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(Article begins on next page)

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1 **Phytoplankton and environmental drivers at a long-term offshore station in the northern**

2 **Adriatic Sea (1988–2018)**

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Abstract

A long term (1988–2018) data set of phytoplankton and physico-chemical parameters was analyzed for the first time in an offshore station of the LTER Senigallia-Susak transect (northern Adriatic Sea) not directly affected by the coastal nutrient input. The mean annual cycle of phytoplankton markedly differed from that observed in the coastal areas, showing the maximum in June and the minimum in November. The main component of phytoplankton community was represented by phytoflagellates, whose trend paralleled that of total phytoplankton. On average, diatoms peaked in July, dinoflagellates in June and coccolithophores in April. The phytoplankton maximum in summer is explained mainly by the allochthonous input of DIN and PO_4 carried by the low salinity waters expanding eastward, during the stratification of water column. Resuspension processes seem to be less effective for PO_4 than for DIN. The most representative phytoplankton taxa for each season as indicated by the IndVal analysis were identified and were only in part similar to those observed in the coastal area. The interannual anomaly trend showed a significant increase of temperature, DIN and phosphates. Although no significant changes were found for the total phytoplankton, a reduction of winter dinoflagellate and coccolithophore abundances was observed.

1. Introduction

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

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

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

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

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

93   composition and (iii) the relationships between phytoplankton and environmental parameters in an

94   offshore area not directly affected by the coastal nutrients input.

95

96   **2. Materials and Methods**

97   *2.1 Sampling*

98   Sampling was carried out at the SG05 station (43°54.460 N, 13°26.941 E) (bottom depth 55 m)

99   located at 15 nM from the Italian coast (Fig.1) on board of several oceanographic vessels (S. Lo

100   Bianco, G. Dallaporta, Tethis, Copernaut Franca, Urania, Alliance, Minerva, Bannock, D'Ancona)

101   since July 1988 to December 2018 with a variable time frequency (from monthly to quarterly) and

102   with periods of interruptions the longest of which was between 2003 and 2012 for phytoplankton

103   sampling. A total of 11,339 samples were collected. Sampling dates for each parameter are indicated

104   in the Table S1.

105   From 1988 to 1991, Conductivity-Temperature-Depth (CTD) data were acquired by a Neil Brown

106   Instrument System (NBIS), while after 1992 with a SeaBird Electronic SBE 911plus. Dissolved

107   oxygen was directly analyzed on board according to Winkler (1888), and samples were immediately

108   fixed and stored in the dark and analyzed within 24 h. From 1988 to 1998 the oxygen concentrations

109   were determined using titration and then after 1998 by potentiometric method (Furuya and Harada,

110   1995).

111   Water samples for determination of dissolved inorganic nutrients (nitrite-NO_2, nitrate-NO_3,

112   ammonia-NH_4, orthophosphate-PO_4 and orthosilicate-$Si(OH)_4$) and for phytoplankton analysis were

113   collected by Rosette system equipped with Niskin bottles. Filtration was done directly on board and

114   significant sample alterations were excluded as: (i) as soon as the Niskin bottle was on board, water

115   was sampled with a closed filtration system without any contact with air, (ii) the filtrated was closed

116   in a 4 ml compressed polyethylene tube and rapidly frozen, (iii) unfreezing phase was as rapidly and

117   the analysis was done directly on the tube, without further pouring. Sampled quotes for nutrients and

118   phytoplankton were: surface (0-5 m layer), 1-2 intermediate depths, generally at the base of mixed

119   layer and the maximum fluorescence depth (between 10 and 40 m) and 1 m above the bottom (54 m).

Samples for nutrient analysis were filtered (GF/F Whatman, 0.7 μm), and stored at −22 °C in

polyethylene vials until analysis. Water samples for phytoplankton analysis were collected in 250 ml

dark glass bottles and preserved by adding 0.8% formaldehyde (prefiltered and neutralized with

hexamethylenetetramine) (Throndsen, 1978) until analysis.
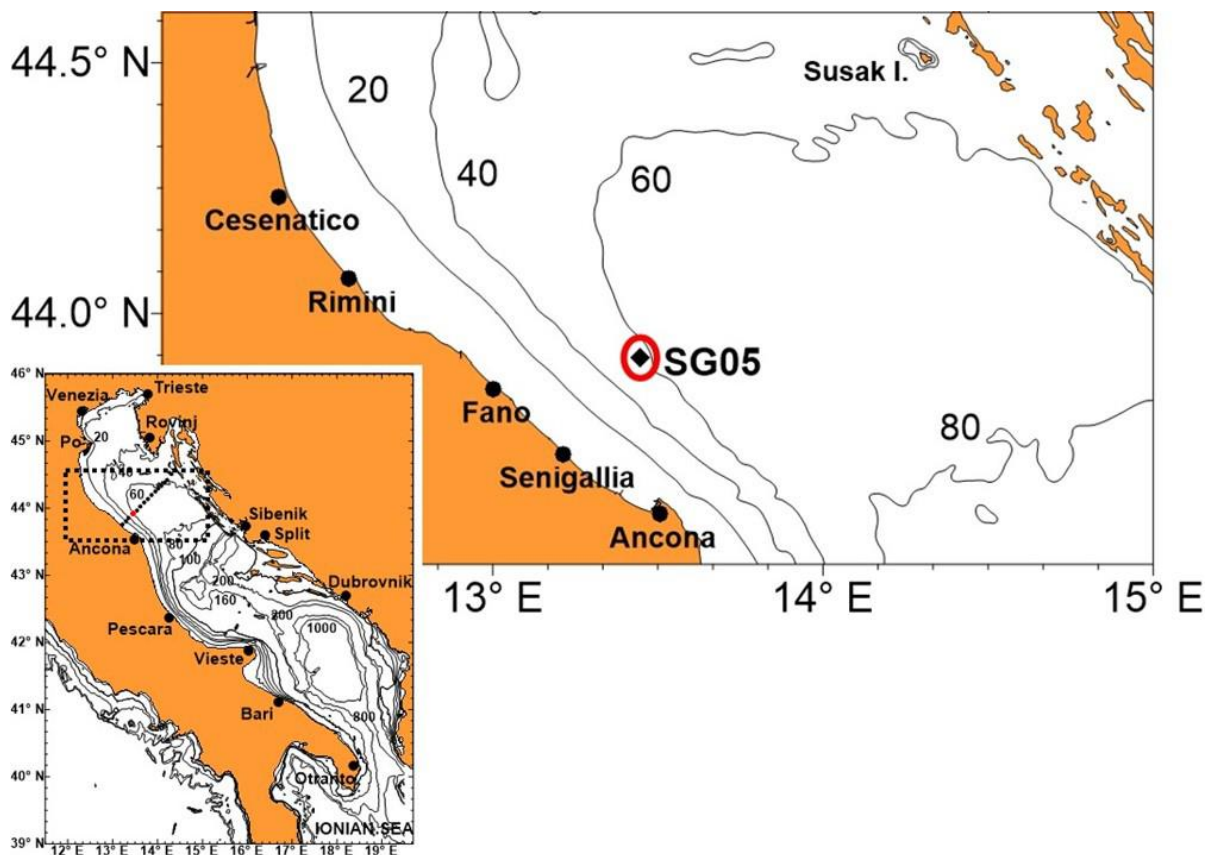
Fig. 1. Map of the study area indicating the position of sampling station. In the small box, the

Senigallia-Susak transect is shown and the study station is indicated by the red color.

2.2 Nutrient analysis

Nutrient analysis was carried out using a Perkin Elmer spectrophotometer 550A model in the period

1988 to 1998, an autoanalyzer TRAACS 800 BRAN+LUEBBE in 1999 to 2005 and an autoanalyzer

QUAATRO Technicon (Strickland and Parsons, 1972) after 2005. Dissolved Inorganic Nitrogen

(DIN) is intended as the sum of NO_2, NO_3 and NH_4 concentrations.

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).
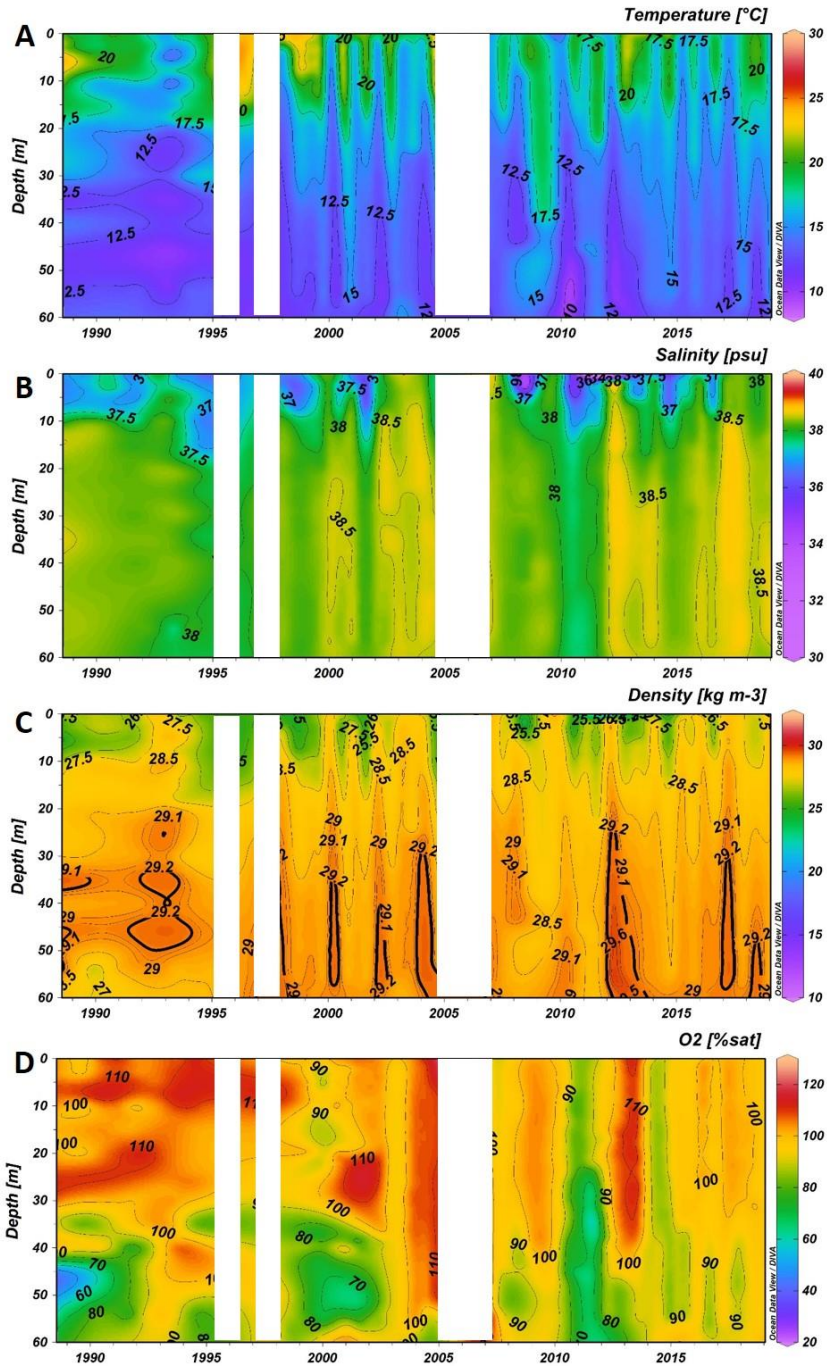
194

195   Fig. 2. Temporal and vertical variations of (A) temperature (°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

Nutrient concentrations in the water column along the study period are shown in Fig. 3. High concentrations of DIN (Fig. 3A) were recorded in correspondence of lower salinity values (Fig. 2B) related to the higher Po River flow (not shown) in November 2010, May 2013, and November 2014. In winter 2012, a cold and high-salinity water mass with high DIN values was observed at 20 m depth (Fig. 3A), and this water mass extended even eastward (data not shown). Time evolution of orthophosphate (Fig. 3B) showed higher values in the last decade. High values were often observed below 40 m depth, and at 0-10 m layer in December 2007 and June 2008, corresponding to relatively low salinity values. Concentrations of silicate were higher (> 3 µM) below the 30 m depth, with peaks in 2001, 2010 and 2014. Only in 1989 a higher value (6 µM) was recorded at 10 m (Fig. 3C).
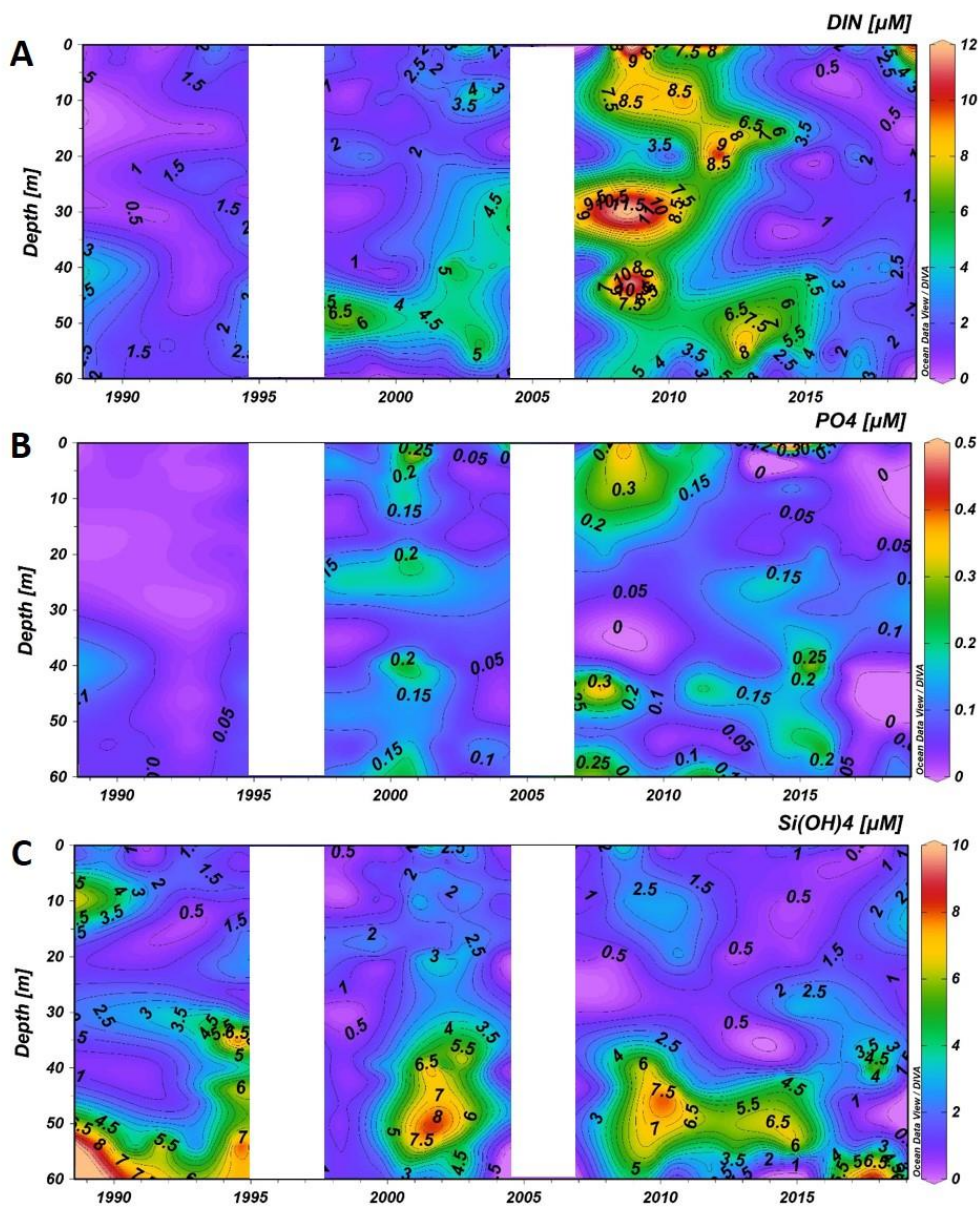
208 Fig. 3. Temporal and vertical variations of (A) Dissolved Inorganic Nitrogen (DIN), (B)

209 orthophosphate (PO_4) and (C) orthosilicate ($Si(OH)_4$) 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 ± 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).
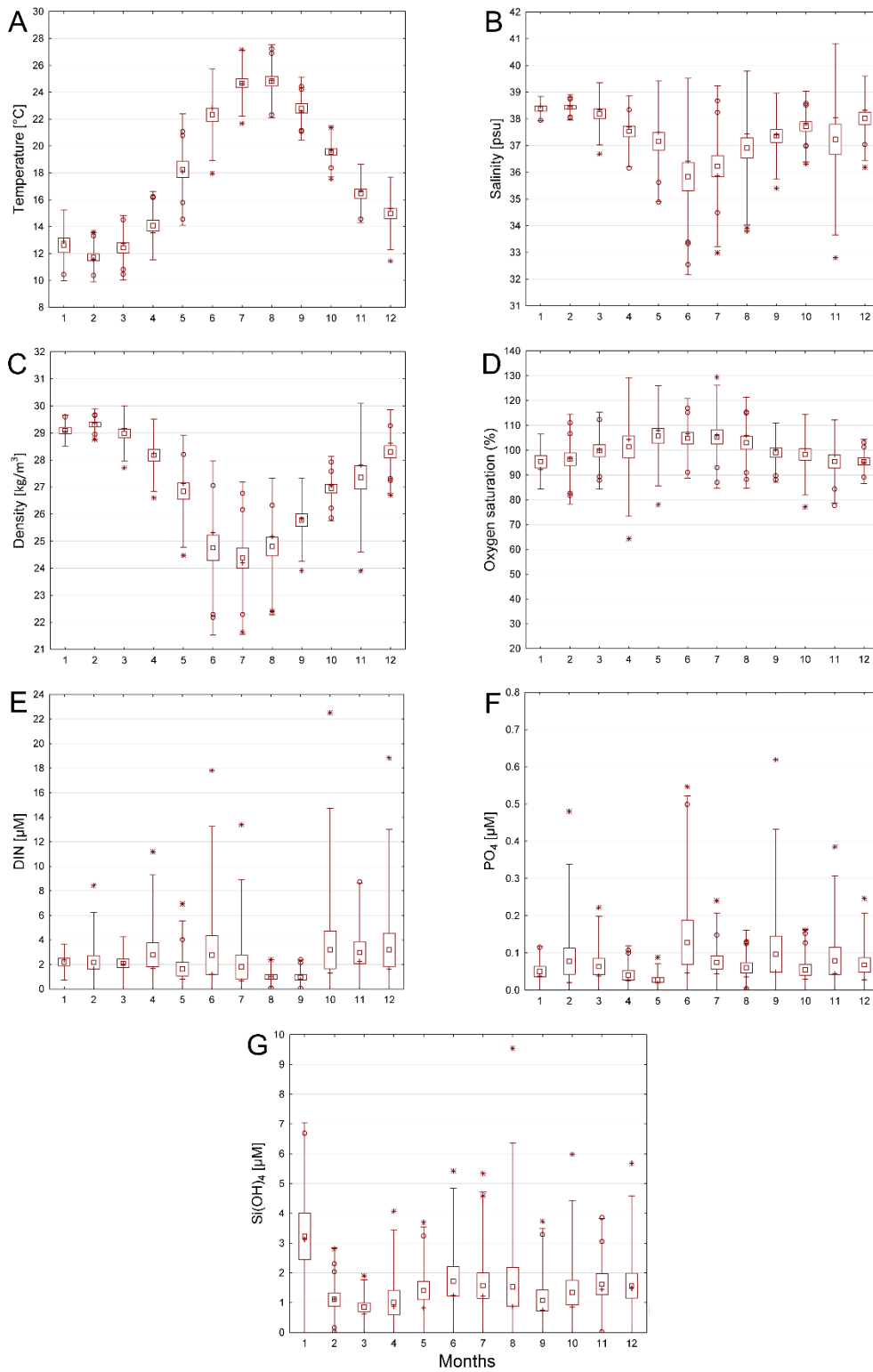
Fig. 4. Mean annual cycle of physico-chemical parameters calculated on a mean monthly basis from 1988 to 2018 in SG05 station at surface: (A) water temperature (°C), (B) salinity, (C) density (kg m^{-3}), (D) oxygen saturation (%), (E) Dissolved Inorganic Nitrogen (DIN, µM), (F) orthophosphate (PO$_4$, 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 (∗).

223   Salinity showed the maximum in February (38.43 ± 0.06), followed by a decreasing trend, reaching

224   the minimum in June (35.84 ± 0.53), after which an increasing trend was observed (Fig. 4B).

225   The mean annual trend of water density at surface showed the highest values in February (29.31 ±

226   0.08 kg m^{-3}), then a decrease was observed in spring and the minimum (24.38 ± 0.38 kg m^{-3}) was

227   recorded in July (Fig. 4C).

228   On average, at surface dissolved oxygen showed oversaturation values (above 100%) from April to

229   August (Fig. 4D) indicating a predominance of oxygen production than consumption.

230   The mean annual cycle of Dissolved Inorganic Nitrogen (DIN) showed the minimum values in

231   September (0.95 ± 0.21 µM), and an increase in spring and autumn, with maximum in October (3.19

232   ± 1.54 µM, Fig. 4E). Orthophosphate showed the minimum in May (0.03 ± 0.01 µM) and maximum

233   in June (0.13 ± 0.06 µM) followed by a decrease in summer and a new peak in September (Fig. 4F).

234   Silicate values ranged from 0.85 ± 0.15 µM in March and 3.23 ± 0.78 µM in January (Fig. 4G).

235   On a mean monthly basis, the N:P ratio showed lower values in summer with the minimum in July

236   (23.42 ± 6.83), whilst the maximum was found in October (143.41 ± 81.23) (Fig. S1A). Regarding

237   the Si:N ratio, the lowest values were observed in winter (minimum in February, 0.66 ± 0.20),

238   followed by an increase in spring with the maximum in June (6.35 ± 3.11), high values in summer

239   and a new peak in October (Fig. S1B).

240

241   3.1.3 Interannual trend

242   The trends of physico-chemical parameters in terms of anomalies are shown in Figure 5. Temperature

243   anomaly (Fig. 5A) showed a significant increase ($p < 0.05$). On the contrary, the salinity trend did

244   not reveal any significant interannual variations (Fig. 5B).

245   Regarding nutrients, both DIN and PO$_4$ (Fig. 5C,D) showed a significantly increasing trend ($p < 0.01$),

246   whilst silicate (Fig. 5E) did not reveal any significant interannual variations. Considering N:P and

247   Si:N ratios (Fig. S1C,D), no significant interannual trend was observed ($p > 0.05$).

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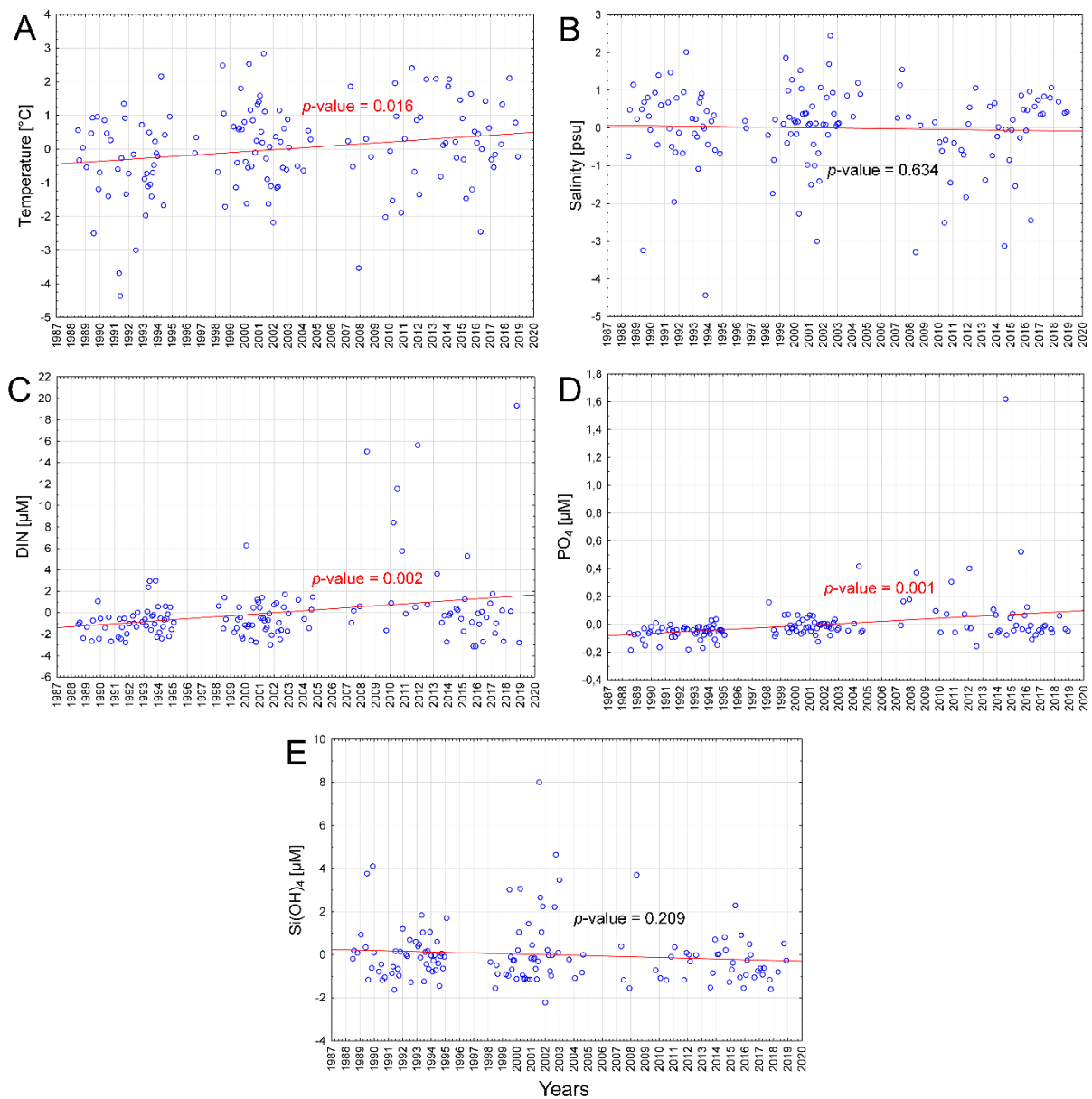
Fig. 5. Trend of anomalies of physical and chemical parameters at surface layers: (A) water temperature (° C), (B) salinity, (C) Dissolved Inorganic Nitrogen concentrations (DIN) (μM), (C) PO$_4$ concentration (μM) and (E) silicate concentration (μM).

3.2 Phytoplankton

3.2.1 Temporal variability in the water column

The vertical distribution of phytoplankton is showed in Figure S2. Diatom abundances (Fig. S2A) showed that in the period 1988 to 1998 higher values were observed in the 0–20 m layer than in the 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 o 45%. Dinoflagellate and

280   coccolithophore contribution ranged from 3 to 5% and from 1 to 5% respectively.

281
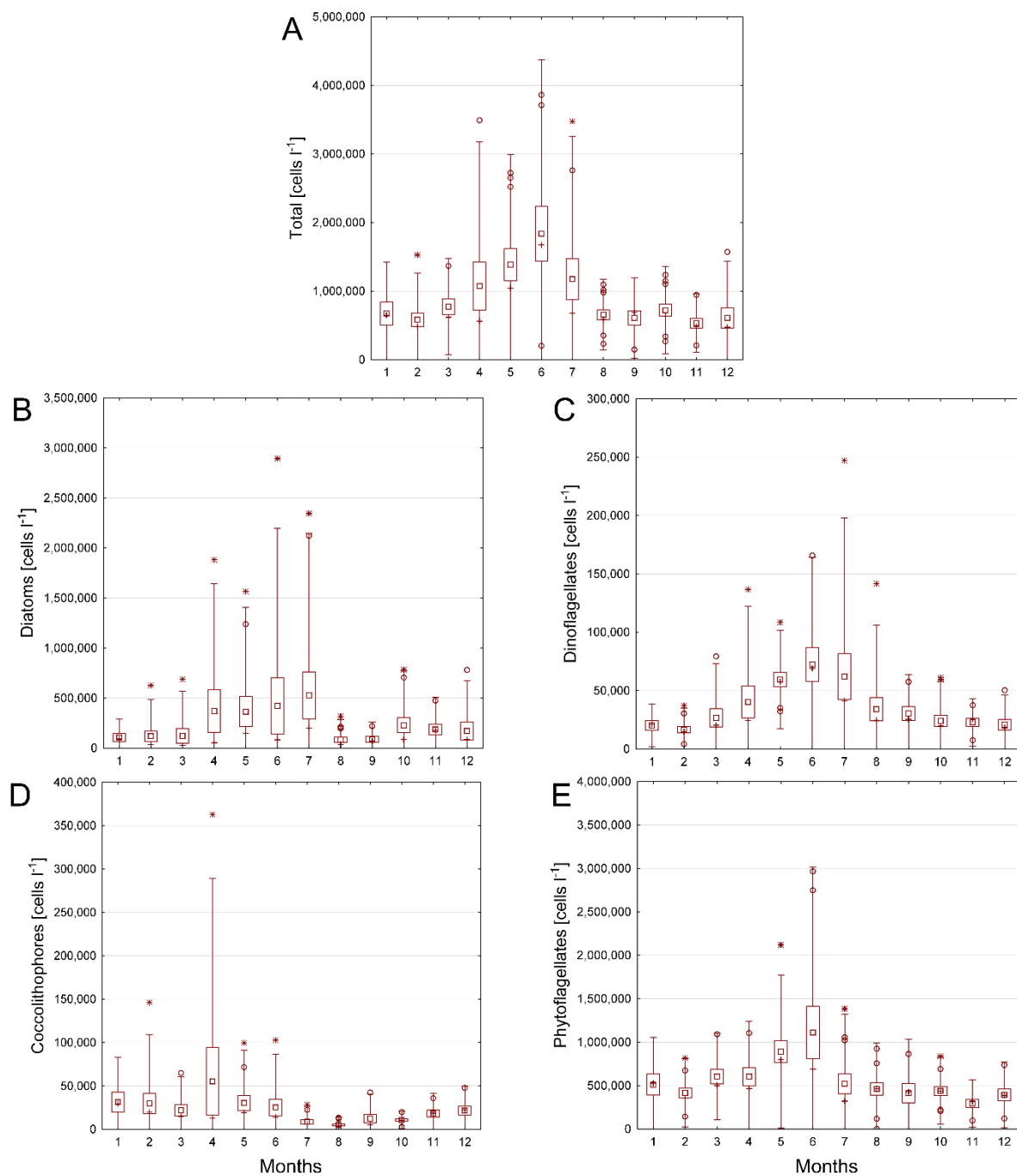
Fig. 6. Mean annual cycle of phytoplankton abundance (cells l^{-1}) calculated on a mean monthly basis from 1988 to 2018 in SG05 station at surface: (A) total phytoplankton, (B) diatoms, (C) dinoflagellates, (D) coccolithophores and (E) phytoflagellates. Box plots report the data distribution with the mean (+), the median (□), the interquartile range (box), the non-outlier range (vertical bars), the outliers (○) and the extremes (∗).

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

290   At the end of summer, values decreased, and in August minimum was observed (83,125 ± 27,492

291   cells l⁻¹). A second peak was found in autumn (October, 228,213 ± 75,201 cells l⁻¹).

292   Dinoflagellate abundances showed minimum values in February (16,496 ± 2,670 cells l⁻¹) and an

293   increase in spring, showing the maximum in June (72,381 ± 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 ± 1,201 cells l⁻¹), an increase in autumn and winter, and the maximum in April (55,192 ± 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 ± 300,909 cells l⁻¹) and the minimum in

299   November (294,238 ± 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 *Emiliania huxleyi*, *Skeletonema marinoi*, *Dactyliosolen phuketensis,*

308   *Pseudosolenia calcar avis* and *Chaetoceros affinis*. In spring, significant taxa were *Dactyliosolen*

309   *fragilissimus*, *Chaetoceros throndsenii*, *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.

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

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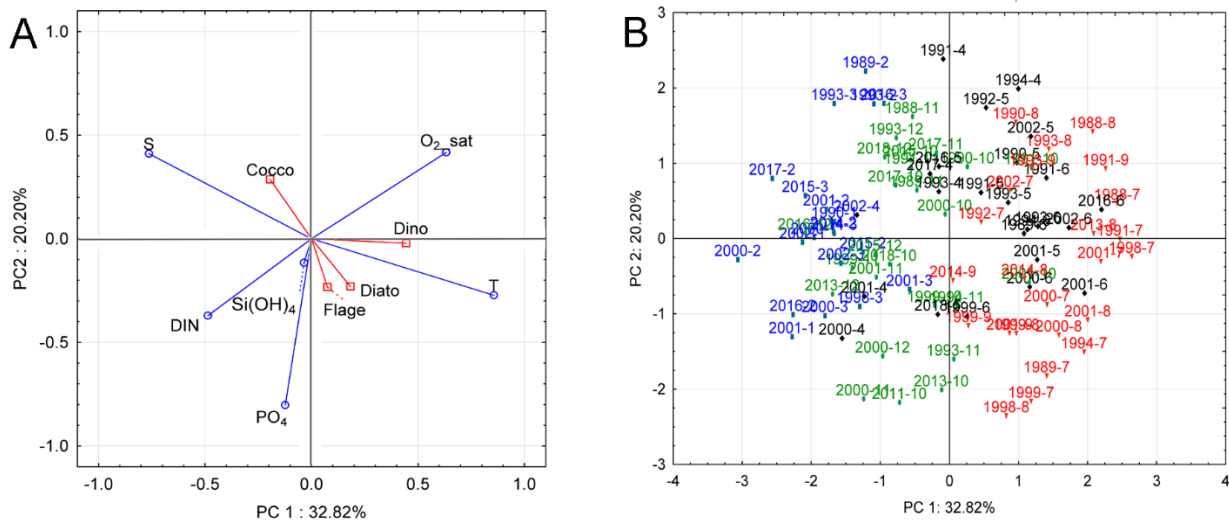
327 phytoplankton).

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 ± standard error (SE) are shown. As: ns, not significant; *

332 $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Parameter	Season	1988-2002 Avg ± SE	2013-2018 Avg ± SE	p-level	Tukey test
Diatoms (cells l^{-1})	Winter	270,576 ± 52,820	112,754 ± 29,515	ns	ns
	Spring	438,586 ± 145,388	155,989 ± 96,916	ns	ns
	Summer	258,821 ± 100,686	108,899 ± 54,128	ns	ns
	Autumn	213,083 ± 51,227	106,662 ± 43,421	ns	ns
Dinoflagellates (cells l^{-1})	Winter	40,017 ± 3,801	27,285 ± 6,612	**	88-02 > 13-18
	Spring	57,554 ± 6,560	60,226 ± 23,258	ns	ns
	Summer	44,532 ± 9,241	30,974 ± 4,161	ns	ns
	Autumn	25,818 ± 3,479	17,767 ± 1,915	ns	ns
Coccolithophores (cells l^{-1})	Winter	23,228 ± 4,348	15,081 ± 4,040	**	88-02 > 13-18
	Spring	38,151 ± 14,430	26,275 ± 15,682	ns	ns
	Summer	8,501 ± 1,766	3,974 ± 1,693	ns	ns
	Autumn	15,640 ± 2,654	17,722 ± 2,843	ns	ns
Flagellates (cells l^{-1})	Winter	552,546 ± 42,610	594,488 ± 104,196	ns	ns
	Spring	846,407 ± 111,278	1,009,411 ± 398,669	ns	ns
	Summer	456,606 ± 59,475	622,905 ± 176,270	ns	ns
	Autumn	354,789 ± 47,219	437,893 ± 27,120	ns	ns
Total (cells l^{-1})	Winter	941,984 ± 79,779	750,418 ± 134,936	ns	ns
	Spring	1,486,528 ± 202,217	1,253,552 ± 528,283	ns	ns
	Summer	839,434 ± 132,973	769,163 ± 128,399	ns	ns
	Autumn	629,423 ± 79,279	580,201 ± 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 PO_4. 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 PO_4 (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, PO_4 = Orthophosphate, $Si(OH)_4$ = silicates, O_2_sat = oxygen saturation, Diato =

353   Diatoms, Dino = Dinoflagellates, Cocco = Coccolithophores, Flage = Flagellates. (B) Score plot

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

355   ● = autumn).

356

357   **4. Discussion**

358   In this study, we highlighted for the first time the intra and interannual variability of phytoplankton,

359   the community composition, and their relationships with the oceanographic and trophic condition, in

360   an offshore area of the northern Adriatic basin based on a long-term dataset.

361

362   *4.1 Mean annual cycle*

363   The mean annual cycle of phytoplankton in an open-water station of the LTER Senigallia-Susak

364   transect (NA) indicated that highest abundances occurred in early summer mainly due to

365   phytoflagellates' and diatoms' maxima. Minimum abundances occurred in autumn and winter.

366   This cycle markedly differed from that recorded both in the western and eastern NA coastal waters.

367   In the E Adriatic coast, spring and autumn peaks are commonly observed mainly related to diatom

368   proliferation (Cerino et al., 2019; Maric et al., 2012; Mozetič et al., 2010). Instead, in the coastal

369   waters of the NW Adriatic Sea, the winter diatom bloom represents the most relevant plankton

370   increase throughout the year (Totti et al., 2019), as already observed in other Mediterranean areas

371   (Goffart et al., 2015), followed by the classic peaks in spring and autumn again due to diatoms and

372   phytoflagellates. The observed differences between the offshore and costal sites of the Senigallia-

373   Susak transect could be explained considering the different oceanographic conditions. In the coastal

374   station, the strong winter bloom is related to the shallowness of water column (11 m depth) that allows

375   phytoplankton to remain in the photic zone even in winter mixing conditions, allowing the bloom of

376   *Skeletonema marinoi*, a small colonial diatom with a marked seasonal behaviour (Totti et al., 1999).

377   On the contrary, in the deeper offshore station, the winter phytoplankton abundance is low, according

378   to the classical Sverdrup model (Sverdrup, 1953). Furthermore, the offshore station is located in the

379   waters beyond the coastal front, not directly affected by the coastal input, except than during the

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

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

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

4.2 Community structure

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

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), whom 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 on 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/PO_4 and $Si(OH)_4/DIN$. The latter has been

468 suggested as able to affect the phytoplankton community, facilating 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 *Emiliania 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 *Emiliania huxleyi* for which the haploid life phase does not bear

483   coccoliths (Frada et al., 2012), and a hypothetical occurrence and proliferation of that phase would

484   surely go completely unnoticed, unless other methodological approaches are used. Regarding the

485   observed decrease of dinoflagellates, we need to evaluate it carefully in a longer data set.

486   The long-time changes in the trophic state and phytoplankton communities have been highlighted in

487   a number of Mediterranean sites (Goffart et al., 2002, 2015; Mercado et al., 2005; Ninčević Gladan

488   et al., 2010; Totti et al., 2019; Zingone et al., 2010b), where to date longer and longer temporal series

489   are becoming available. In the Adriatic Sea, long-term studies sometimes have highlighted tendencies

490   apparently contrasting, depending on the studied area and/or on the considered starting and ending

491   points to trace the traits. For example, a number of studies highlighted a tendency to oligotrophication

492   (Cabrini et al., 2012; Djakovac et al., 2012; Mozetič et al., 2010), whereas others did not (Bernardi

493   Aubry et al., 2012; Totti et al., 2019). In any case, all agree that the main forcing that is responsible

494   for this variability is the rate of the Po River outflow, i.e., the rain regime. In that sense, it has recently

495   been highlighted that an increase in the frequency of intense rains and extremely high Po River

496   discharges have occurred during the last decade, and that could have affected the annual availability

497   of nutrients, particularly of DIN, in the NA (Grilli et al., 2020; Totti et al., 2019).

498

499   **5. Conclusions**

500   In this study, the phytoplankton communities in their oceanographic scenario in an LTER-offshore

501   station not directly affected by riverine inputs, were highlighted over 30 years. The mean monthly

502   cycle of the phytoplankton was found to be highly dependent on the oceanographic conditions of the

503   water column, as during water stratification the spreading of the fresher and nutrient-rich waters

504   beyond the coastal front allows the phytoplankton annual peak. This study confirmed the P limiting

505   conditions of the N Adriatic Sea, suggesting the importance of organic P sources and remineralization

506   processes along the water column. Instead, the resuspension processes seemed less effective for P

507   than for DIN. Species composition in the offshore station was found to reflect only in part the coastal

508   community, as many taxa were significant only in the offshore site or related to a different season.

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

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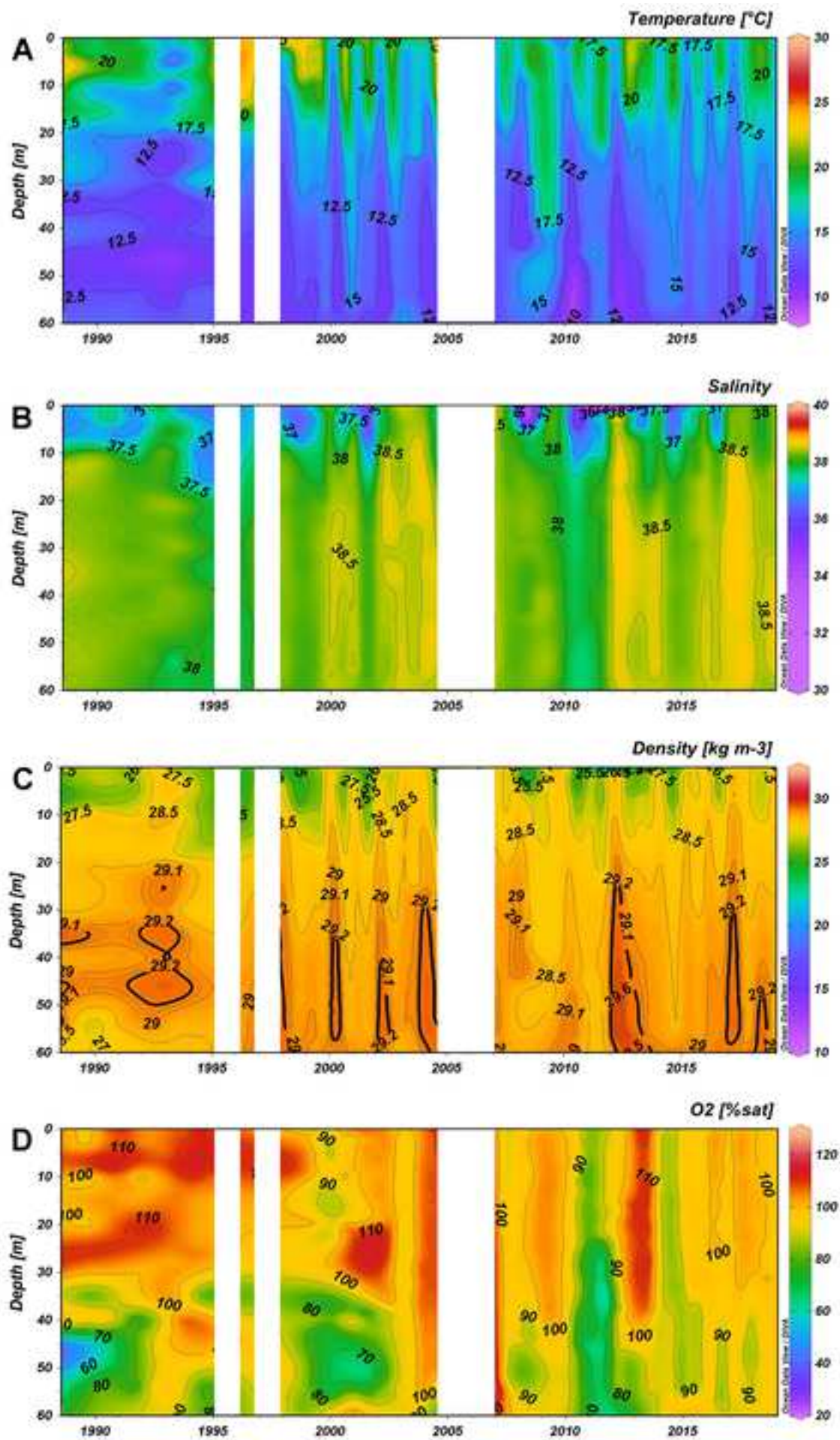
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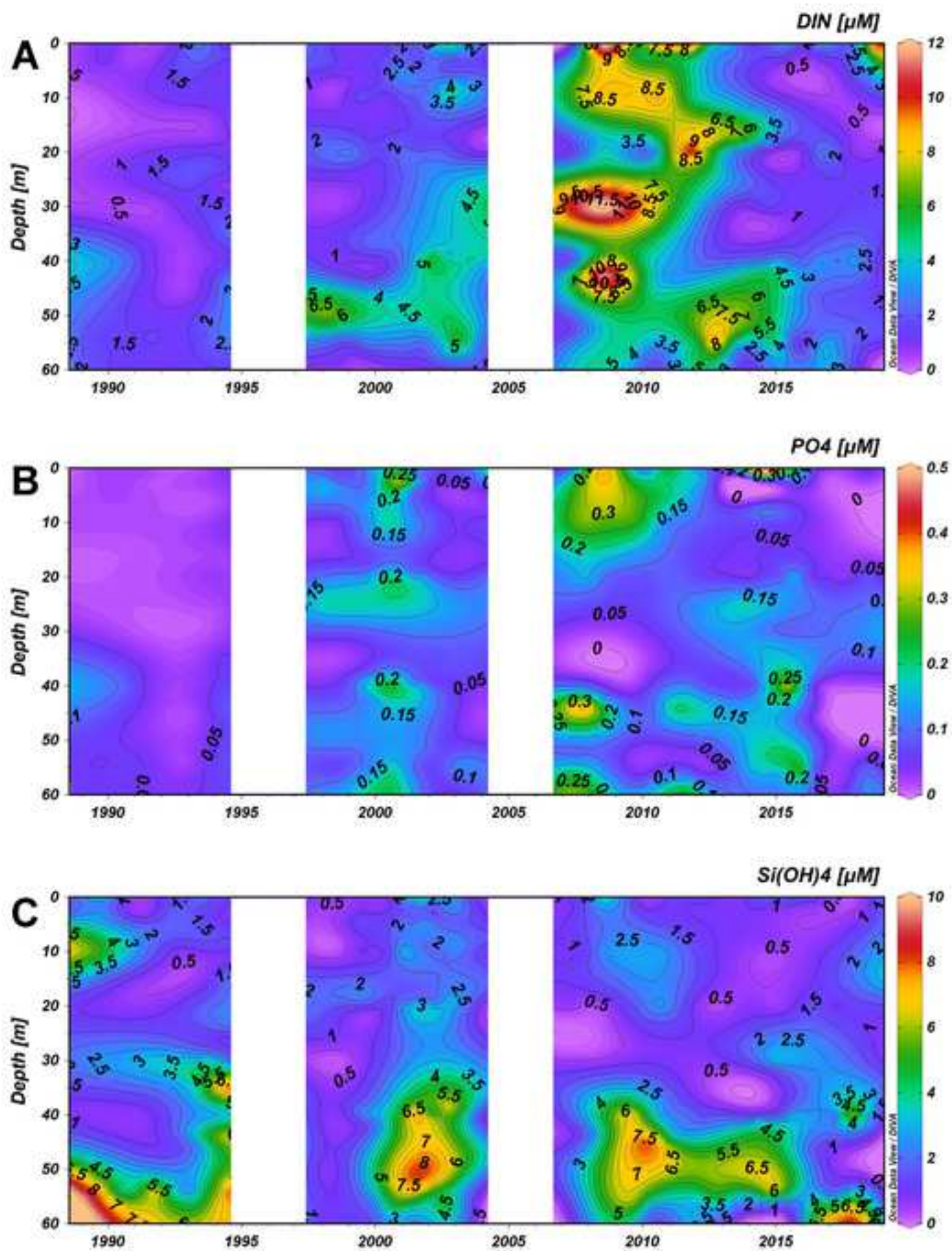
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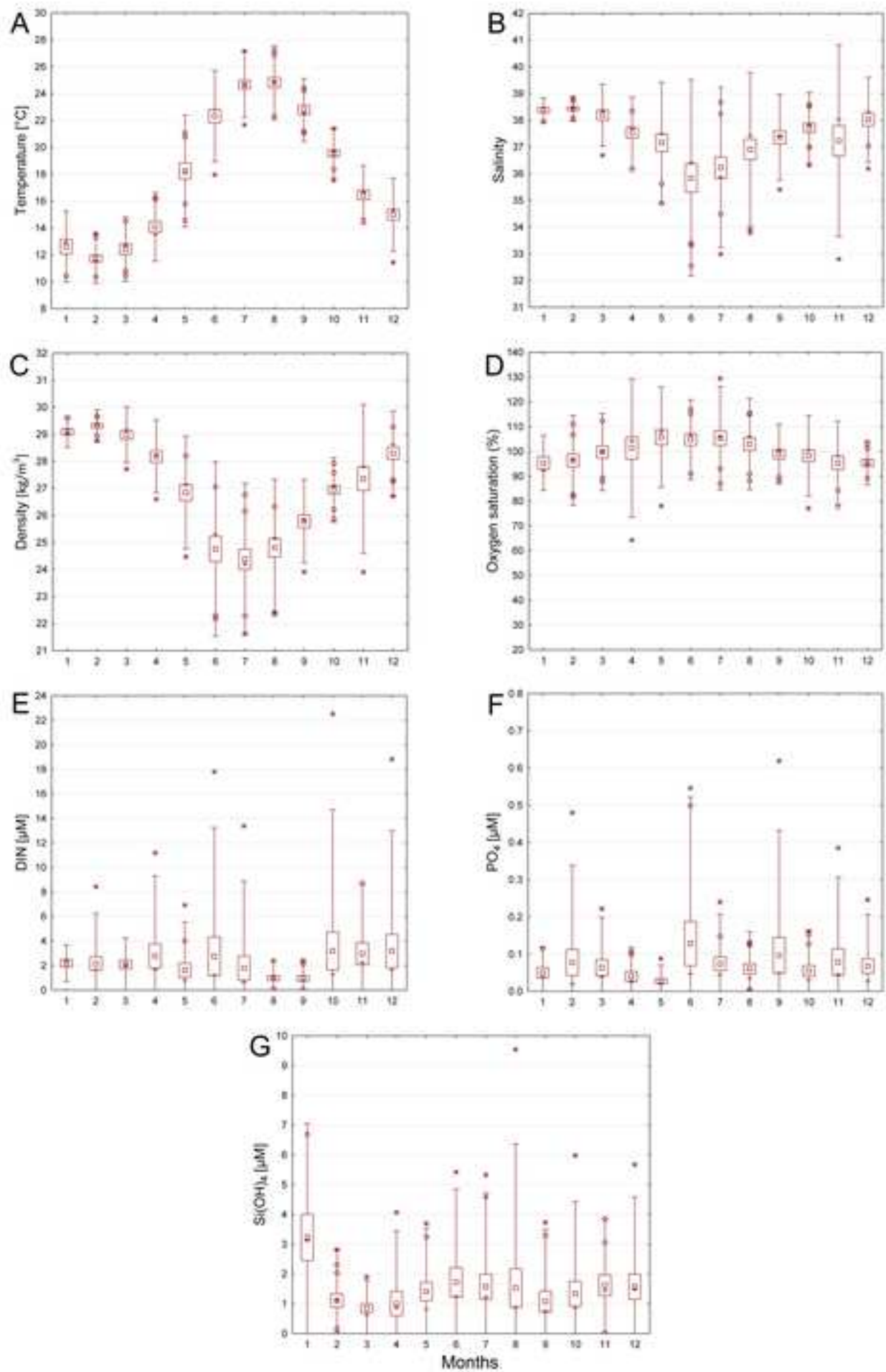
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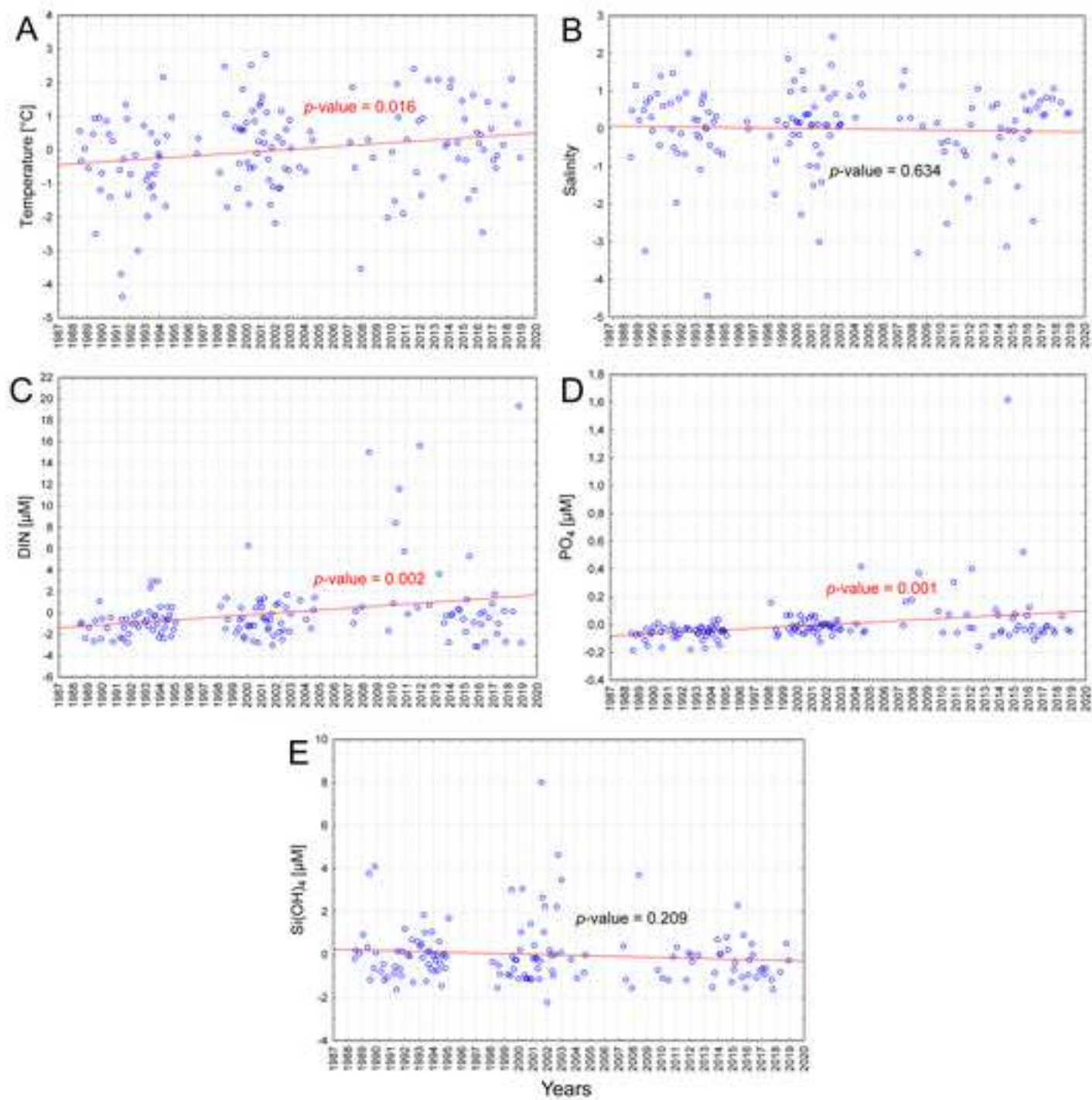
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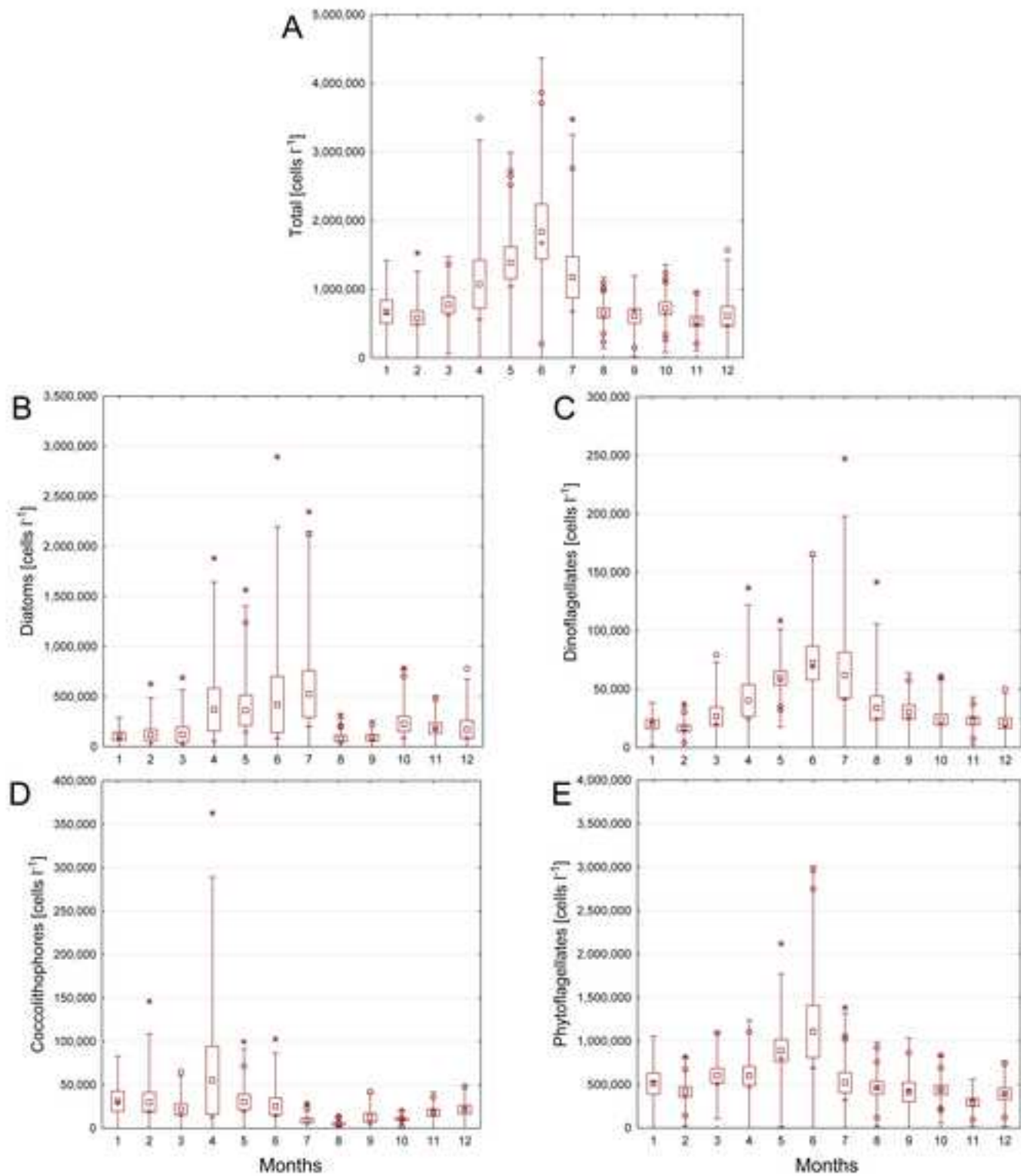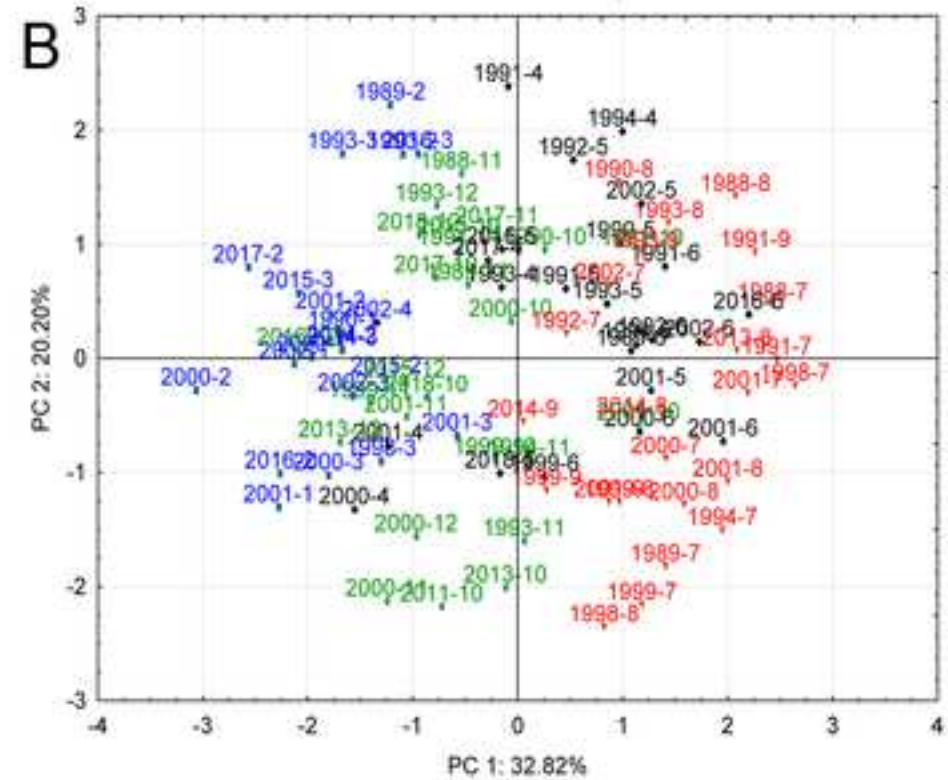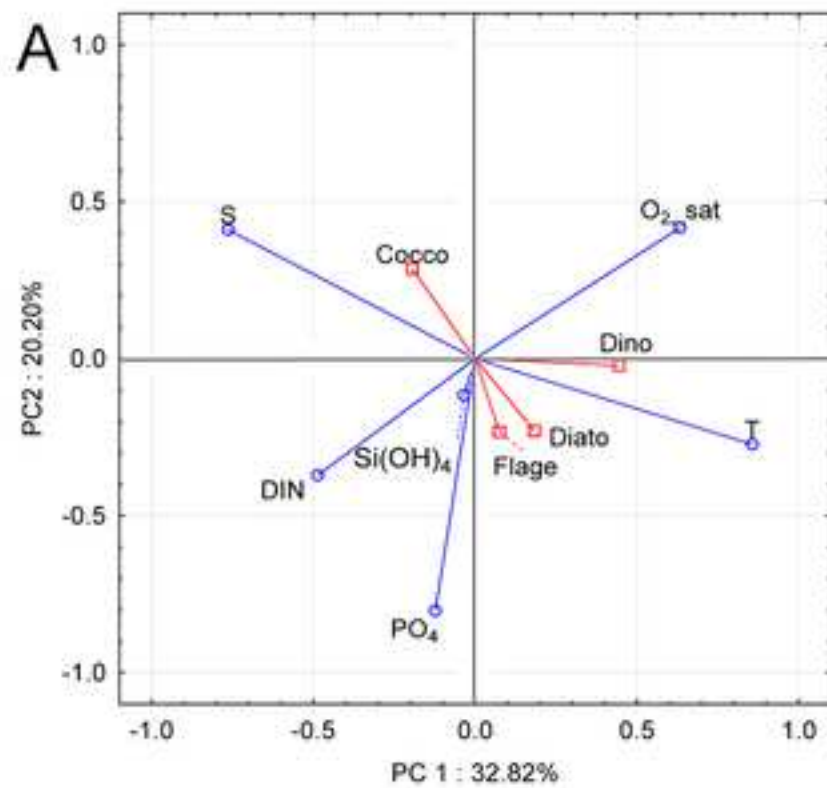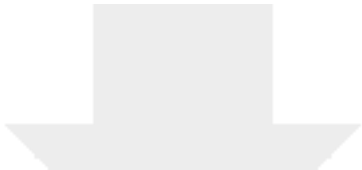
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Figure 1

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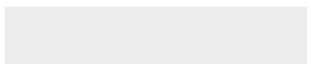Click here to access/download;Figure;Fig. 7.tif ☒
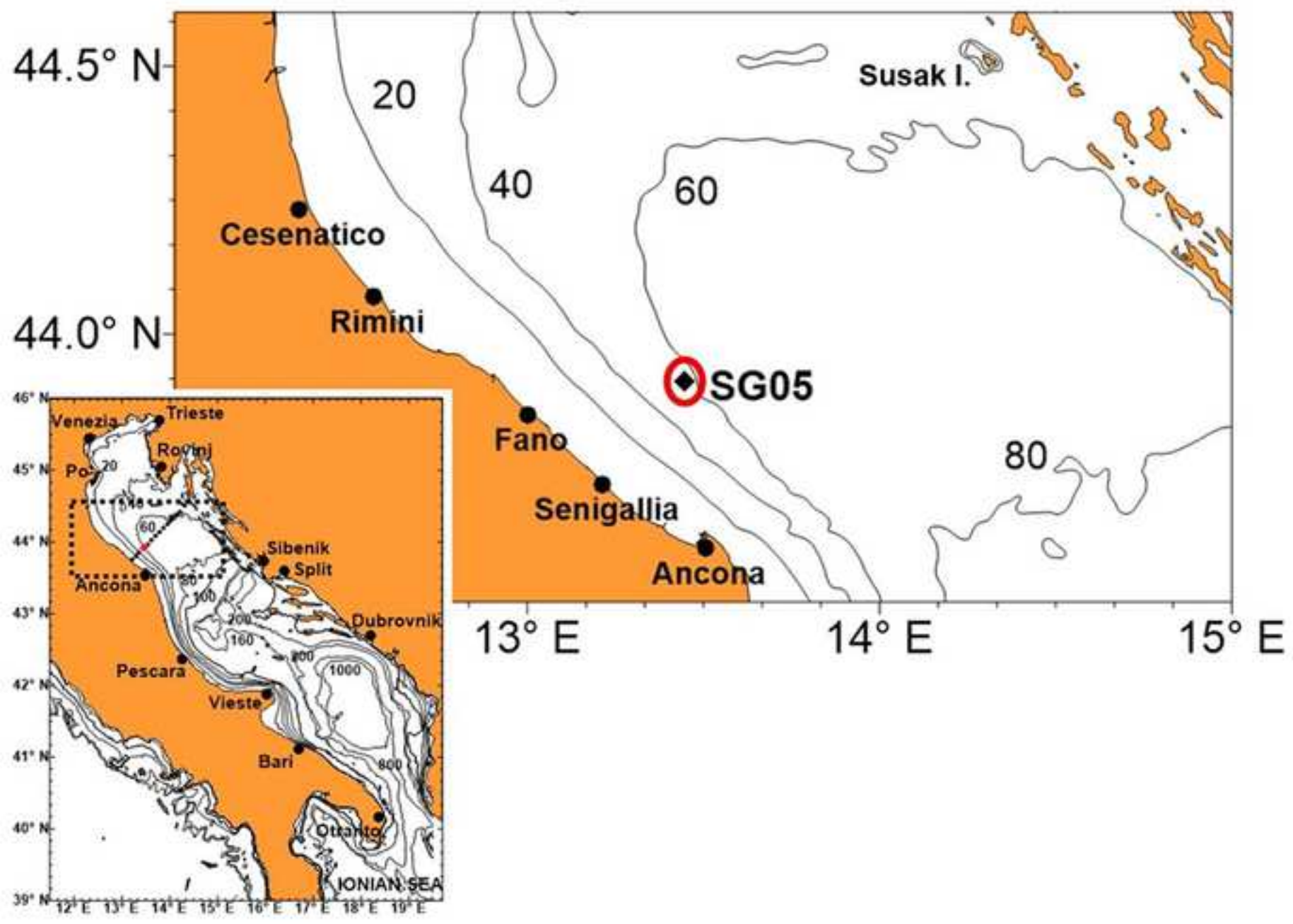
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