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Phytoplankton and environmental drivers at a long-term offshore station in the northern Adriatic Sea (1988–2018)

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21 **Abstract**

22 A long term (1988–2018) data set of phytoplankton and physico-chemical parameters was analyzed
23 for the first time in an offshore station of the LTER Senigallia-Susak transect (northern Adriatic Sea)
24 not directly affected by the coastal nutrient input. The mean annual cycle of phytoplankton markedly
25 differed from that observed in the coastal areas, showing the maximum in June and the minimum in
26 November. The main component of phytoplankton community was represented by phytoflagellates,
27 whose trend paralleled that of total phytoplankton. On average, diatoms peaked in July,
28 dinoflagellates in June and coccolithophores in April. The phytoplankton maximum in summer is
29 explained mainly by the allochthonous input of DIN and PO₄ carried by the low salinity waters
30 expanding eastward, during the stratification of water column. Resuspension processes seem to be
31 less effective for PO₄ than for DIN. The most representative phytoplankton taxa for each season as
32 indicated by the IndVal analysis were identified and were only in part similar to those observed in
33 the coastal area. The interannual anomaly trend showed a significant increase of temperature, DIN
34 and phosphates. Although no significant changes were found for the total phytoplankton, a reduction
35 of winter dinoflagellate and coccolithophore abundances was observed.

36

37 **Keywords**

38 Phytoplankton, interannual variability, LTER-long-term ecosystem research, biodiversity,
39 biological oceanography

40 **1. Introduction**

41 Phytoplankton community dynamics are considered as a key indicator to detect changes in the marine
42 ecosystems as they show a rapid turnover, and they are directly related to the abiotic parameters. For
43 these reasons, phytoplankton diversity and temporal changes are included in the Marine Strategy
44 Framework Directive (MSFD) as an indicator for the Good Environmental Status assessment (MSFD,
45 2008/56/EC). In the light of this, long-term studies focusing on the status of biological communities
46 and environmental conditions in pelagic habitats are strategic and precious tools, as they allow to
47 detect changes and trends and they serve as baseline for the GES definition.

48 The Northern Adriatic (NA), one of the most productive areas of the Mediterranean (D'Ortenzio and
49 Ribera d'Alcalà, 2009), is highly influenced by river floods that affect both the circulation and the
50 trophic state (Campanelli et al., 2011; Cozzi and Giani, 2011), by introducing a large amount of both
51 inorganic and organic nutrients (Brush et al., 2021; Degobbis et al., 2000; Marini et al., 2008). These
52 low-salinity and nutrient-rich waters affect the NA trophic state in different ways depending on the
53 stability conditions of water column: during the stratification regime (spring-summer), the Po River
54 outflow spreads horizontally at the surface layer forming a plume that affect a great part of the NA
55 (Viličić et al., 2013), while during mixing regime (autumn-winter) the plume is less pronounced, and
56 the freshwater input is mainly deviated southward by the Western Adriatic Current (WAC). As a
57 result, the position of frontal system, separating the low salinity eutrophic waters from the high
58 salinity oligotrophic ones, is highly dynamic and dependent on the above-mentioned conditions
59 (Franco and Michelato, 1992). Another factor affecting the nutrient cycle in the NA is the general
60 water masses' circulation that is strongly influenced by winds, with significant differences between
61 winter, mainly Sirocco (SE) and Bora (NE) and summer mainly Mistral (NW) (Russo and Artegiani,
62 1996). In particular, the cold Bora wind, occurring frequently in fall and winter seasons, plays a
63 significant role in determining the cooling and mixing of the water column and in the formation of
64 Northern Adriatic Dense Water (NAdDW), flowing then southward (Bergamasco et al., 1999; Marini
65 et al., 2006; Vilibić and Supić, 2005).

66 In the Adriatic Sea, four subregions have been recognized so far, based on their differences in terms
67 of trophic state, phytoplankton biomass and annual trend (Fonda Umani, 1996; Franco and Michelato,
68 1992): (i) the NA, including a narrow coastal belt along the western coast, which is characterized by
69 high production, (ii) the open waters characterized by low production, (iii) the eastern coastal waters
70 with moderate production and (iv) the zones (lagoons and embayments) under strong coastal
71 influences.

72 The bulk of knowledge about phytoplankton trends and community composition of the NA is
73 concentrated in coastal areas of both western (Bernardi Aubry et al., 2004, 2006, 2012; Penna et al.,
74 2004; Pugnetti et al., 2004, 2008; Totti et al., 1999, 2005; 2019; Zoppini et al., 1995) and eastern
75 (Cabrini et al., 2012; Cerino et al., 2019; Fuks et al., 2012; Mozetič et al., 1998, 2010, 2012; Viličić
76 et al., 2009) sides, where monitoring activities are easier and more frequent than offshore, where data
77 are collected with an irregular frequency. The phytoplankton annual cycle varies among the NA areas.
78 Along the northwestern coast, there is an intense diatom winter bloom, which is responsible for the
79 highest values of abundance and biomass of the year, followed by classic spring and autumn blooms
80 (Bernardi Aubry et al., 2004; Totti et al., 2019). Instead, the eastern part of the NA is characterized
81 by typical spring and autumn blooms (Cerino et al., 2019; Marić et al., 2012). Anyway, at interannual
82 scale, several studies have reported changes in the phytoplankton annual maximum and shifts in the
83 seasonality of blooms in the entire NA (Cerino et al., 2019; Marić et al., 2012; Mozetič et al., 2010;
84 Totti et al., 2019).

85 The Senigallia-Susak transect (SS), located in the lower part of the NA, represents a LTER (Long-
86 Term Ecosystem Research) site, where physico-chemical parameters and phytoplankton abundance,
87 biomass and species composition have been recorded since 1988. In the SS, the WAC is sharper than
88 in the northernmost part of the NA (Russo and Artegiani, 1996), and the position of the frontal system
89 separating nutrient rich coastal waters from oligotrophic offshore waters is located near the 10 nM.
90 In this study, we analysed a long-term data set referred to a station located at 15 nM, beyond the
91 border of the WAC, with the aim to depict (i) the temporal variability of physico-chemical parameters
92 and phytoplankton both at an intra-annual and interannual scale, (ii) the phytoplankton community

composition and (iii) the relationships between phytoplankton and environmental parameters in an offshore area not directly affected by the coastal nutrients input.

2. Materials and Methods

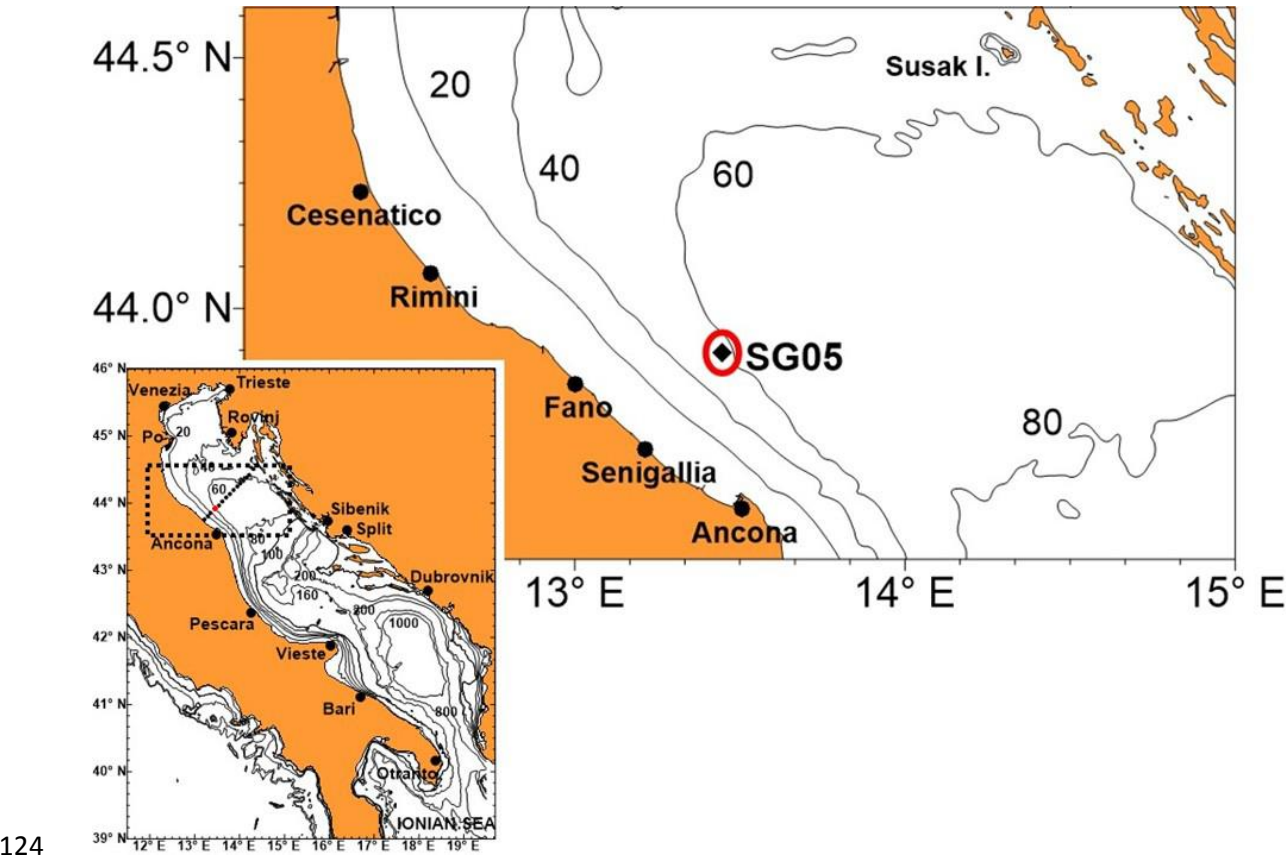
2.1 Sampling

Sampling was carried out at the SG05 station (43°54.460 N, 13°26.941 E) (bottom depth 55 m) located at 15 nM from the Italian coast (Fig.1) on board of several oceanographic vessels (S. Lo Bianco, G. Dallaporta, Tethis, Copernaut Franca, Urania, Alliance, Minerva, Bannock, D'Ancona) since July 1988 to December 2018 with a variable time frequency (from monthly to quarterly) and with periods of interruptions the longest of which was between 2003 and 2012 for phytoplankton sampling. A total of 11,339 samples were collected. Sampling dates for each parameter are indicated in the Table S1.

From 1988 to 1991, Conductivity-Temperature-Depth (CTD) data were acquired by a Neil Brown Instrument System (NBIS), while after 1992 with a SeaBird Electronic SBE 911plus. Dissolved oxygen was directly analyzed on board according to Winkler (1888), and samples were immediately fixed and stored in the dark and analyzed within 24 h. From 1988 to 1998 the oxygen concentrations were determined using titration and then after 1998 by potentiometric method (Furuya and Harada, 1995).

Water samples for determination of dissolved inorganic nutrients (nitrite-NO₂, nitrate-NO₃, ammonia-NH₄, orthophosphate-PO₄ and orthosilicate-Si(OH)₄) and for phytoplankton analysis were collected by Rosette system equipped with Niskin bottles. Filtration was done directly on board and significant sample alterations were excluded as: (i) as soon as the Niskin bottle was on board, water was sampled with a closed filtration system without any contact with air, (ii) the filtrate was closed in a 4 ml compressed polyethylene tube and rapidly frozen, (iii) unfreezing phase was as rapidly and the analysis was done directly on the tube, without further pouring. Sampled quotes for nutrients and phytoplankton were: surface (0-5 m layer), 1-2 intermediate depths, generally at the base of mixed layer and the maximum fluorescence depth (between 10 and 40 m) and 1 m above the bottom (54 m).

120 Samples for nutrient analysis were filtered (GF/F Whatman, 0.7 μm), and stored at $-22\text{ }^{\circ}\text{C}$ in
 121 polyethylene vials until analysis. Water samples for phytoplankton analysis were collected in 250 ml
 122 dark glass bottles and preserved by adding 0.8% formaldehyde (prefiltered and neutralized with
 123 hexamethylenetetramine) (Throndsen, 1978) until analysis.



125 Fig. 1. Map of the study area indicating the position of sampling station. In the small box, the
 126 Senigallia-Susak transect is shown and the study station is indicated by the red color.

128 2.2 Nutrient analysis

129 Nutrient analysis was carried out using a Perkin Elmer spectrophotometer 550A model in the period
 130 1988 to 1998, an autoanalyzer TRAACS 800 BRAN+LUEBBE in 1999 to 2005 and an autoanalyzer
 131 QUAATRO Technicon (Strickland and Parsons, 1972) after 2005. Dissolved Inorganic Nitrogen
 132 (DIN) is intended as the sum of NO_2 , NO_3 and NH_4 concentrations.

134 2.3 Phytoplankton analysis

135 Identification and counting of phytoplankton were carried out using an inverted microscope (ZEISS
136 Axiovert 135) equipped with phase contrast, following the Utermöhl method (Edler and Elbrachter,
137 2010). Counting was carried out at 400x magnification, along transects or in random visual fields,
138 depending on cell abundance, to count a minimum of 200 cells. Moreover, a half of the Utermöhl
139 chamber was analyzed at 200x magnification for a more precise estimation of less abundant
140 microphytoplanktonic taxa.

141 Phytoplankton taxa were grouped into major groups (diatoms, dinoflagellates, coccolithophores,
142 phytoflagellates and others), and abundances were expressed as cells l⁻¹. Biomass measurements
143 through biovolumes did not cover all the sampling period, therefore they were not considered in this
144 study. Dinoflagellates were considered as a taxonomical group and both autotrophic and heterotrophic
145 species were included in counting. Phytoflagellates are an informal group that includes haptophytes
146 (except coccolithophores), cryptophytes, chrysophytes, dictyochophytes, raphidophytes,
147 chlorophytes and euglenophytes. Others include cyanophytes and *incertae sedis*.

148

149 2.4 Statistical analysis

150 The surface layer (0-5 m) was considered for the statistical analysis. The interannual anomalies of
151 physico-chemical parameters were calculated by the difference between each monthly value and the
152 mean monthly value of the entire series. Then linear regression was calculated by means of the
153 ordinary least square method. Unfortunately, due to the interruption in the phytoplankton sampling
154 from 2003 to 2012, we could not analyze the interannual variability of phytoplankton using the same
155 method as for physico-chemical parameters. Therefore, we compared, in terms of phytoplankton
156 abundances, two periods (1988–2002 and 2013–2018), by a one-way analysis of variance (ANOVA).
157 Abundances were ranked transformed prior to analysis. When significant differences for the main
158 effect were observed ($p < 0.05$), a Tukey's pairwise comparison test was also performed.

159 Relationships between environmental parameters and phytoplankton group abundances were studied
160 through Principal Component Analysis (PCA) performed on a correlation matrix of ranked monthly
161 values of physico-chemical parameters, as active variables, and phytoplankton group abundances, as

162 supplementary variables. Furthermore, Pearson's correlations were performed on the same ranked-
163 transformed variables.

164 The above statistical analyses were conducted using Statistica 12 (StatSoft Inc., Tulsa, OK, USA)
165 software.

166 To identify phytoplankton key species, the Indicator Value (IndVal) was applied, which combines
167 the relative abundance of a species with its relative frequency of occurrence in a given period
168 (Dufrêne and Legendre, 1997). The IndVal was here calculated on a seasonal basis, considering
169 climatological seasons, as done in other oceanographic studies (Bernardi-Aubry et al., 2006; Grilli et
170 al., 2005, 2020; Totti et al., 2019): winter = January, February, March; spring = April, May, June;
171 summer = July, August, September; autumn = October, November, December.

172

173 **3. Results**

174 *3.1 Physico-chemical parameters*

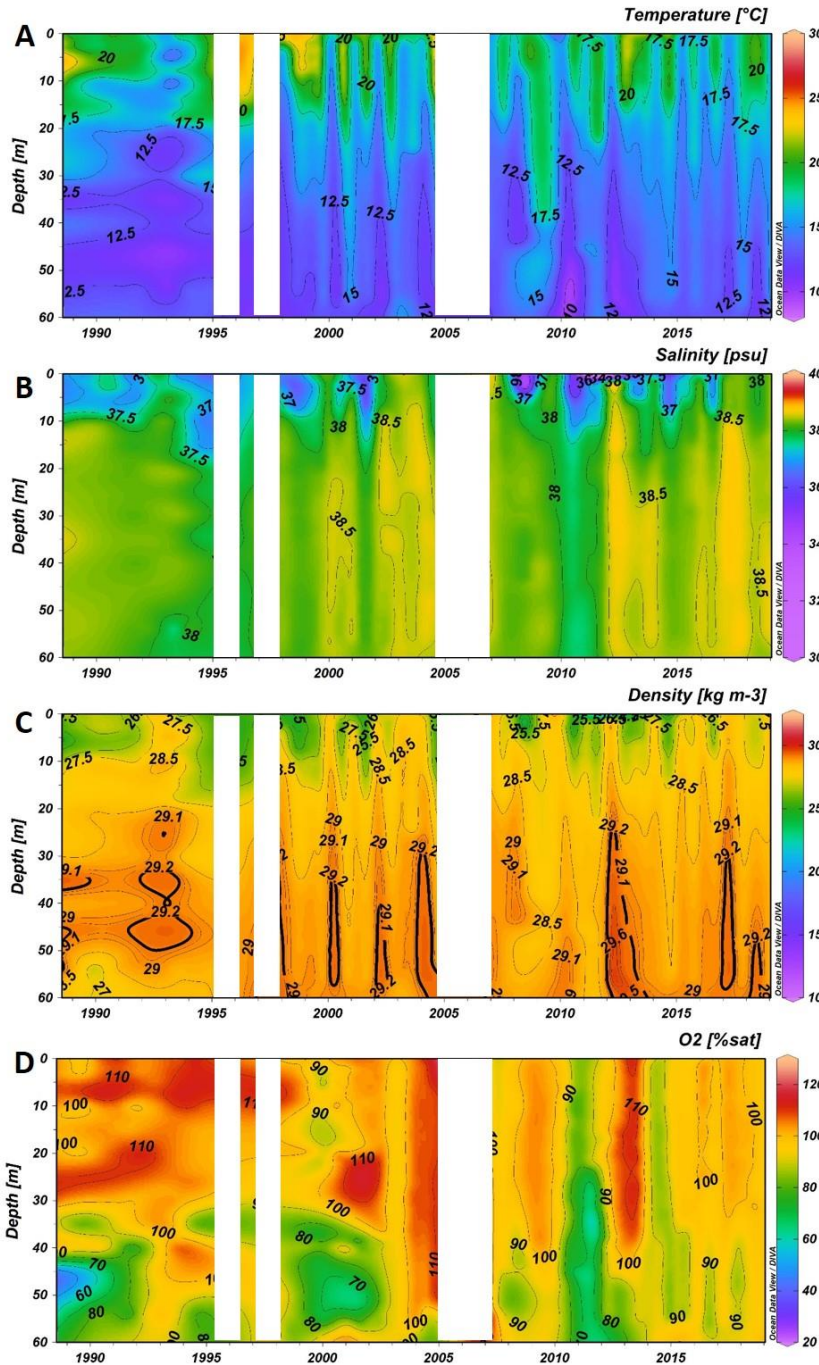
175 3.1.1 Temporal variability in the water column

176 The time evolution of physico-chemical parameters in the water column from 1988 to 2018 at the
177 station SG05 is shown in Figs. 2 and 3.

178 Temperature (Fig. 2A) showed a strong interannual signal. An overall increase of temperature can be
179 visually detected, with prolonged cooler periods in the bottom layer at the beginning of the series
180 (e.g., 1988–1994) and shorter periods of warmer temperatures more frequent in the second part of the
181 series (e.g., 2000–2001, 2007, 2014 and 2017). Maximum values (around 26–27 °C) at the surface
182 were reached in summer 1998, 2000 and 2012. Regarding the salinity, prolonged periods of high
183 salinity (i.e., >38) were observed below 10 m depth (e.g., 1988–1991, 2002–2007 and 2012–2013,
184 2015–2018), interrupted by shorter periods of lower salinity values (e.g., 1996, 1998–1999, 2001 and
185 2009–2011, 2014) (Fig. 2B).

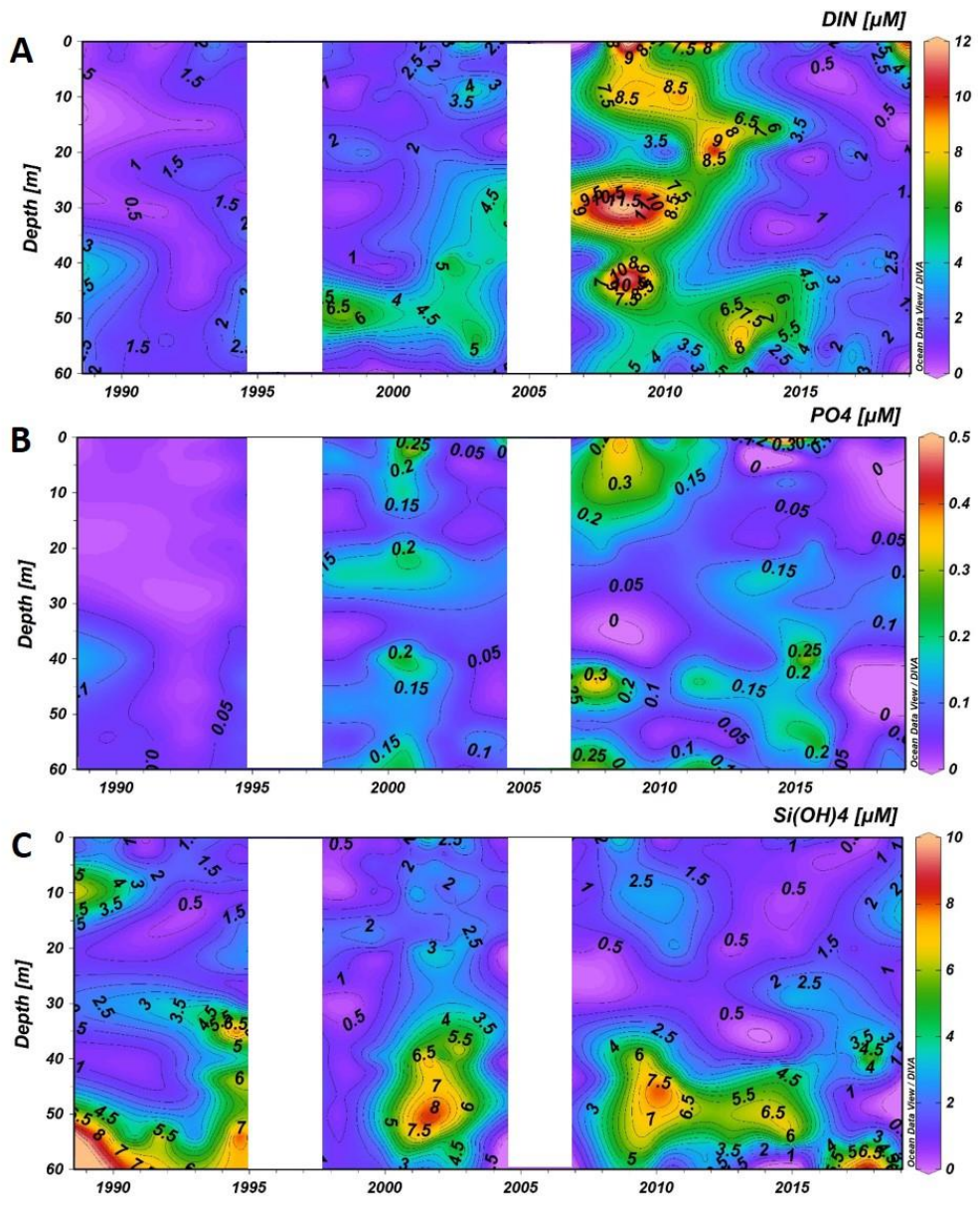
186 Seawater density (Fig. 2C) showed values higher than 29.2 kg m⁻³ (considered the threshold for the
187 Adriatic dense waters, Artegiani et al., 1989) measured from 30 m down to the bottom in winter 1993,
188 1998, 2000, 2002, 2004, 2017 and 2018. The highest value (>29.6 kg m⁻³ corresponding to NAdDW)

189 was recorded at the bottom layer in 2012 in accordance with the well documented exceptional dense
 190 water formation on the Adriatic shelf (Mihanović et al., 2013).
 191 Oxygen saturation showed values under 100% in the bottom layer of the entire series and along the
 192 water column in 1999, 2010-2011 and 2013-2014. The deeper part of the station SG05 with lower
 193 dissolved oxygen values (Fig. 2D) was characterized by higher concentration of Si(OH)_4 (Fig. 3D).



194
 195 Fig. 2. Temporal and vertical variations of (A) temperature ($^{\circ}\text{C}$), (B) salinity, (C) density (kg m^{-3}),
 196 where the 29.2 kg m^{-3} isoline is thicker marked, and (D) dissolved oxygen (% sat) at station SG05.
 197

198 Nutrient concentrations in the water column along the study period are shown in Fig. 3. High
 199 concentrations of DIN (Fig. 3A) were recorded in correspondence of lower salinity values (Fig. 2B)
 200 related to the higher Po River flow (not shown) in November 2010, May 2013, and November 2014.
 201 In winter 2012, a cold and high-salinity water mass with high DIN values was observed at 20 m depth
 202 (Fig. 3A), and this water mass extended even eastward (data not shown). Time evolution of
 203 orthophosphate (Fig. 3B) showed higher values in the last decade. High values were often observed
 204 below 40 m depth, and at 0-10 m layer in December 2007 and June 2008, corresponding to relatively
 205 low salinity values. Concentrations of silicate were higher ($> 3 \mu\text{M}$) below the 30 m depth, with peaks
 206 in 2001, 2010 and 2014. Only in 1989 a higher value ($6 \mu\text{M}$) was recorded at 10 m (Fig. 3C).



208 Fig. 3. Temporal and vertical variations of (A) Dissolved Inorganic Nitrogen (DIN), (B)
209 orthophosphate (PO_4) and (C) orthosilicate ($\text{Si}(\text{OH})_4$) concentrations (μM).
210

211 3.1.2 Mean annual cycle

212 The annual cycle of physico-chemical parameters at the surface layer is shown in Fig. 4, where mean
213 monthly values (mean \pm standard error) are shown.

214 Seawater temperature showed minimum mean values in February (11.7 ± 0.3 °C) and maximum mean
215 values in August (24.8 ± 0.4 °C) (Fig. 4A).

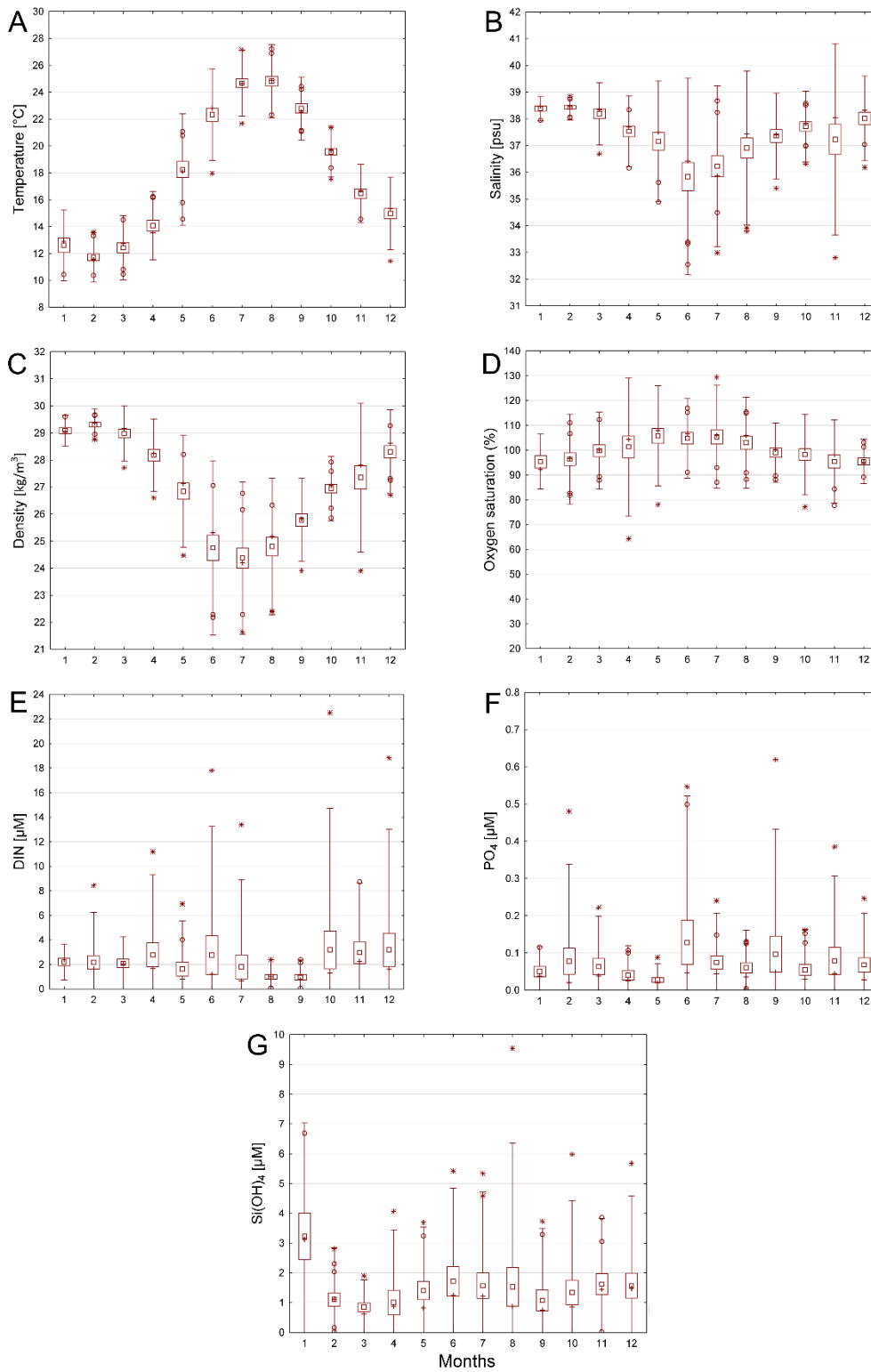


Fig. 4. Mean annual cycle of physico-chemical parameters calculated on a mean monthly basis from 1988 to 2018 in SG05 station at surface: (A) water temperature (°C), (B) salinity, (C) density (kg m⁻³), (D) oxygen saturation (%), (E) Dissolved Inorganic Nitrogen (DIN, μM), (F) orthophosphate (PO₄, μM) and (G) silicate concentrations (Si(OH)₄, μM). Box plots report the data distribution with the mean (+), the median (□), the interquartile range (box), the non-outlier range (vertical bars), the outliers (○) and the extremes (*).

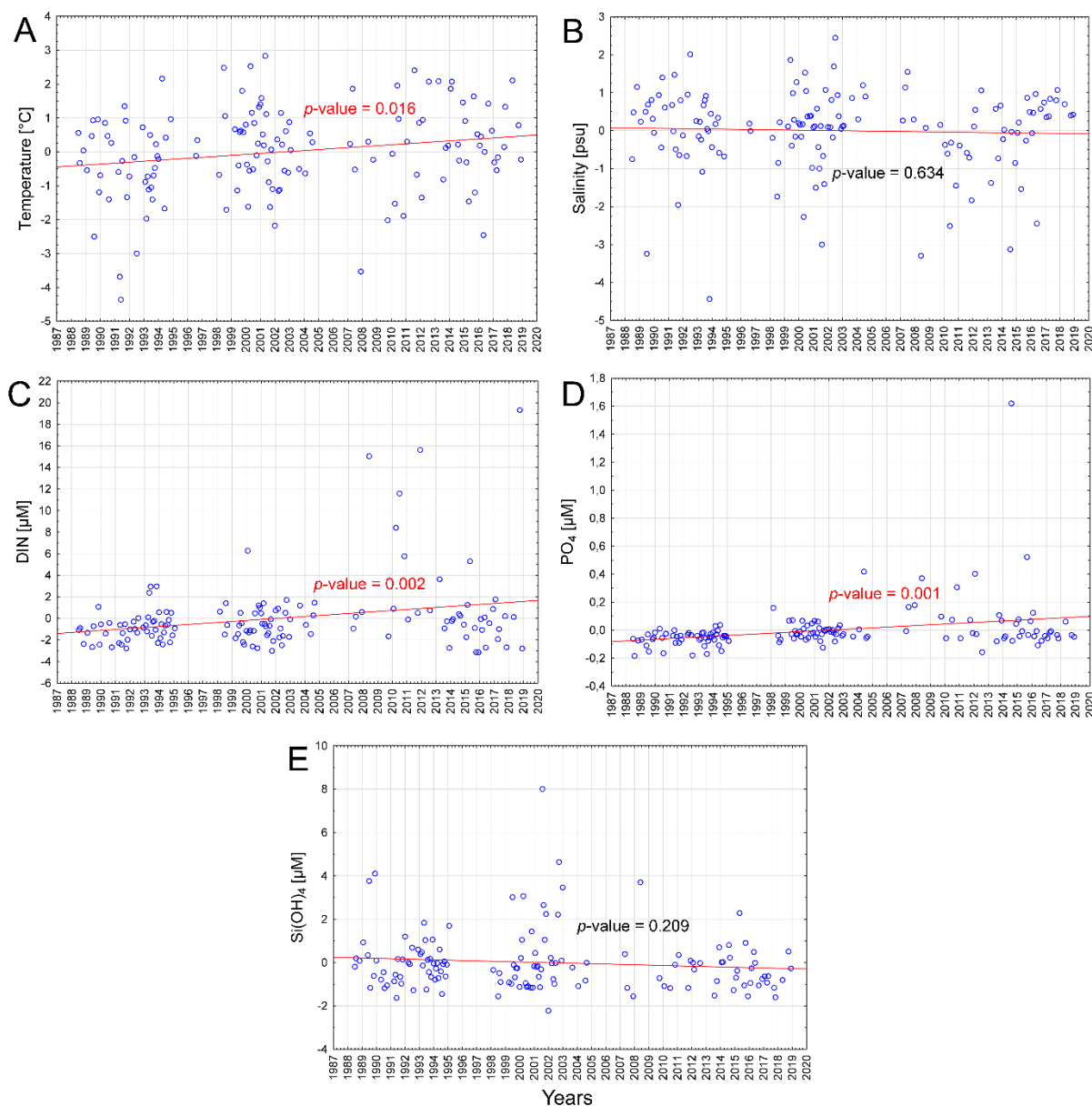
Salinity showed the maximum in February (38.43 ± 0.06), followed by a decreasing trend, reaching the minimum in June (35.84 ± 0.53), after which an increasing trend was observed (Fig. 4B). The mean annual trend of water density at surface showed the highest values in February ($29.31 \pm 0.08 \text{ kg m}^{-3}$), then a decrease was observed in spring and the minimum ($24.38 \pm 0.38 \text{ kg m}^{-3}$) was recorded in July (Fig. 4C). On average, at surface dissolved oxygen showed oversaturation values (above 100%) from April to August (Fig. 4D) indicating a predominance of oxygen production than consumption. The mean annual cycle of Dissolved Inorganic Nitrogen (DIN) showed the minimum values in September ($0.95 \pm 0.21 \text{ }\mu\text{M}$), and an increase in spring and autumn, with maximum in October ($3.19 \pm 1.54 \text{ }\mu\text{M}$, Fig. 4E). Orthophosphate showed the minimum in May ($0.03 \pm 0.01 \text{ }\mu\text{M}$) and maximum in June ($0.13 \pm 0.06 \text{ }\mu\text{M}$) followed by a decrease in summer and a new peak in September (Fig. 4F). Silicate values ranged from $0.85 \pm 0.15 \text{ }\mu\text{M}$ in March and $3.23 \pm 0.78 \text{ }\mu\text{M}$ in January (Fig. 4G). On a mean monthly basis, the N:P ratio showed lower values in summer with the minimum in July (23.42 ± 6.83), whilst the maximum was found in October (143.41 ± 81.23) (Fig. S1A). Regarding the Si:N ratio, the lowest values were observed in winter (minimum in February, 0.66 ± 0.20), followed by an increase in spring with the maximum in June (6.35 ± 3.11), high values in summer and a new peak in October (Fig. S1B).

240

241 3.1.3 Interannual trend

The trends of physico-chemical parameters in terms of anomalies are shown in Figure 5. Temperature anomaly (Fig. 5A) showed a significant increase ($p < 0.05$). On the contrary, the salinity trend did not reveal any significant interannual variations (Fig. 5B). Regarding nutrients, both DIN and PO_4 (Fig. 5C,D) showed a significantly increasing trend ($p < 0.01$), whilst silicate (Fig. 5E) did not reveal any significant interannual variations. Considering N:P and Si:N ratios (Fig. S1C,D), no significant interannual trend was observed ($p > 0.05$).

248



249

250 Fig. 5. Trend of anomalies of physical and chemical parameters at surface layers: (A) water
 251 temperature ($^{\circ}\text{C}$), (B) salinity, (C) Dissolved Inorganic Nitrogen concentrations (DIN) (μM), (C)
 252 PO_4 concentration (μM) and (E) silicate concentration (μM).

253

254 3.2 Phytoplankton

255 3.2.1 Temporal variability in the water column

256 The vertical distribution of phytoplankton is showed in Figure S2. Diatom abundances (Fig. S2A)
 257 showed that in the period 1988 to 1998 higher values were observed in the 0–20 m layer than in the
 258 underlying water column, while in the period 2013–2018 high abundances were concentrated in a

259 narrower layer (i.e., 0–10 m). Subsurface maxima were found in the layer 20–30 m in June 2002 and
260 in December 2018. Lower abundances were observed in years 2013 and 2015.

261 Dinoflagellates showed higher values in the upper water column than in the underlying layers,
262 particularly in the 1988–2002 period and in 2018 (Fig. S2B). A subsurface maximum (20 m) was
263 observed in June 2002. The lowest values were found below 35 m since 2015 (minimum in autumn
264 2017, 40m).

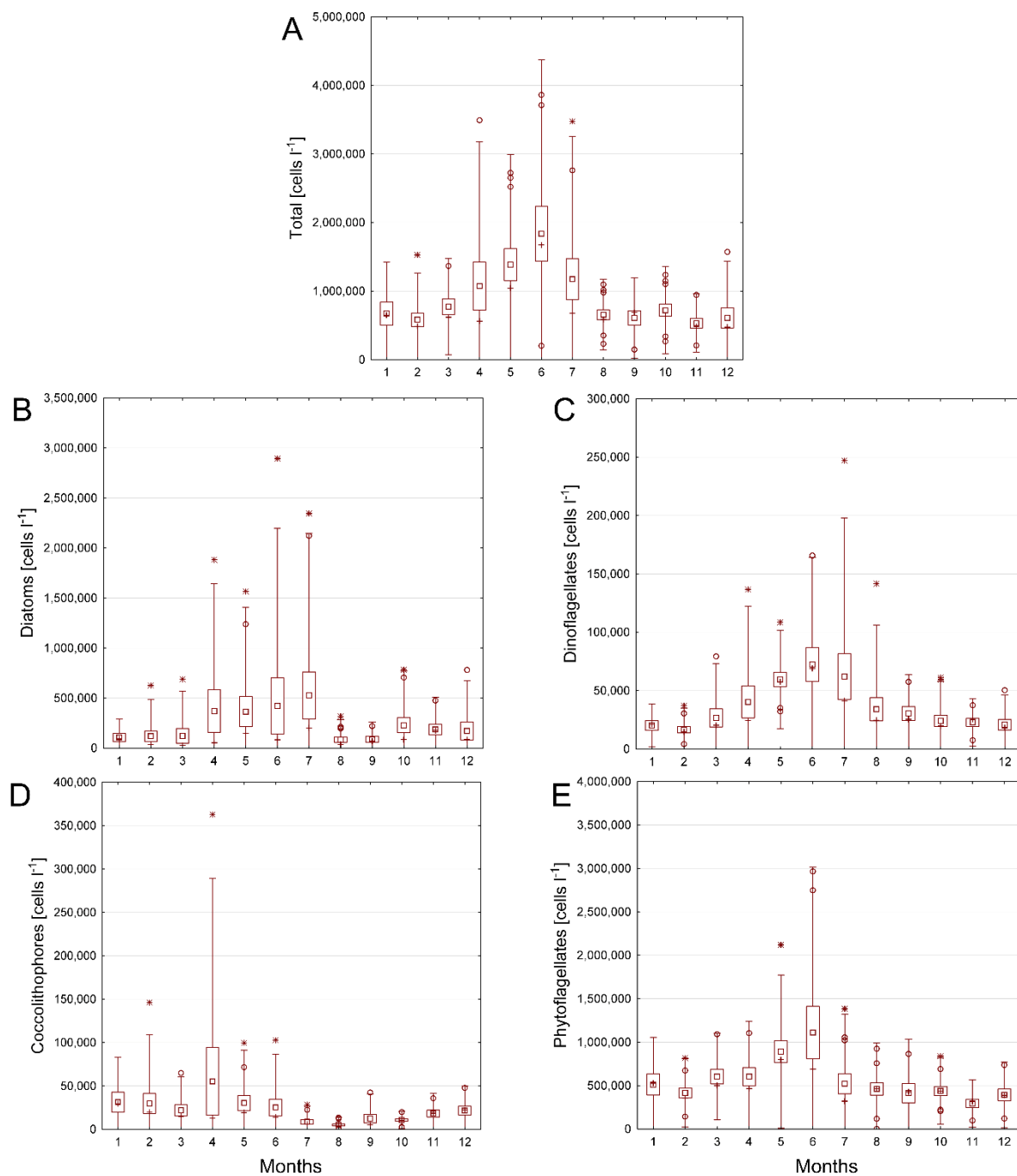
265 The vertical distribution of coccolithophores abundance showed that the highest values were observed
266 in the mid water column and at bottom, from 1988 to 2000 (Fig. S2C). In the second period, very low
267 values were found from 2013 to 2015, particularly at bottom, and from 2016 coccolithophore maxima
268 occurred at surface.

269 Phytoflagellates (Fig. S2D) were always the most abundant group. Their vertical distribution showed
270 several subsurface maxima that occurred with those of diatoms.

271

272 3.2.2 Mean annual cycle

273 The annual cycle (on a monthly basis) of phytoplankton groups calculated at the surface layer from
274 1988 to 2018 in SG05 station is shown in Fig. 6. Considering the total phytoplankton (Fig. 6A), the
275 annual maximum was observed in June ($1,838,830 \pm 400,360$ cells l^{-1}), whilst the minimum was
276 recorded in November ($533,496 \pm 71,092$ cells l^{-1}). Phytoflagellates were the most abundant group
277 throughout the year representing from 44 to 78 % of the total community. The minimum contribution
278 of phytoflagellates was observed in July when diatom maximum occurred. Diatoms represented the
279 second group with a percent contribution ranging from 13 to 45%. Dinoflagellate and
280 coccolithophore contribution ranged from 3 to 5% and from 1 to 5% respectively.



281

282 Fig. 6. Mean annual cycle of phytoplankton abundance (cells l^{-1}) calculated on a mean monthly basis
 283 from 1988 to 2018 in SG05 station at surface: (A) total phytoplankton, (B) diatoms, (C)
 284 dinoflagellates, (D) coccolithophores and (E) phytoflagellates. Box plots report the data distribution
 285 with the mean (+), the median (\square), the interquartile range (box), the non-outlier range (vertical bars),
 286 the outliers (\circ) and the extremes (*).

287

288 Observing the mean annual trend of diatoms (Fig. 6B), low values were observed in winter months,
 289 then abundances increased in spring reaching their maximum in July ($526,278 \pm 234,168$ cells l^{-1}).

290 At the end of summer, values decreased, and in August minimum was observed ($83,125 \pm 27,492$
291 cells l^{-1}). A second peak was found in autumn (October, $228,213 \pm 75,201$ cells l^{-1}).
292 Dinoflagellate abundances showed minimum values in February ($16,496 \pm 2,670$ cells l^{-1}) and an
293 increase in spring, showing the maximum in June ($72,381 \pm 14,495$ cells l^{-1}) (Fig. 6C).
294 The mean annual cycle of coccolithophores was characterize by minimum values in summer (August,
295 $5,060 \pm 1,201$ cells l^{-1}), an increase in autumn and winter, and the maximum in April ($55,192 \pm 38,987$
296 cells l^{-1}) (Fig. 6D).
297 Being the dominant group in the total community, phytoflagellates' trend paralleled that of total
298 phytoplankton, with the annual peak in June ($1,110,694 \pm 300,909$ cells l^{-1}) and the minimum in
299 November ($294,238 \pm 45,046$ cells l^{-1}) (Fig. 6E).

300

301 3.2.3 Phytoplankton community composition

302 In the whole study period, the number of identified taxa was 320: 153 diatoms, 112 dinoflagellates,
303 32 haptophytes (among which 28 coccolithophores), 3 cryptophytes, 5 chrysophytes, 5
304 dictyochophytes, 2 raphidophytes, 5 chlorophytes, 2 euglenophytes, 1 cyanophyte (Table S2). The
305 IndVal analysis, calculated for the surface layer, revealed the taxa that were significant for each
306 season in terms of both abundance and frequency of occurrence (Table 1). In winter, significantly
307 high values were observed for *Emiliana huxleyi*, *Skeletonema marinoi*, *Dactyliosolen phuketensis*,
308 *Pseudosolenia calcar avis* and *Chaetoceros affinis*. In spring, significant taxa were *Dactyliosolen*
309 *fragilissimus*, *Chaetoceros thronsenii*, *C. simplex*, and *Cyclotella* spp. In summer, the most
310 representative taxa were *Calycomonas vangoorii*, *Pseudoscourfieldia marina* and *Chaetoceros*
311 *anastomosans*. *Nitzschia gobbii* showed a high Ind-Val although not significant. In autumn, a high
312 number of taxa resulted significant: *Guinardia striata*, *Lioloma pacificum*, *Pseudo-nitzschia* cfr.
313 *delicatissima*, *Chaetoceros danicus*, *C. curvisetus*, *C. costatus*, *C. socialis*, *Asterionellopsis glacialis*,
314 *Pleurosigma* sp., *Cylindrotheca closterium*, *Navicula* spp.

315 Table 1. List of phytoplankton taxa characterized by the highest IndVal for each season calculated
 316 for the surface layer. Values indicated in italic are significant at $p < 0.05$, those in bold italic are
 317 significant at $p < 0.01$, those in bold italic and underlined are significant at $p < 0.001$. The shades of
 318 colour are proportional to the IndVal values, from dark green to white, in decreasing order.

	Winter	Spring	Summer	Autumn
<i>Emiliania huxleyi</i>	42.54	24.83	0.733	17.999
<i>Skeletonema marinoi</i>	38.423	3.191	0	0.327
<i>Chaetoceros affinis</i>	26.577	0	3.153	1.228
<i>Dactyliosolen phuketensis</i>	26.404	0	0	6.432
<i>Pseudosolenia calcar avis</i>	26.262	0	5.616	0.084
<i>Syracosphaera pulchra</i>	16.486	1.948	6.68	10.168
<i>Dactyliosolen fragilissimus</i>	0.486	46.141	21.095	3.983
<i>Chaetoceros thronsenii</i>	0.109	32.254	0.091	0.547
<i>Cyclotella</i> spp.	0.046	28.971	2.227	0.165
<i>Prorocentrum micans</i>	0.268	26.011	6.895	0.894
<i>Diplopsalis</i> group	0.315	23.196	0.339	0.049
<i>Prorocentrum compressum</i>	0.173	18.47	0.591	0.033
<i>Chaetoceros</i> cfr. <i>simplex</i>	0.069	18.417	0.146	0.327
<i>Tripos furca</i>	0.04	17.647	0.832	0.92
<i>Thalassionema nitzschioides</i>	3.082	16.79	0.794	3.42
<i>Tripos muelleri</i>	3.543	16.375	0.435	0.281
<i>Prorocentrum cordatum</i>	1.95	15.826	1.469	0.493
cfr. <i>Chrysochromulina</i> sp.	3.418	14.561	2.491	1.363
<i>Pseudokephyrion</i> spp.	1.703	14.216	0.686	5.267
cfr. <i>Scrippsiella</i> sp.	0	13.659	7.549	0
<i>Thalassiosira</i> spp.	3.597	13.622	0.071	0.11
<i>Leptocylindrus minimus</i>	0	12.803	0.923	0.646
<i>Nitzschia longissima</i>	0.36	11.303	1.711	1.684
<i>Protoperidinium</i> cfr. <i>steinii</i>	0.473	10.693	1.434	4.161
<i>Calycomonas vangoorii</i>	0.086	7.657	62.851	5.02
<i>Proboscia alata</i>	0.169	2.547	52.331	5.089
<i>Cerataulina pelagica</i>	2.135	7.275	50.861	5.035
<i>Pseudoscurfieldia marina</i>	0	11.864	46.203	0.185
<i>Nitzschia gobbii</i>	1.477	0.1	30.879	4.731
<i>Hemiaulus hauckii</i>	7.971	0.065	24.689	7.097
<i>Guinardia flaccida</i>	8.291	0.121	24.549	8.497
<i>Leptocylindrus danicus</i>	0.248	4.516	23.768	17.729
<i>Rhizosolenia</i> spp.	6.738	0.005	22.26	2.866
<i>Chaetoceros anastomosans</i>	0	0.317	21.996	0.223
<i>Chaetoceros</i> spp.	1.668	20.953	20.985	7.397
<i>Pseudo-nitzschia</i> cfr. <i>pseudodelicatissima</i>	0.678	12.362	20.647	10.208
<i>Rhabdosphaera clavigera claviger</i>	0.432	1.485	18.817	9.875
<i>Gymnodinium</i> spp.	1.099	4.14	17.411	3.576
<i>Bacteriastrum</i> spp.	1.005	1.622	12.364	4.518
<i>Guinardia striata</i>	3.971	0	3.882	49.879
<i>Lioloma pacificum</i>	0	0	2.426	45.529
<i>Pseudo-nitzschia</i> cfr. <i>delicatissima</i>	0.495	2.995	0.164	38.253
<i>Chaetoceros danicus</i>	2.916	0	0.036	36.487
<i>Asterionellopsis glacialis</i>	4.717	0	0	34.209
<i>Cylindrotheca closterium</i>	1.951	1.937	22.752	31.752
<i>Pleurosigma</i> spp.	8.545	0	0.165	30.085
<i>Chaetoceros curvisetus</i>	13.009	0	0.088	27.995
<i>Chaetoceros lorenzianus</i>	0.953	5.228	6.099	27.339
<i>Navicula</i> spp.	10.499	0.146	0.299	26.138
<i>Chaetoceros socialis</i>	1.317	0	0.239	22.027
<i>Dictyocha fibula</i>	10.262	0.076	0	21.645
<i>Chaetoceros costatus</i>	2.42	0	0	21.037
<i>Hemiaulus sinensis</i>	2.606	1.613	0.077	18.444
<i>Calciosolenia brasiliensis</i>	2.258	0.97	1.525	18.203
<i>Pseudo-nitzschia</i> spp.	2.605	0.57	0.725	17.913
<i>Calciosolenia murrayi</i>	0.012	1.061	1.404	17.077
<i>Euglena</i> spp.	3.231	2.6	0.86	16.967

320 3.2.4 Interannual variations

321 Due to the interruption in the phytoplankton sampling from 2003 to 2012, to assess if changes
 322 occurred in the phytoplankton communities along the study period, we compared the mean seasonal
 323 abundances at surface between two periods 1988-2002 and 2013-2018, through one-way ANOVA
 324 test. Results highlighted that the winter abundances of dinoflagellates and coccolithophores were
 325 significantly higher ($p < 0.01$ and $p < 0.05$, respectively) in first period than in the second one (Table
 326 2), while no significant differences were observed for the other groups and for the total
 327 phytoplankton).

328

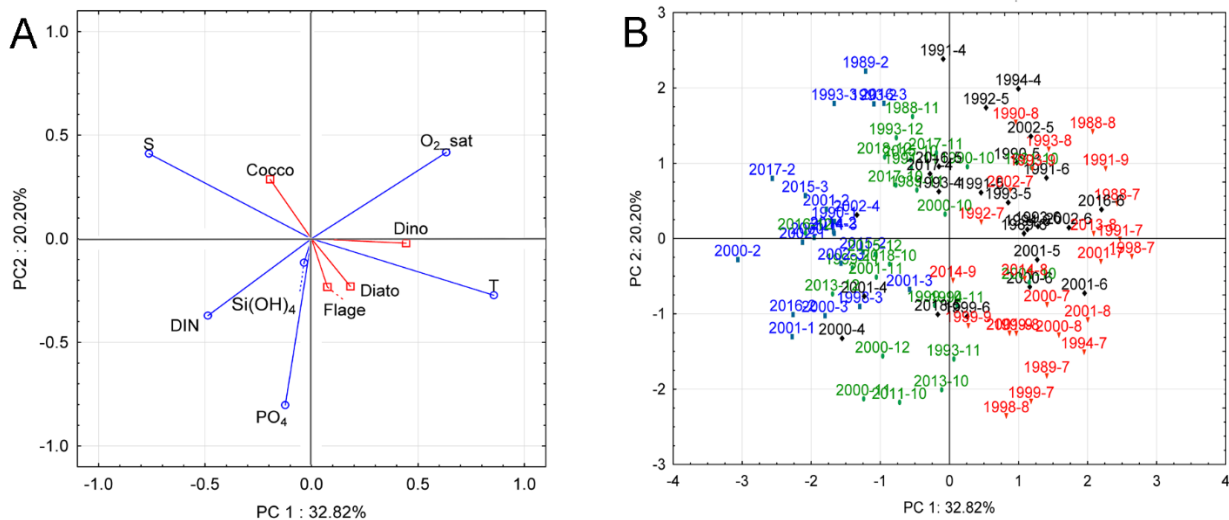
329 Table 2. Results of ANOVA and Tukey's tests performed on the abundances (cells l^{-1}) of diatoms,
 330 dinoflagellates, coccolithophores, flagellates and total phytoplankton in the period 1988–2002 and
 331 2013-2018 at surface layer. Mean values \pm standard error (SE) are shown. As: ns, not significant; *
 332 $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Parameter	Season	1988-2002	2013-2018	p-level	Tukey test
		Avg \pm SE	Avg \pm SE		
Diatoms (cells l^{-1})	Winter	270,576 \pm 52,820	112,754 \pm 29,515	ns	ns
	Spring	438,586 \pm 145,388	155,989 \pm 96,916	ns	ns
	Summer	258,821 \pm 100,686	108,899 \pm 54,128	ns	ns
	Autumn	213,083 \pm 51,227	106,662 \pm 43,421	ns	ns
Dinoflagellates (cells l^{-1})	Winter	40,017 \pm 3,801	27,285 \pm 6,612	**	88-02 > 13-18
	Spring	57,554 \pm 6,560	60,226 \pm 23,258	ns	ns
	Summer	44,532 \pm 9,241	30,974 \pm 4,161	ns	ns
	Autumn	25,818 \pm 3,479	17,767 \pm 1,915	ns	ns
Coccolithophores (cells l^{-1})	Winter	23,228 \pm 4,348	15,081 \pm 4,040	**	88-02 > 13-18
	Spring	38,151 \pm 14,430	26,275 \pm 15,682	ns	ns
	Summer	8,501 \pm 1,766	3,974 \pm 1,693	ns	ns
	Autumn	15,640 \pm 2,654	17,722 \pm 2,843	ns	ns
Flagellates (cells l^{-1})	Winter	552,546 \pm 42,610	594,488 \pm 104,196	ns	ns
	Spring	846,407 \pm 111,278	1,009,411 \pm 398,669	ns	ns
	Summer	456,606 \pm 59,475	622,905 \pm 176,270	ns	ns
	Autumn	354,789 \pm 47,219	437,893 \pm 27,120	ns	ns
Total (cells l^{-1})	Winter	941,984 \pm 79,779	750,418 \pm 134,936	ns	ns
	Spring	1,486,528 \pm 202,217	1,253,552 \pm 528,283	ns	ns
	Summer	839,434 \pm 132,973	769,163 \pm 128,399	ns	ns
	Autumn	629,423 \pm 79,279	580,201 \pm 50,405	ns	ns

333

334 3.3 Relationships between phytoplankton and environmental parameters

335 The PCA analysis, performed for the surface layer, showed a 53.02% of explained variance of the
 336 first two components (Fig. 7). The first component (PC1) showed salinity opposite to temperature,
 337 highlighting the seasonal behaviour of the site. The second axis (PC2) was determined by nutrient
 338 concentrations. Diatom abundances were directly related to temperature and inversely related to
 339 salinity. On the contrary, coccolithophores were found to be directly associated to salinity and
 340 inversely related to temperature and PO₄. Flagellates and dinoflagellates were found both to be
 341 associated to temperature and oxygen saturation, being inversely related to salinity.
 342 The analysis of correlation between environmental parameters and phytoplankton abundances
 343 highlighted a negative correlation between salinity and diatoms ($r = -0.38, p < 0.05$), dinoflagellates
 344 ($r = -0.47, p < 0.05$) and phytoflagellates ($r = -0.27, p < 0.05$). A positive and negative correlation
 345 was detected between temperature and dinoflagellates ($r = 0.32, p < 0.05$), and temperature and
 346 coccolithophores ($r = -0.37, p < 0.05$), respectively. Furthermore, a negative correlation between
 347 coccolithophores and PO₄ ($r = -0.23, p < 0.05$) was found.



348
 349 Fig. 7. Principal Component Analysis (PCA) based on correlation matrix of ranked environmental
 350 variables and main phytoplankton group abundance values, used as supplementary variables,
 351 performed for the surface layer. (A) Loading plot (T = temperature, S = salinity, DIN = Dissolved
 352 Inorganic Nitrogen, PO₄ = Orthophosphate, Si(OH)₄ = silicates, O₂_sat = oxygen saturation, Diato =
 353 Diatoms, Dino = Dinoflagellates, Cocco = Coccolithophores, Flage = Flagellates. (B) Score plot

(colours and markers represent the seasons: blue ■ = winter, black ◆ = spring, red ▲ = summer, green ● = autumn).

4. Discussion

In this study, we highlighted for the first time the intra and interannual variability of phytoplankton, the community composition, and their relationships with the oceanographic and trophic condition, in an offshore area of the northern Adriatic basin based on a long-term dataset.

4.1 Mean annual cycle

The mean annual cycle of phytoplankton in an open-water station of the LTER Senigallia-Susak transect (NA) indicated that highest abundances occurred in early summer mainly due to phytoflagellates' and diatoms' maxima. Minimum abundances occurred in autumn and winter.

This cycle markedly differed from that recorded both in the western and eastern NA coastal waters. In the E Adriatic coast, spring and autumn peaks are commonly observed mainly related to diatom proliferation (Cerino et al., 2019; Maric et al., 2012; Mozetič et al., 2010). Instead, in the coastal waters of the NW Adriatic Sea, the winter diatom bloom represents the most relevant plankton increase throughout the year (Totti et al., 2019), as already observed in other Mediterranean areas (Goffart et al., 2015), followed by the classic peaks in spring and autumn again due to diatoms and phytoflagellates. The observed differences between the offshore and costal sites of the Senigallia-Susak transect could be explained considering the different oceanographic conditions. In the coastal station, the strong winter bloom is related to the shallowness of water column (11 m depth) that allows phytoplankton to remain in the photic zone even in winter mixing conditions, allowing the bloom of *Skeletonema marinoi*, a small colonial diatom with a marked seasonal behaviour (Totti et al., 1999). On the contrary, in the deeper offshore station, the winter phytoplankton abundance is low, according to the classical Sverdrup model (Sverdrup, 1953). Furthermore, the offshore station is located in the waters beyond the coastal front, not directly affected by the coastal input, except than during the

380 stratification period (see below). In the study area the nutrient source is partly autochthonous, i.e.
381 represented by the regeneration processes occurring in the water column and by the release from
382 bottom sediments (Baric et al., 2002), and partly allochthonous due to the spreading south-eastward
383 of the floods of the Po River in stratified conditions as demonstrated by several recorded events of
384 plume extension beyond the midline (Campanelli et al., 2011; Grilli et al., 2020).

385 As regards as DIN, the allochthonous source is highlighted by the June peak coinciding with the
386 minimum of salinity, while the autochthonous (i.e. resuspension) due to the thermocline rupture, is
387 highlighted by the October peak. The same peaks were observed for the silicates, suggesting the
388 importance of both allochthonous and autochthonous (i.e. weathering of rocks and sediments) source
389 also for this nutrient. The Si:N ratio in the study area was higher than in other areas of the NA (Cozzi
390 et al., 2020; Giani et al., 2012). N:P values were comparable to those recorded in other NA areas
391 (Accoroni et al., 2015; Cozzi et al., 2020; Degobbis et al., 2005; Djakovac et al., 2012; Giani et al.,
392 2012), highlighting the P-limitation condition typical of the NA. Concentrations of orthophosphate
393 in the study area were often below 0.2 μM as typically recorded in all the long-term data records in
394 the Adriatic Sea (Grilli et al., 2020). The high P concentrations often recorded below 40 m depth
395 suggests that the main inorganic P source in the study area would be the remineralization processes
396 at the bottom, although even an allochthonous source was revealed by low salinity values at surface.
397 The fact that the autumn peak was observed for the DIN but not for the PO_4 would suggest that the
398 resuspension process is less effective for P than for DIN, probably due to phosphate absorption into
399 particles (Boldrin et al., 2009; Tengberg et al., 2003). In warm months, the water stratification was
400 often associated to the decrease of salinity and the increase of nutrients' concentration (mainly P),
401 indicating the spreading eastwards of the riverine waters. Such conditions in which nutrients became
402 available in a stratified photic zone represent optimal conditions for phytoplankton to proliferate.
403 Similar conditions in which phytoplankton was influenced by riverine plumes were observed in other
404 studies (Diaz et al., 2008; O'Connor et al. 2016; Tang et al., 2004).

405 In our study, almost all phytoplankton groups showed their maximum abundances in early summer
406 and multivariate statistical analysis highlighted an inverse relation between their abundances and
407 salinity. These maxima were pointed out by oxygen oversaturation during the same period. Diatoms
408 and phytoflagellates represented the dominant groups, while dinoflagellates showed abundances one
409 order of magnitude lower than the others. This behaviour agrees with that observed in the coastal
410 areas of the NA Sea (Cerino et al., 2019; Totti et al., 2019). On the contrary, coccolithophores' mean
411 annual cycle showed the peak in April, while in the coastal station they occurred with high
412 abundances even from February to April (Totti et al., 2019).

413

414 4.2 Community structure

415 The phytoplankton community composition in the offshore area of the Senigallia-Susak transect has
416 been characterized for the first time in this study. Despite the low abundances, the winter community
417 is characterized by taxa expressing a marked seasonal behaviour, such as the coccolithophore
418 *Emiliania huxleyi*, and the diatom *Skeletonema marinoi*, which have been reported in winter even in
419 the coastal station (Totti et al., 2019), as well as in other Mediterranean coastal areas (Cerino et al.,
420 2019; Turkoglu, 2010; Zingone et al., 2010a). The occurrence of *E. huxleyi* together with rapid
421 growth/small-chained diatoms as *S. marinoi* would indicate the aptitude of that species to proliferate
422 in mixing in turbulence regime, as already observed in other areas (Guerreiro et al., 2013).
423 Surprisingly even some large-sized diatom species, i.e., *Pseudosolenia calcar avis*, and *Dactyliosolen*
424 *phuketensis* resulted significant for winter. Spring communities were dominated by phytoflagellates
425 and only a few indicator taxa were observed, e.g. some nanoplanktonic species belonging to
426 *Chaetoceros* and *Cyclotella* genera and the large sized *Dactyliosolen fragilissimus*. The composition
427 of summer communities was characterized by small nanoplanktonic species as expected in
428 oligotrophic conditions, such as the chrysophyte *Calycomonas vangoorii*, the prasinophyte
429 *Pseudoscourfieldia marina* and the diatom *Chaetoceros anastomosans*. In autumn, a number of taxa
430 resulted significant, including some autumn species also typical in coastal stations, such as

431 *Asterionellopsis glacialis* and *Chaetoceros curvisetus* (Bernardi Aubry et al., 2004; Totti et al., 2019).
432 The presence of *Pseudo-nitzschia delicatissima* species complex as autumn taxa was surprising, as in
433 coastal areas it is typical of late winter (Bernardi Aubry et al., 2004; Giulietti et al., 2021a; Totti et al.,
434 2019). However, we should consider that the *Pseudo-nitzschia delicatissima* species complex actually
435 includes several cryptic species, each expressing different seasonal behaviour also in geographically
436 close areas (Turk Dermastia et al., 2020; Giulietti et al., 2021b).
437 On the whole, the community of the offshore site reflected only in part that of the coastal area and
438 only some species were found to be key taxa of a certain season in both stations (e.g. *Skeletonema*
439 *marinoi* in winter and *Chaetoceros costatus* in autumn), whilst many taxa were found to be indicative
440 of a different season (e.g. *Guinardia striata* for the summer and autumn community in the coastal
441 and offshore site, respectively) or significant only in the offshore site (e.g. *Chaetoceros thronsenii*
442 in spring, *C. anastomosans* in summer, *C. danicus* in autumn).

443

444 4.3 Interannual variations

445 The interannual trend of the physico-chemical parameters highlighted a significant increase in the
446 temperature values, as previously reported in the NA basin (Vilibić et al., 2019). On the contrary,
447 salinity did not show significant trends. A significant increase was observed even for inorganic
448 nitrogen, as already observed by Grilli et al. (2020) through a basin-scale interannual analysis. This
449 could be explained by the increasing inputs of anthropogenic nitrogen in the drainage basin of the Po
450 River, which cause high nitrogen loads during those years of high river waters discharge (Cozzi et
451 al., 2018; Cozzi and Giani, 2011; Viaroli et al., 2018) coupled with a limited N use by a P-limited
452 phytoplankton community. Although in coastal areas of the NA, silicates showed an increasing trend
453 (Cozzi et al., 2020; Totti et al., 2019), in the offshore station of the SS they did not show a significant
454 trend, as already observed by Grilli et al. (2020). The temporal trend of inorganic phosphorous
455 concentrations in the study area increased significantly, as already found by Totti et al. (2019) in the
456 coastal station of the same transect. However, Grilli et al. (2020) did not find any significant trend

457 for orthophosphate at a whole sub-basin scale, highlighting that the evaluation of P trend is
458 particularly controversial. In fact, the NA is in an almost permanent P-deficient status (Brush et al.,
459 2021), and the availability of phosphate, particularly in offshore waters not directly affected by the
460 riverine runoff, depends mainly on the regenerative processes in the water column and on the
461 sediment resuspension (see above). Moreover, it is becoming increasingly clear that an important
462 source of phosphorus is its organic form (not investigated in this study), whose availability has been
463 proved in many studies by high values of alkaline phosphatase activity in sea water (Ivančić et al.,
464 2012, 2016; Tanković et al., 2018). In addition, Baturin (2003) suggested that phosphorus cycling is
465 tightly connected to carbon dioxide in the atmosphere. An increase in carbon dioxide led to warming
466 and the rise of the phosphorus input into the sea, an accelerated rate of sinking of organic matter and
467 phosphorus. No significant trends were found for DIN/PO₄ and Si(OH)₄/DIN. The latter has been
468 suggested as able to affect the phytoplankton community, facilitating flagellate growth at the expense
469 of diatoms (Gilpin et al., 2004; Turner et al., 1998). This could be one reason why no changes in the
470 study periods were detected in terms of phytoflagellate and diatom abundances.

471 Unfortunately, in this study the interannual variations in the phytoplankton communities in the whole
472 period was not represented, as data in the period 2002-2013 were too irregular. Comparing abundance
473 values of 1988-2002 period with 2013-2018 on a seasonal basis, we did not observe significant
474 changes. However, considering groups, we observed significant lower values in winter during the
475 second period for both dinoflagellates and coccolithophores, in agreement with that reported in the
476 coastal area of the transect (Totti et al., 2019). The major reason of the coccolithophore decrease is
477 the decrease of *Emiliana huxleyi* abundance in winter. The observed decreasing trend of
478 coccolithophores is difficult to explain. Coccolithophores are a complex group with diverse life
479 strategies and, although they have been traditionally associated to oligotrophic conditions, they also
480 proliferate in very diverse trophic conditions (Balch, 2018; Guerreiro et al., 2013; Moita et al., 2010).
481 Moreover, their haplodiplontic life cycle complicates a correct estimation of their occurrence. This is
482 particularly problematic with *Emiliana huxleyi* for which the haploid life phase does not bear

coccoliths (Frada et al., 2012), and a hypothetical occurrence and proliferation of that phase would surely go completely unnoticed, unless other methodological approaches are used. Regarding the observed decrease of dinoflagellates, we need to evaluate it carefully in a longer data set.

The long-time changes in the trophic state and phytoplankton communities have been highlighted in a number of Mediterranean sites (Goffart et al., 2002, 2015; Mercado et al., 2005; Ninčević Gladan et al., 2010; Totti et al., 2019; Zingone et al., 2010b), where to date longer and longer temporal series are becoming available. In the Adriatic Sea, long-term studies sometimes have highlighted tendencies apparently contrasting, depending on the studied area and/or on the considered starting and ending points to trace the traits. For example, a number of studies highlighted a tendency to oligotrophication (Cabrini et al., 2012; Djakovac et al., 2012; Mozetič et al., 2010), whereas others did not (Bernardi Aubry et al., 2012; Totti et al., 2019). In any case, all agree that the main forcing that is responsible for this variability is the rate of the Po River outflow, i.e., the rain regime. In that sense, it has recently been highlighted that an increase in the frequency of intense rains and extremely high Po River discharges have occurred during the last decade, and that could have affected the annual availability of nutrients, particularly of DIN, in the NA (Grilli et al., 2020; Totti et al., 2019).

498

499 **5. Conclusions**

In this study, the phytoplankton communities in their oceanographic scenario in an LTER-offshore station not directly affected by riverine inputs, were highlighted over 30 years. The mean monthly cycle of the phytoplankton was found to be highly dependent on the oceanographic conditions of the water column, as during water stratification the spreading of the fresher and nutrient-rich waters beyond the coastal front allows the phytoplankton annual peak. This study confirmed the P limiting conditions of the N Adriatic Sea, suggesting the importance of organic P sources and remineralization processes along the water column. Instead, the resuspension processes seemed less effective for P than for DIN. Species composition in the offshore station was found to reflect only in part the coastal community, as many taxa were significant only in the offshore site or related to a different season.

509 Temperature, DIN and P showed a significant increase, highlighting long-term changes in the
510 environmental conditions in this offshore station as highlighted in other Mediterranean sites. A
511 decreasing of winter diatoms and dinoflagellates was observed, although more data and a longer time
512 series would be necessary to better understand the phytoplankton trends.

513

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533

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Figure 1

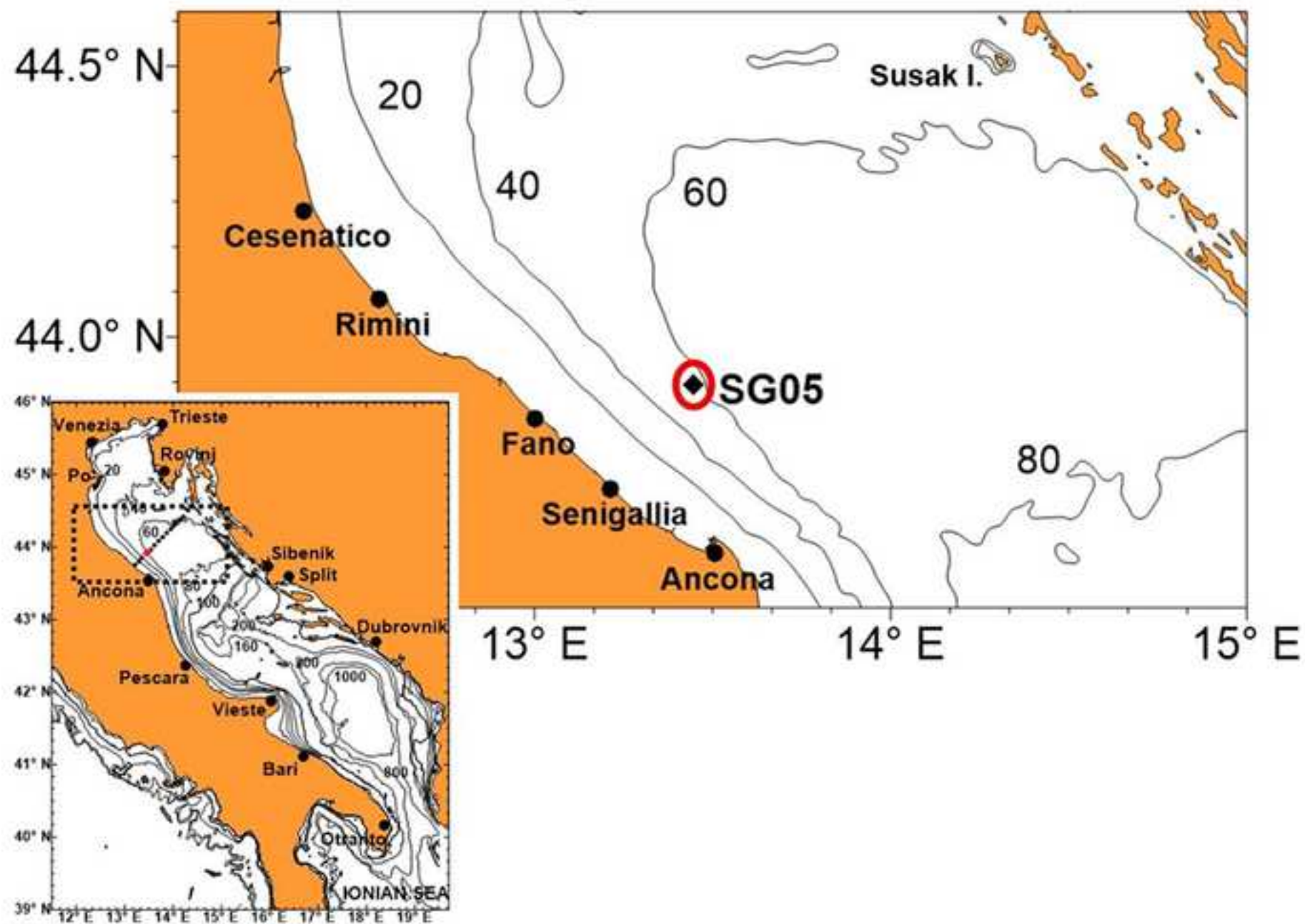
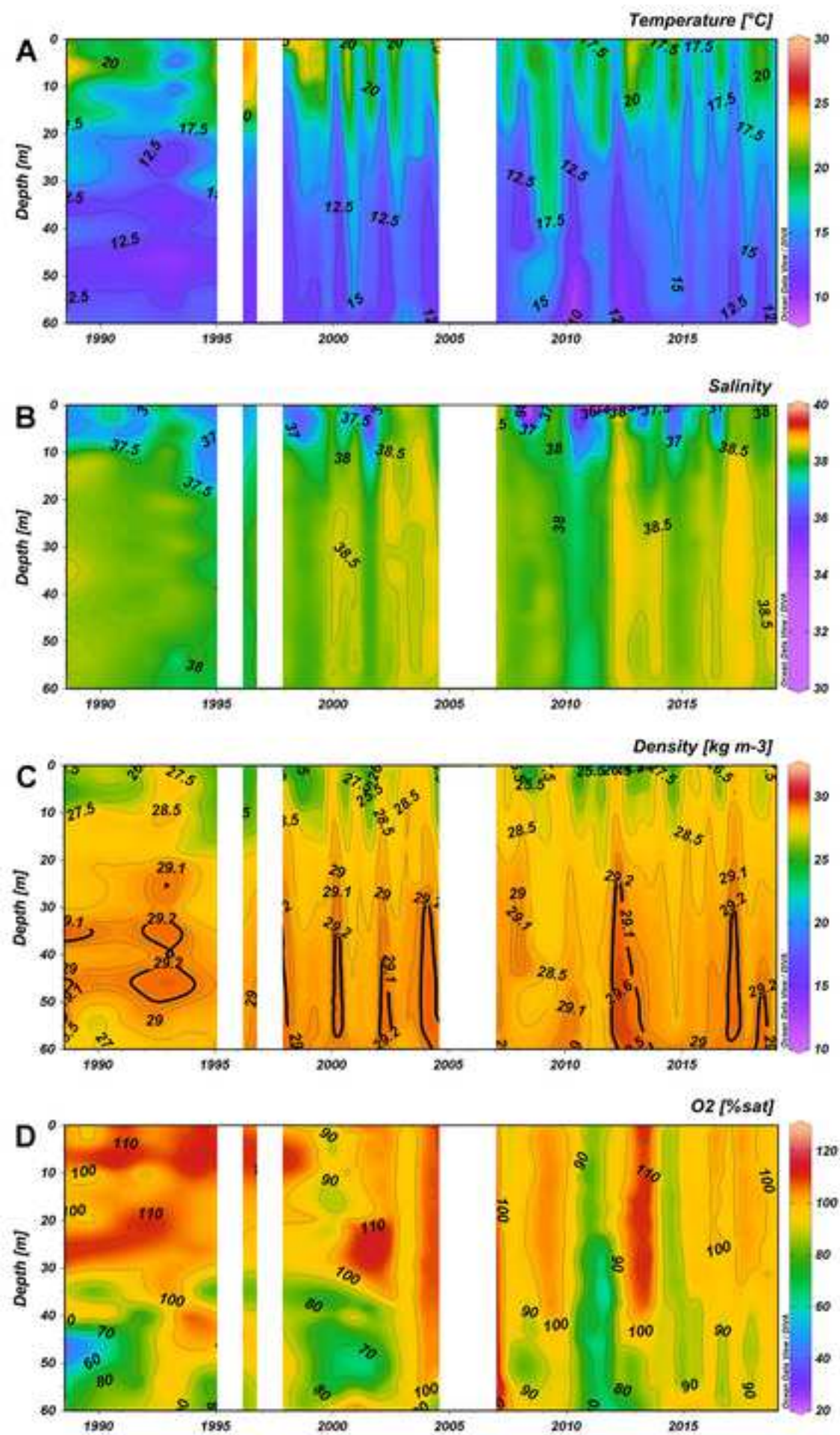


Figure 2

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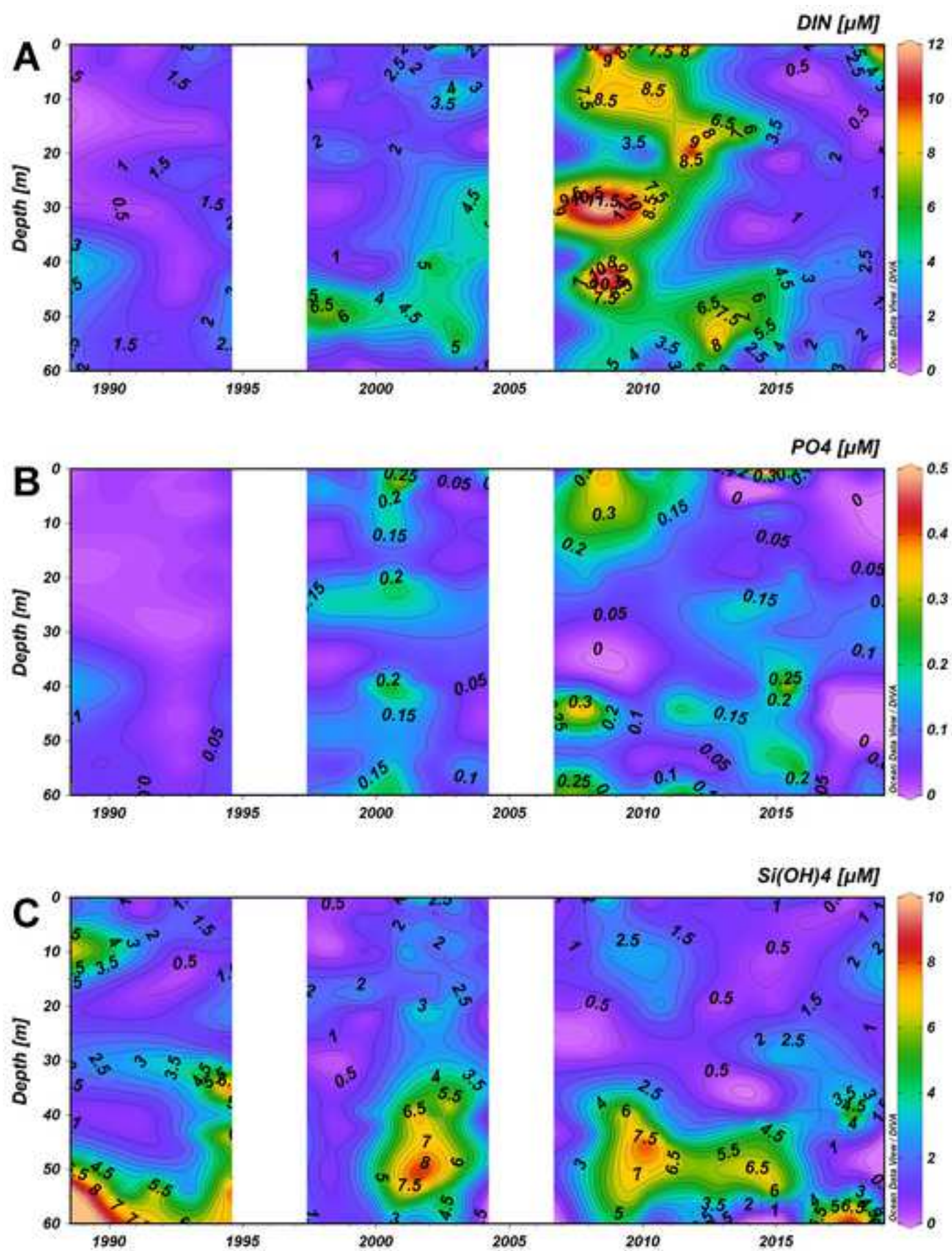


Figure 4

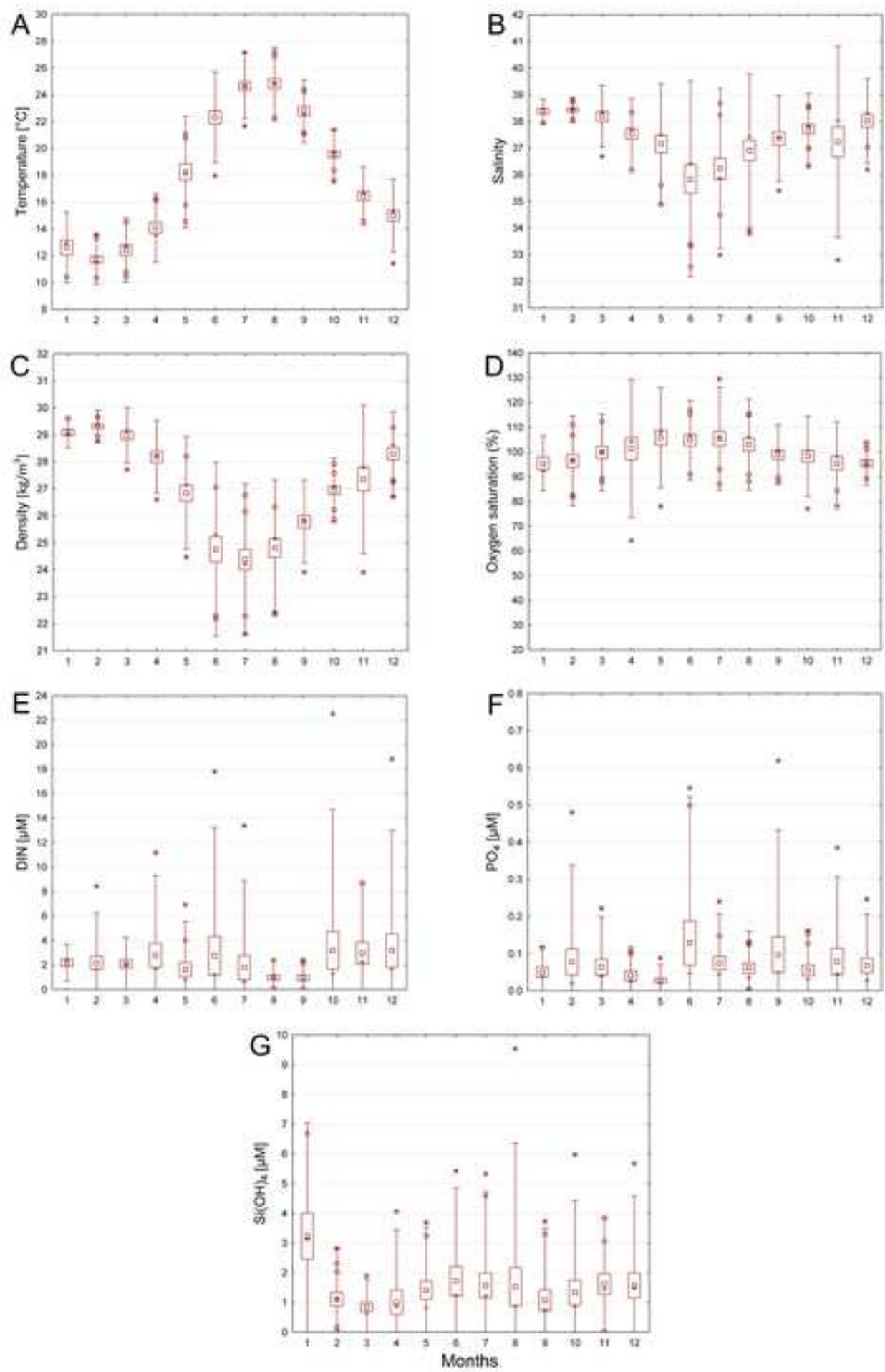
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Figure 5

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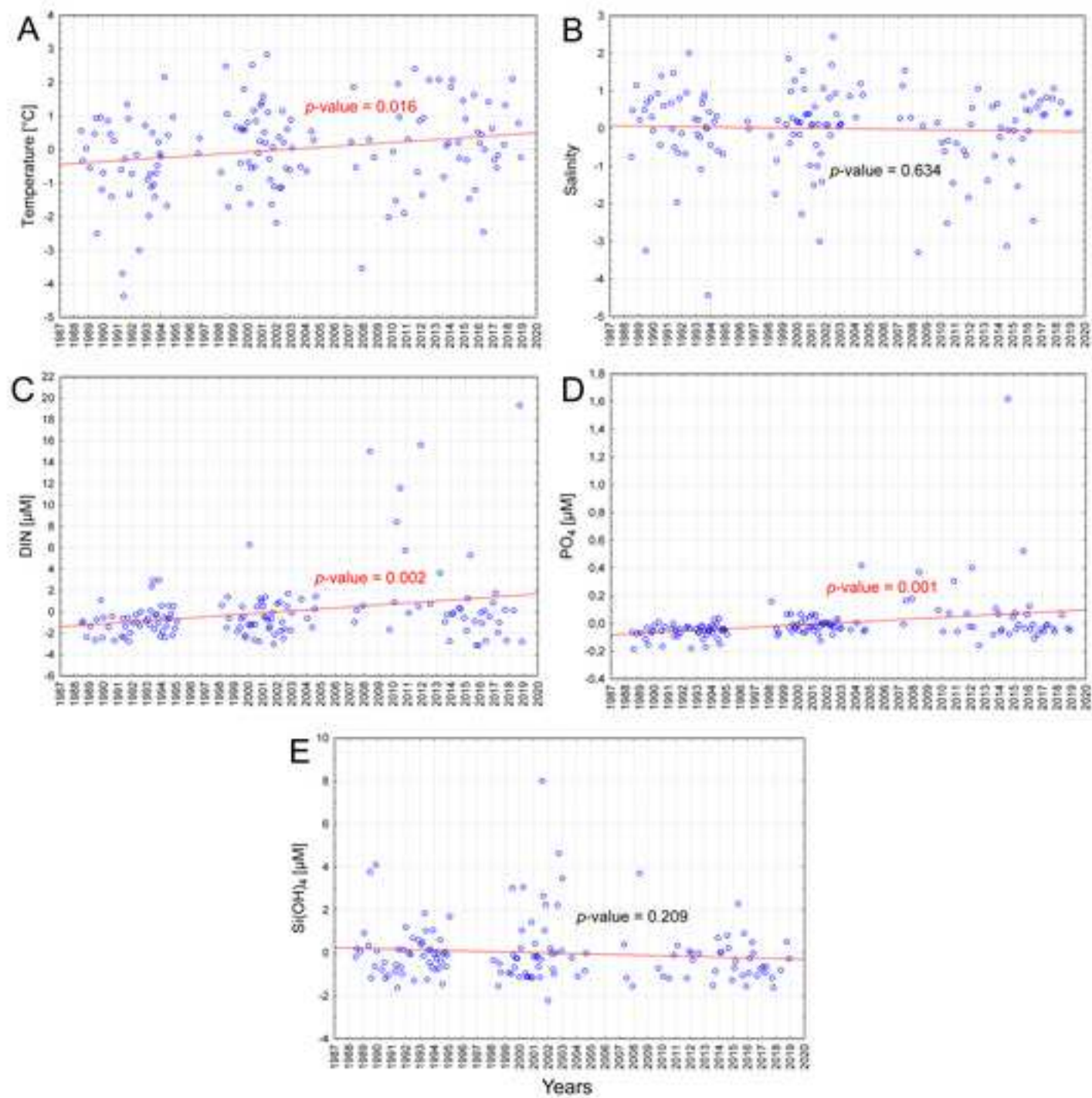


Figure 6

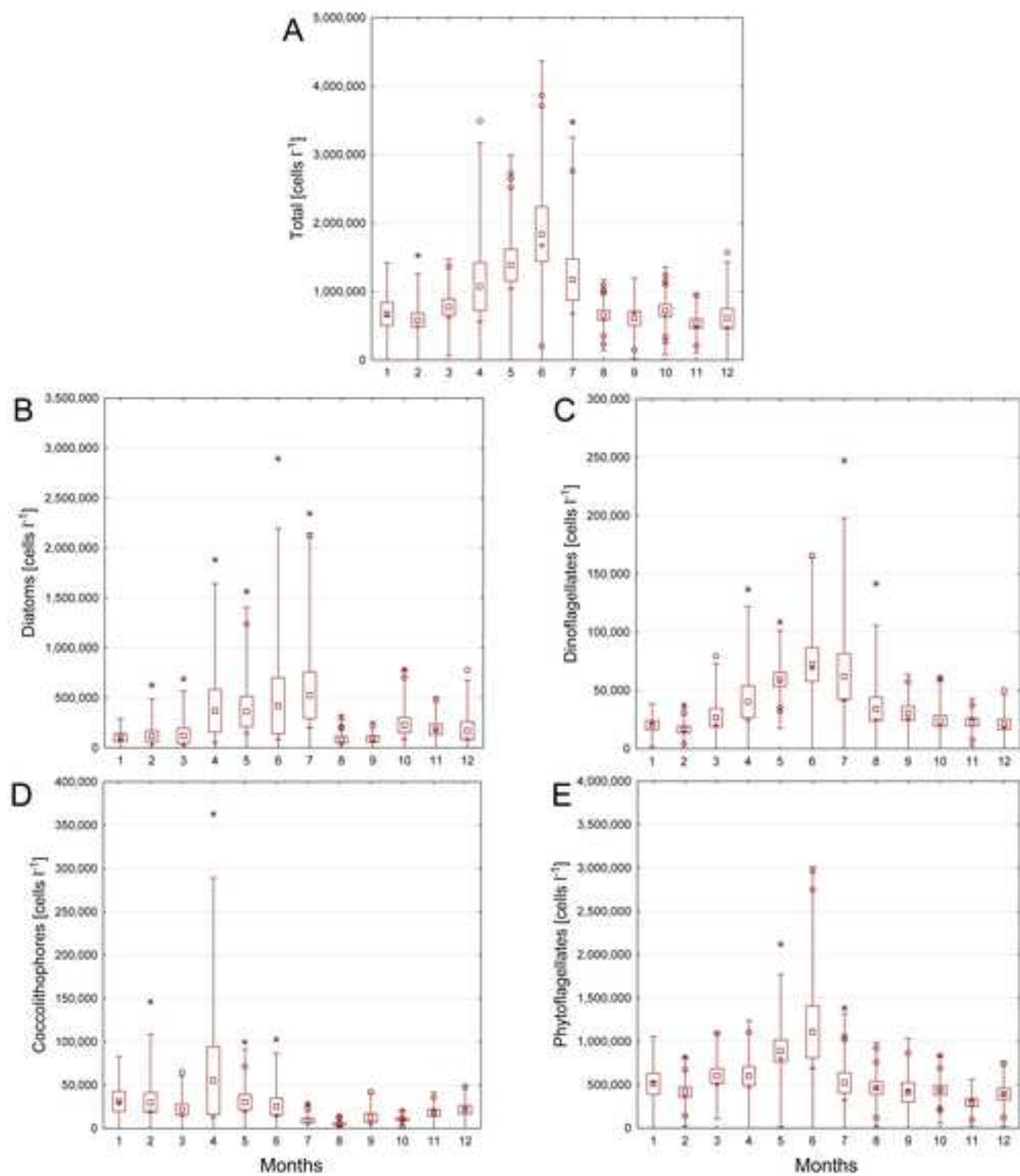
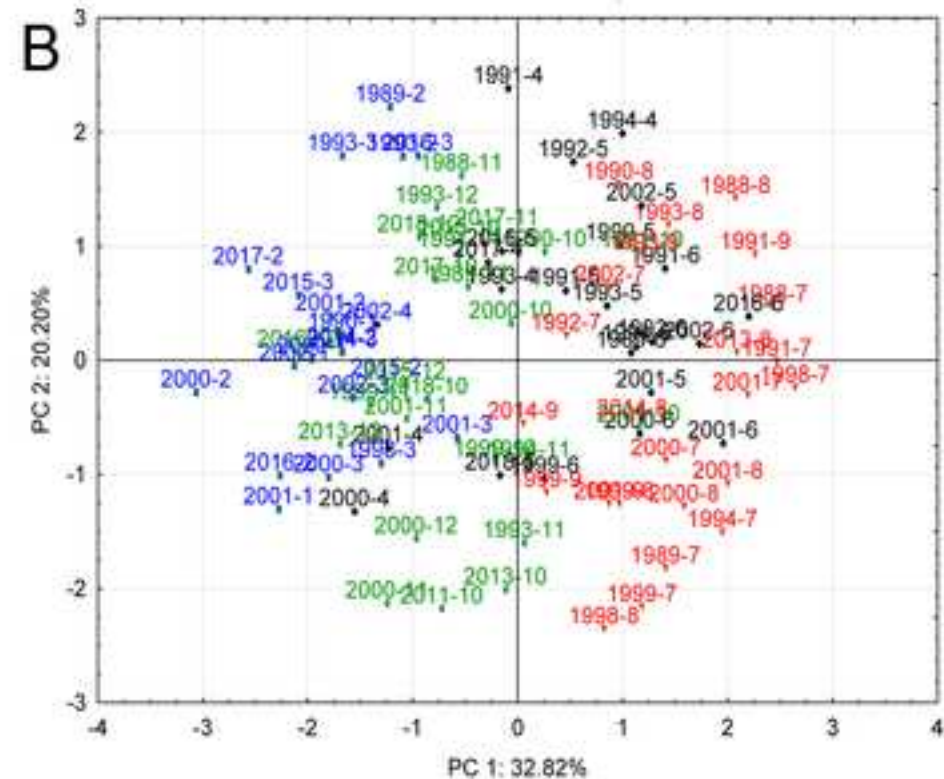
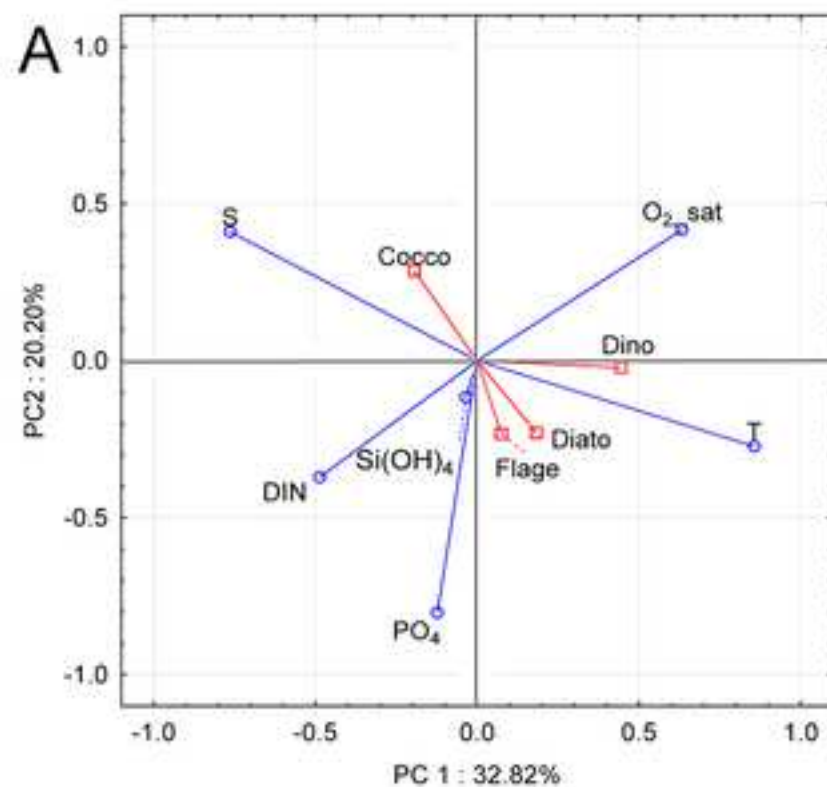


Figure 7

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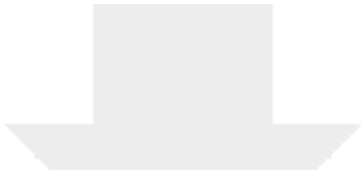




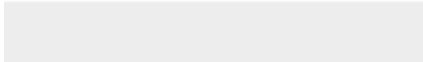
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