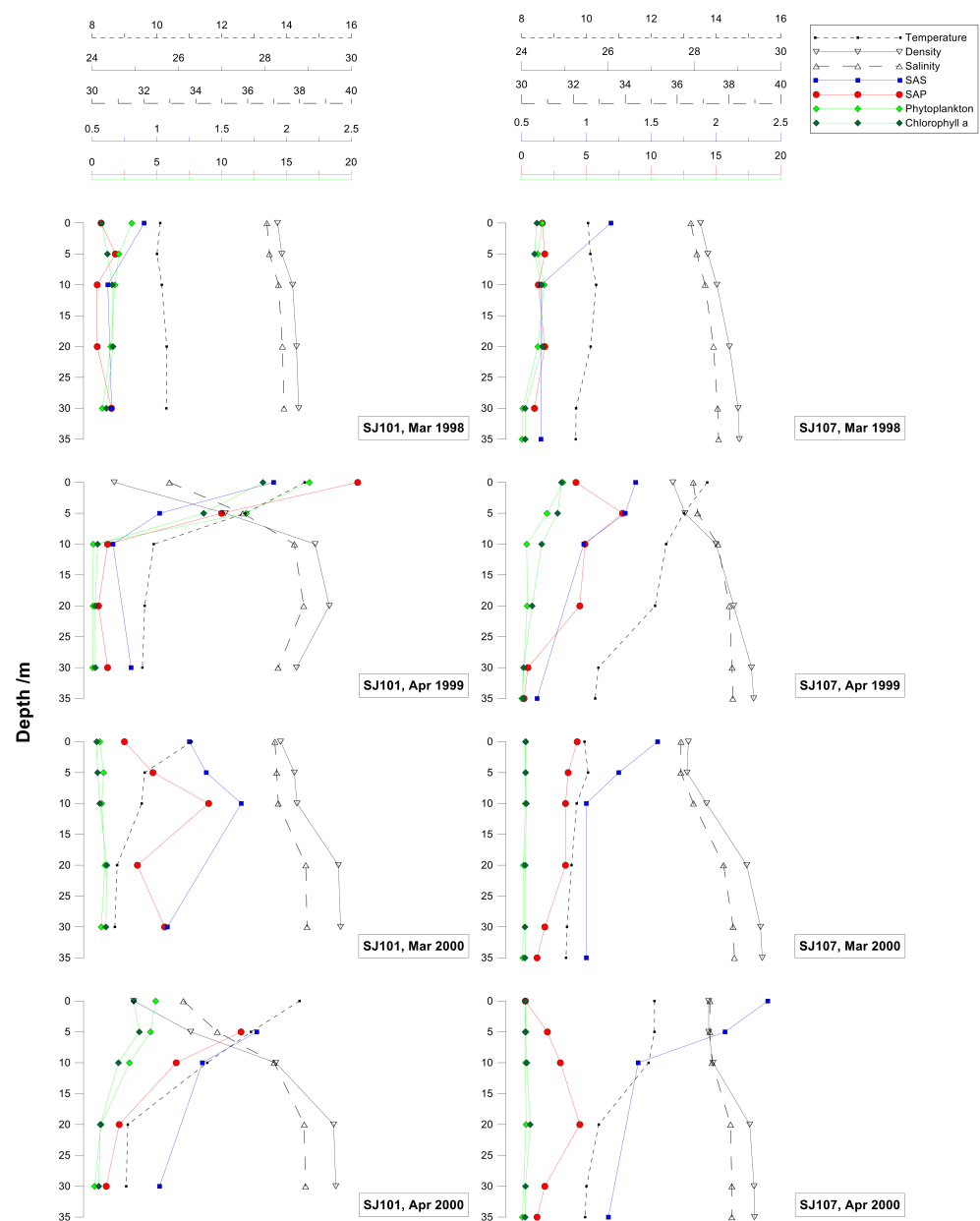


Figure 5. Vertical distribution of SAP (10^6 L^{-1}), SA (mg L^{-1}), phytoplankton (10^6 L^{-1}), chlorophyll-*a* ($\mu\text{g L}^{-1}$), temperature ($^\circ\text{C}$), density (kg m^{-3}), and salinity (‰) at stations SJ101 and SJ107 in March 1998, April 1999, March 2000, and April 2000.

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, 0 m, 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 1.68 × 10⁷ cells L⁻¹, chlorophyll-*a* 13.19 µg L⁻¹, SAP 2.1 × 10⁷ L⁻¹, and SA 1.9 mg L⁻¹, while the values from 10 m to the bottom were much lower (1 × 10⁵ cells L⁻¹, 0.4 µg L⁻¹, 1.2 × 10⁶ L⁻¹, and 0.8 mg L⁻¹, respectively). The 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.

In March 2000, we note that high concentrations of SAP and SA at 10 m depth at SJ101 (9 × 10⁶ L⁻¹ and 1.7 mg L⁻¹, respectively) were accompanied by rather low phytoplankton and chlorophyll-*a* (7.5 × 10⁵ 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: 5 × 10⁶ L⁻¹, 3.7 µg L⁻¹, 6.5 × 10⁶ 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 × 10⁶ L⁻¹ at the surface to 4.5 × 10⁶ L⁻¹ at 20 m depth and a decrease to 1.2 × 10⁶ 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).

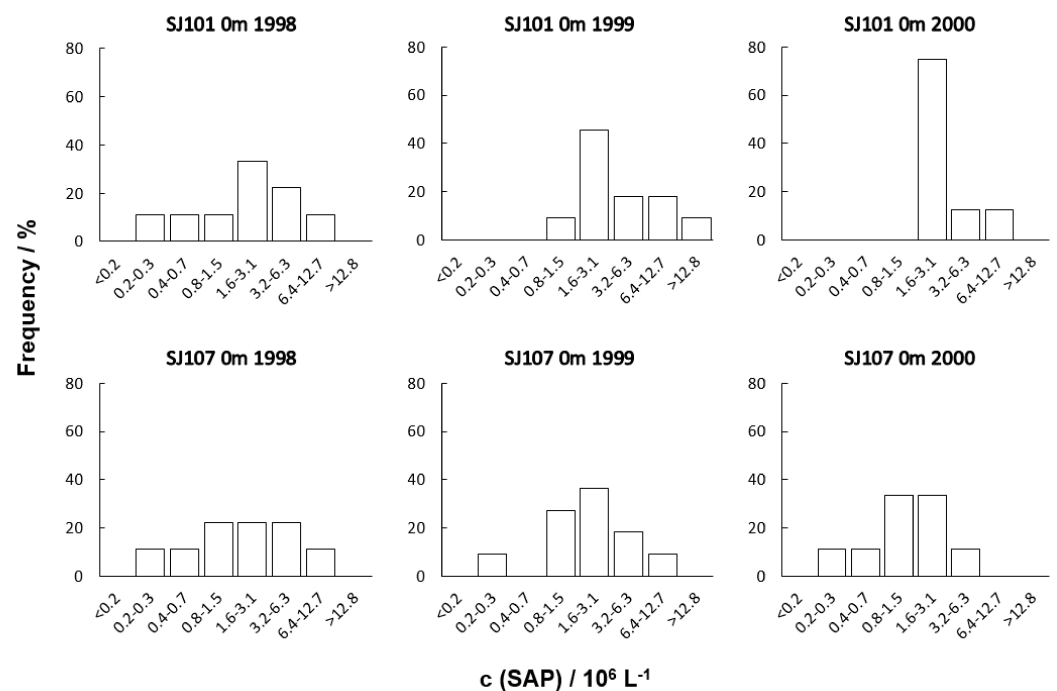


Figure 6. Frequency distribution (%) of concentration of SAP (10⁶ L⁻¹) at 0 m depth from SJ101 and SJ107 in 1998, 1999, and 2000.

At SJ101, the concentrations of SAP from 1998 to 2000 were mainly $1.6\text{--}3.1 \times 10^6 \text{ L}^{-1}$ with an increasing abundance from 33 to 45 and 75%. The low SAP concentrations ($<1.5 \times 10^6 \text{ L}^{-1}$) on SJ 101 were not detected. In contrast, a wide variety of concentrations were detected on SJ107. For SJ107, the distribution was 22% and evenly represented in three concentration classes, 0.8–1.5, 1.6–3.1, and $3.2\text{--}6.3 \times 10^6 \text{ L}^{-1}$, 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 ($1.5 \times 10^5 \text{ cells L}^{-1}$), while in March 1998 in SJ101 and in February and March in SJ107, diatom *Nitzschia delicatissima* complex (*N. delicatissima* complex) was the dominant diatom (5.9×10^4 , 9.3×10^4 , and $1.2 \times 10^5 \text{ cells L}^{-1}$, respectively).

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 of at least 80%.

Station	Date	Depth	Species	Species Abundance	Total Abundance	Species Contribution	Cumulative (%)
SJ101	25 Feb 1998	0	<i>Skeletonema marinoi</i>	748,140	1,146,260	65.3	65.3
			<i>Nitzschia delicatissima</i> complex	290,820		25.4	90.6
		5	<i>Nitzschia delicatissima</i> complex	928,700	1,735,300	53.5	53.5
<i>Skeletonema marinoi</i>	715,580	41.2	94.8				
SJ101	17 Mar 1998	0	<i>Nitzschia delicatissima</i> complex	59,570	61,050	97.6	97.6
		5	<i>Nitzschia delicatissima</i> complex	39,590		41,810	94.7
SJ101	15 Feb 1999	0	<i>Skeletonema marinoi</i>	85,470	125,800	67.9	67.9
		<i>Nitzschia delicatissima</i> complex	28,120	22.4		90.3	
SJ101	24 Apr 1999	0	<i>Skeletonema marinoi</i>	6,926,400	15,258,800	45.4	45.4
			<i>Nitzschia delicatissima</i> complex	5,220,700		34.2	79.6
		<i>Chaetoceros</i> sp.	1,713,100	11.2		90.8	
SJ101	21 Feb 2000	5	<i>Nitzschia delicatissima</i> complex	657,860	990,120	66.4	66.4
			<i>Skeletonema marinoi</i>	227,920		23.0	89.5
		10	<i>Skeletonema marinoi</i>	14,430	31,080	46.4	46.4
<i>Nitzschia delicatissima</i> complex	13,690	44.0	90.5				
SJ101	21 Mar 2000	0	<i>Chaetoceros</i> sp.	10,730	21,460	50.0	50.0
			<i>Skeletonema marinoi</i>	4440		20.7	70.7
		5	<i>Chaetoceros</i> sp.	14,060		38,110	36.9
<i>Skeletonema marinoi</i>	8510	22.3	59.2				
<i>Nitzschia delicatissima</i> complex	7400	19.4	78.6				
<i>Chaetoceros curvisetus</i>	6660	17.5	96.1				
SJ101	17 Apr 2000	0	<i>Nitzschia delicatissima</i> complex	29,230	48,840	59.8	59.8
			<i>Skeletonema marinoi</i>	10,360		21.2	81.1
		<i>Chaetoceros curvisetus</i>	5180	10.6		91.7	
SJ101	17 Apr 2000	0	<i>Nitzschia delicatissima</i> complex	102,490	113,960	89.9	89.9
		5	<i>Nitzschia delicatissima</i> complex	156,140		95.5	95.5
		10	<i>Nitzschia delicatissima</i> complex	33,670		39,220	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 Feb 1998	0	<i>Nitzschia delicatissima</i> complex	93,240	124,320	75.0	75.0
			<i>Rhizosolenia fragilissima</i>	13,320		10.7	85.7
		5	<i>Nitzschia delicatissima</i> complex	386,280	481,000	80.3	80.3
			<i>Rhizosolenia fragilissima</i>	86,580		18.0	98.3
		10	<i>Nitzschia delicatissima</i> complex	123,210	140,970	87.4	87.4
SJ107	17 Mar 1998	0	<i>Nitzschia delicatissima</i> complex	123,580	127,280	97.1	97.1
		5	<i>Nitzschia delicatissima</i> complex	66,970	71,780	93.3	93.3
		10	<i>Nitzschia delicatissima</i> complex	94,720	99,160	95.5	95.5
SJ107	15 Feb 1999	0	<i>Skeletonema marinoi</i>	3700	12,210	30.3	30.3
			<i>Chaetoceros affinis</i>	1480		12.1	42.4
			<i>Chaetoceros curvisetus</i>	1480		12.1	54.5
			<i>Nitzschia tenuirostris</i>	1480		12.1	66.7
		5	<i>Skeletonema marinoi</i>	8880	13,320	66.7	66.7
			<i>Nitzschia tenuirostris</i>	2220	16.7	83.3	
	10	<i>Skeletonema marinoi</i>	14,060	17,390	80.9	80.9	
SJ107	24 Apr 1999	0	<i>Chaetoceros</i> sp.	364,450	836,200	43.6	43.6
			<i>Nitzschia delicatissima</i> complex	223,850		26.8	70.4
		5	<i>Nitzschia delicatissima</i> complex	246,420	695,600	35.4	35.4
			<i>Skeletonema marinoi</i>	155,400	22.3	57.8	
			<i>Chaetoceros socialis</i>	138,380	19.9	77.7	
			<i>Chaetoceros curvisetus</i>	76,220	11.0	88.6	
		10	<i>Nitzschia delicatissima</i> complex	109,520	204,240	53.6	53.6
<i>Skeletonema marinoi</i>	47,360		23.2	76.8			
SJ107	21 Feb 2000	0	<i>Chaetoceros curvisetus</i>	492	1476	33.3	33.3
			<i>Nitzschia delicatissima</i> complex	246		16.7	50.0
			<i>Nitzschia tenuirostris</i>	246		16.7	66.7
			<i>Prorocentrum micans</i>	246		16.7	83.3
		5	<i>Nitzschia tenuirostris</i>	555	1850	30.0	30.0
			<i>Melosira dubia</i>	370	20.0	50.0	
			<i>Nitzschia delicatissima</i> complex	370	20.0	70.0	
			<i>Achmanthes</i> sp.	185	10.0	80.0	
		10	<i>Nitzschia tenuirostris</i>	246	861	28.6	28.6
			<i>Achmanthes</i> sp.	123	14.3	42.9	
			<i>Mastogloia</i> sp.	123	14.3	57.1	
			<i>Navicula</i> sp.	123	14.3	71.4	
			<i>Nitzschia delicatissima</i> complex	123	14.3	85.7	
SJ107	17 Mar 2000	0	<i>Skeletonema marinoi</i>	75,110	105,080	71.5	71.5
			<i>Chaetoceros</i> sp.	17,760		16.9	88.4
		5	<i>Skeletonema marinoi</i>	81,770	92,500	88.4	88.4
	10	<i>Skeletonema marinoi</i>	36,260	45,140	80.3	80.3	
SJ107	14 Apr 2000	0	<i>Nitzschia delicatissima</i> complex	555	1110	50.0	50.0
			<i>Prorocentrum micans</i>	370		33.3	83.3
		5	<i>Nitzschia delicatissima</i> complex	1353	1353	100.0	100.0
10	<i>Nitzschia delicatissima</i> complex	1110	1665	66.7	66.7		
	<i>Glenodinium</i> sp.	185	11.1	77.8			
	<i>Gymnodinium</i> sp.	185	11.1	88.9			

In February 1999, *S. marinoi* was dominant in SJ101 (8.5×10^4 cells L⁻¹), while the phytoplankton community in SJ107 had no particular dominant species and total abundance was low (3.7×10^3 cells L⁻¹). In April 1999, an exceptionally intense diatom bloom was observed in SJ101, where the *N. delicatissima* complex and *S. marinoi* (5.2×10^6 and 6.9×10^6 cells L⁻¹, respectively) were jointly dominant. In comparison, the

abundance of the diatom genus *Chaetoceros* was much lower in SJ107 but also significant (3.6×10^5 cells L⁻¹).

High abundance of *S. marinoi* was observed at SJ101 in February 2000 (1.3×10^6 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 $7\text{--}8 \times 10^4$ cells L⁻¹ in March 2000.

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 show 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 meteorological 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 a massive amount of 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 anticyclonic eddies that occur under these stable conditions in NA and act as “hotspots” for macroaggregate formation [60].

Mucilage event features the 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 originates from diatoms [27,60–64] and is formed during phosphorus-limited growth (e.g., [62]) 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. On the 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 ($\sim 2 \times 10^7 \text{ L}^{-1}$) is reached, a phase transition from dispersed microparticles to a gel state occurs when supported by meteorological and hydrodynamic conditions [22]. Our results showed that the critical concentration of SAP was reached in April 1999 and that no mucilage event did occur (Figure 5). In contrast, the concentration of SAP reached only $1.15 \times 10^7 \text{ L}^{-1}$ in April 2000, but a 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, a 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 provided further confirmation of the hypothesis that diatoms contribute significantly to the formation of macroaggregates [70].

It should be noted that phytoplankton populations contribute significantly to the DOM pool through their activity and decay as shown by Marty and 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, the 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 the abundance has declined, which are 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 in the NA. Based on the reported evidence and our results, the mucilage event in the NA could be predicted by (i) specific oceanographic conditions that maintain stratification and limited water exchange in winter, (ii) a winter diatom bloom that secretes polysaccharides, and (iii) a concentration of SAP that is an order of magnitude higher 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.

Finally, 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 has 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. There are numerous scientific efforts to use modeling approaches to monitor climate changes that affect water quality and ecosystem changes [83–87]. These methods could potentially be applied to the mucilage preconditioning phase as well. The

integration of a comprehensive data set consisting of experimental data, mathematical models, meteorological data, oceanographic conditions, circulation patterns, and satellite data may contribute to a better understanding of the dynamics of organic matter in aquatic systems applicable to marine environmental protection and coastal management.

5. Conclusions

For the first time, the spatio-temporal distribution of surface-active particles in the northern Adriatic was studied over a 3-year period. The results show that the concentration of SAP is subject to seasonal variations and usually is higher in spring/summer and early fall, probably because of the increased abundance of phytoplankton and favorable growing temperature. Additionally, the concentration of SAP is usually higher in the western (SJ108, SJ101) than the eastern sampling stations (SJ107, RV001), probably related to the inflow from the Po River Delta. In addition, an increase in surfactant activity and concentration of SAP was observed in the subsurface layer and in the stratified column before the appearance of mucilage. This increase can be associated with a very high abundance of *Skeletonema marinoi*, a diatom characteristic of the winter bloom in the northern Adriatic. 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 concentration of SAP in seawater that is an order of magnitude than typically observed in late winter/early spring.

Author Contributions: R.K. and N.I.D. contributed equally to the work. All authors have read and agreed to the published version of the manuscript.

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; (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 Robert Precali. Special thanks to 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 Vera Žutić and Vesna Svetličić for coordinating the study of organic microparticles within 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.

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