Phytoplankton communities in the northwestern Adriatic Sea: interdecadal variability over a 30-years period (1988-2016) and relationships with meteoclimatic drivers

Cecilia Totti[1], Tiziana Romagnoli[1], Stefano Accoroni[1], Alessandro Coluccelli[1], Marco Pellegrini[1,2], Alessandra Campanelli[3], Federica Grilli[3], Mauro Marini[3]

[1]Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona (Italy)

[2]LIF srl, Via di Porto 159 – 50018 Scandicci (Firenze), Italy

[3]National Research Council, CNR-IRBIM, Largo Fiera della Pesca, 2, 60125, Ancona, Italy

Corresponding Author:

Prof. Cecilia Totti

Dipartimento di Scienze della Vita e dell'Ambiente

Università Politecnica delle Marche

via Brecce Bianche, 60131 Ancona (Italy)

c.totti@univpm.it

+ 39 071 2204647

1   **Abstract**

2   In this study the interannual variability of phytoplankton over a ~30-years period in the coastal site of LTER-

3   Senigallia transect (N Adriatic Sea) was investigated to document patterns potentially related to

4   environmental/climatic drivers. Comparing physical and chemical data of the periods 1988-2002 and 2007-

5   2016 periods, we showed that phytoplankton abundance and biomass and inorganic nutrient

6   concentrations increased in the last decade, indicating that the tendency to oligotrophication due to the

7   drop of the Po River outflow in the years 2002-2007 was reversed in the period 2007-2016. The typical P-

8   limited conditions of the N Adriatic Sea seem to have been attenuated in the study area. P levels were not

9   explained by the P concentrations in the Po River waters, suggesting the possible influence of other local P

10   sources that could be related to the anomalous meteorological events (intense rainfalls) that took place in

11   the 2007-2016 period. In the last decade, the community structure and seasonality of phytoplankton

12   markedly changed, as highlighted by the different indicator species for each season: the blooms of

13   *Skeletonema marinoi* shifted from winter to spring. A significant decrease of coccolithophores was

14   observed particularly in winter months in the 2007-2016 period: some indicator species among the most

15   relevant in the 1988-2002 period (such as *Emiliania huxleyi* in winter, and *Syracosphaera pulchra* in spring)

16   have lost this role in 2007-2016. Dinoflagellate abundances decreased, except in spring when the

17   occasional proliferation of large sized species caused biomass peaks. The phytoplankton annual cycle

18   became irregular with sudden diatom blooms, reflecting the variability of meteorological events in recent

19   years. It is noteworthy that in the last decade, an allochthonous species, i.e. the diatom *Pseudo-nitzschia*

20   *multistriata*, became a regular inhabitant of the autumn phytoplankton communities of the NW Adriatic

21   Sea.

22

23   **Keywords**: northern Adriatic Sea; LTER-Long-Term Ecosystem Research; nutrients; coccolithophore decline;

24   *Emiliania huxleyi*, winter bloom

25

1. Introduction

The Adriatic Sea is a continental basin of the Mediterranean Sea, located between the Italian peninsula and the Slovenian–Croatian–Montenegrin–Albanian coasts. Its major axis is oriented in the NW–SE direction, showing marked longitudinal and transversal asymmetries in its hydrological and geomorphological features. The northern Adriatic is characterized by shallow waters (mean depth about 35 m), a weak bathymetric gradient along the main axis and a high riverine input, due mainly to the discharge of Italian rivers. Its lower limit (formerly considered the Ancona-Zadar transect) is now regarded as the 100 m isobath, based on a long term hydrological/climatological analysis, which corresponds to the Giulianova-Sibenik transect (Artegiani et al., 1997a).

The Po River represents the major buoyancy input, with an annual mean discharge rate of 1500–1700 m^3/s, accounting for about one third of the total riverine freshwater input into the Adriatic Sea (Campanelli et al., 2011; Marini et al., 2008; Raicich, 1996). The riverine input affects both the circulation regime (Artegiani et al., 1997b) and the trophic status (Degobbis et al., 2000) of the northwestern Adriatic basin, and markedly increases its productivity with respect to the general oligotrophic condition of the Mediterranean Sea (D'Ortenzio and Ribera d'Alcalà, 2009; Fonda Umani, 1996). The western Adriatic coast is characterized by a frontal system separating the coastal low-salinity and nutrient-rich waters from the oligotrophic offshore waters. The position of the frontal zone is highly dynamic and is affected by the circulation and the vertical structure of the water column. Namely, in stratified conditions the plume spread eastward, while in mixing conditions the diluted waters are conveyed southward in the Western Adriatic Current (WAC) along the western coast (Grilli et al., 2005; Jeffiries and Lee, 2007), which flows cyclonically ~~around~~ in the Adriatic basin.

The northern Adriatic is probably one of the most studied seas in the world because it is considered one of the most productive basin of the Mediterranean Sea (Fonda Umani, 1996), but especially because it experienced in different periods several striking phenomena more or less directly related to planktonic communities, such as harmful algal blooms, sometimes associated with water discoloration (Artegiani et al., 1985; Boni, 1983; Boni et al., 1983; Cucchiari et al., 2008; Facca et al., 2014; Fonda Umani et al., 2004;

3

52 Giacobbe et al., 1998; Honsell et al., 1992; Mozetič et al., 2017; Penna et al., 2006), hypoxia of bottom layer

53 (Bernardi Aubry et al., 1999; Degobbis et al., 1979; Djakovac et al., 2015), intense mucilage formation

54 (Flander-Putrle and Malej, 2008; Giani et al., 2005; Turk et al., 2010), jellyfish outbreaks (Piraino et al.,

55 2014) and, more recently, benthic toxic dinoflagellate blooms (Accoroni et al., 2012; Monti et al., 2007;

56 Pfannkuchen et al., 2012).

57 Plankton communities are a key component in the functioning of marine ecosystems and have been

58 included among the indicators for the definition of Good Environmental Status (GES), as required by the

59 European Marine Strategy Framework Directive (MSFD). Due to their rapid turnover and fast responses to

60 environmental changes, plankton is considered a valid proxy suitable to highlight either environmental

61 changes circumscribed in space and/or time or wider climatic variations.

62 The spatial distribution and the seasonal variability of phytoplankton communities in the northwestern

63 Adriatic Sea are mainly driven by the major river discharge (and therefore by the rainfall regime), in

64 combination with the stratification/mixing condition (Revelante and Gilmartin, 1976). A decrease in both

65 biomass and cell size range along a north–south and coastal–offshore gradients is commonly reported for

66 Adriatic plankton communities (Fonda Umani, 1996). On a temporal basis, the phytoplankton annual cycle

67 in the coastal areas of the Adriatic Sea is characterized by an intense winter bloom due to *Skeletonema*

68 *marinoi*, a small colonial diatom with a pronounced seasonal behavior that in the northwestern basin

69 causes the most significant biomass increase during the year (Bernardi Aubry et al., 2004; Totti et al., 2005).

70 Diatom blooms also occur in spring and autumn with variable intensity, depending on rainfall regimes. In

71 periods between these diatoms blooms, phytoplankton communities are dominated by heterogeneous

72 communities of small phytoflagellates, while dinoflagellates increase only in spring-summer, with

73 abundance peaks 1-2 orders of magnitude lower than those of diatoms. Coccolithophorids are a minor but

74 persistent component of winter communities (Bernardi Aubry et al., 2004; Totti et al., 2005).

75 Intensive monitoring programs started since the years 70s-80s, as well as regular sampling activities in

76 selected stations over the years, let to reconstruct the variability of planktonic communities on an

77 interannual or even on an interdecadal basis in a number of coastal sites of the northern Adriatic Sea

4

78 (Bernardi Aubry et al., 2004, 2006, 2012; Cabrini et al., 2012; Degobbis et al., 2000; Fuks et al., 2012;

79 Pugnetti et al., 2004; Regione Emilia Romagna, 1978-2016; Solic et al., 1997). All these studies highlighted

80 that long-term pattern in phytoplankton composition and seasonal trends reflected major environmental

81 changes and/or climatic trends. In this regard, several studies documented a decrease in the phytoplankton

82 biomass that occurred in the northern Adriatic in the years 2000-2009 related to the decrease of Po River

83 outflow causing a strong oligotrophication (Djakovac et al., 2012; Giani et al., 2012; Maric et al., 2012;

84 Mozetič et al., 2010).

85 The Senigallia-Susak (SS) transect is located in the lower part of the northern Adriatic sub-basin, where the

86 Western Adriatic Current (WAC) become sharper (Russo and Artegiani, 1996), clearly separating nutrient

87 rich coastal waters from oligotrophic offshore waters and affecting the horizontal spatial variability of

88 phytoplankton communities. The SS transect represents a LTER (Long-Term Ecosystem Research) site,

89 where physical parameters, nutrient concentration and phytoplankton abundance and biomass along a

90 trophic gradient have been recorded since 1988. In the western coastal section of the transect, the

91 hydrological features and phytoplankton communities are mainly influenced by the inflow of continental

92 waters from the northern Adriatic (Totti and Artegiani, 2001), which are conveyed by the WAC flowing

93 southward along the Italian coast, which reduce salinity and increase nitrate and silicate concentrations and

94 shows high values of phytoplankton abundance and biomass. Offshore (i.e., 10 nM from the Italian coast),

95 the transect is crossed by the NAdDW (Northern Adriatic Deep Water) formed during winter in the

96 northernmost part and flowing southward as bottom current (Artegiani et al., 1999), and presents the

97 oligotrophic characteristics of the Adriatic open waters, with abundance maximum in summer (Totti and

98 Artegiani, 2001).

99 The aims of this study are to analyze the interannual variability of phytoplankton over a ~30-years period in

100 the western coastal site of the SS transect, in order to unravel patterns potentially related to

101 environmental/climatic drivers.

102

103   **2. Materials and Methods**

5

104   *2.1. Study area and general datasets*

105   The sampling station is located in the southern part of the northern Adriatic basin (SG01, 43° 45.86' N, 13°

106   13.00' E, Fig. 1) at 1.2 nM from the Italian coastline (bottom depth 12 m) and included in the LTER Italian

107   sites.

108   In order to evaluate the variations on decadal scale of the winter bloom and of the mean annual cycles of

109   phytoplankton, two datasets (1988-2002 and 2007-2016) were analyzed and compared. Data from 2002 to

110   2007 (seawater physico-chemical parameters and phytoplankton) were excluded from this study because

111   too incomplete.

112   Seasons were defined on the basis of the classical meteorological subdivision, as follows: winter (January-

113   March), spring (April-June), summer (July-September), autumn (October-December).

114

115   *2.2. Meteorological data*

116   Air temperature was measured at the weather station of Torrette (20 km distance from Senigallia) using a

117   platinum resistance temperature detector (PT100), with measurement range from -30 °C to +60 °C with

118   ±0.2 °C accuracy. Rainfall regime was measured at the weather station of Senigallia, using a ETG/R102

119   software-corrected tipping-bucket rain gauge, compliant to WMO requirements (rainfall intensity

120   measurement range: 0-300 mm/h; rainfall amount resolution: 0.2 mm; overall accuracy: ±3%). Both

121   temperature and rain sensors are part of the equipment of the Marche Region meteorological and

122   hydrological monitoring network. Data are available at http://app.protezionecivile.marche.it/sol.

123   The atmospheric pressure in the coastal Senigallia area was measured using the ECMWF ERA-Interim

124   reanalysis dataset (Dee et al., 2011).

125   Temperature and surface pressure were reported in terms of seasonal anomalies, where the reference

126   mean is the seasonal average over a period of 38 years from 1979 to 2016.

127   Unfortunately, wind data (both intensity and direction) were not available for the entire study period.

128

129   *2.3. Sampling*

130 Data were collected from 1988 to 2016 in the framework of several research projects on board of several

131 oceanographic vessels (S. Lo Bianco, Tecnopesca 2, G. Dallaporta, Tethis, Copernaut Franca, Urania,

132 Alliance, Minerva, Bannock, D'Ancona, Actea). Sampling was carried out with approximately a monthly

133 frequency.

134 Conductivity-Temperature-Depth data were collected by CTD instrument: from 1988 to 1991 with a Neil

135 Brown Instrument System (NBIS) (accuracy: Conductivity ± 0.0006 S/m, Temperature ± 0.002 °C, Pressure ±

136 0.05% of full scale range), and after 1992 with a SeaBird Electronic SBE 911plus (accuracy: Conductivity ±

137 0.0003 S/m, Temperature ± 0.001 °C, Pressure ± 0.015% of full scale range). Data were acquired and

138 processed according to UNESCO (1988) standards, obtaining pressure-averaged data (0.5 db interval).

139 Water samples for determination of dissolved inorganic nutrients (nitrite-NO_2, nitrate-NO_3, ammonia-NH_4, -

140 orthophosphate-PO_4 and orthosilicate-$Si(OH)_4$ and for phytoplankton analysis were collected at surface,

141 bottom and intermediate depths by Niskin bottles according to the thermohaline structure. Then, samples

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

143 analysis, whereas water samples for phytoplankton analysis were collected in 250 ml dark glass bottles and

144 preserved by adding 0.8% formaldehyde, prefiltered and neutralized with hexamethylenetetramine

145 (Throndsen, 1978) and stored at 4 °C until analysis. For this study, only the surface layer (i.e., the 0-4 m

146 layer) was considered.

147

148   *2.4. Nutrient analysis*

149 Nutrient concentrations were measured using a Perkin Elmer spectrophotometer 550A model in the period

150 1988-1998, an autoanalyzer TRAACS 800 BRAN+LUEBBE in 1999-2002 and then an autoanalyzer QUAATRO

151 Technicon, following Strickland and Parsons (1972). For both autoanalyzer models, accuracy was ±0.02

152 µmol l^{-1} for ±NO_2, ±NO_3, ±NH_4 and $Si(OH)_4$ and ±0.03 µmol l^{-1} for PO_4. A calibration curve was made with 5

153 levels of Merck® standards and the accuracy was tested using a standard as sample. The precision was

154 tested on 10 replicates of the standard and were: ±0.006 µmol l^{-1} (NO_2), ±0.005 µmol l^{-1} (NO_3), ±0.001 µmol

7

155 l^{-1} (NH$_4$), ±0.001 µmol l^{-1} (PO$_4$) and ±0.055 µmol l^{-1} (Si(OH)$_4$). Dissolved Inorganic Nitrogen (DIN)

156 concentration is intended as the sum of NO$_2$, NO$_3$ and NH$_4$, concentrations.

157

158 *2.5. Phytoplankton analysis*

159 Identification and counting was carried out using an inverted microscope (ZEISS Axiovert 135) equipped

160 with phase contrast, following the Utermöhl method (Hasle, 1978). Counting was carried out at 400x

161 magnification along transects or in random visual fields, depending on cell abundance, to count a minimum

162 of 200 cells. Moreover, a half of the Utermöhl chamber was analyzed at 200x magnification for a more

163 precise estimation of less abundant microphytoplanktonic taxa.

164 Phytoplankton taxa were finally grouped into major groups (diatoms, dinoflagellates, coccolithophores,

165 phytoflagellates and others), and abundances were expressed as cells l^{-1}. Dinoflagellates were considered

166 as a whole taxonomical group and both autotrophic and heterotrophic species were included in counting.

167 Phytoplankton biomass was estimated through cell biovolume: single cells were measured using a

168 micrometric ocular approximating cell shapes to geometrical figures (Hillebrand et al., 1999). Then, the

169 carbon content for each taxon was derived by the mean biovolume, following (Menden-Deuer and Lessard,

170 2000) and biomass was expressed as µg C l^{-1}.

171

172

173 *2.6. Statistical analysis*

174 Differences in seasonal abundance of temperature, salinity nutrients and abundance and biomass of

175 phytoplankton groups between different study periods were assessed through a one-way analysis of variance

176 (ANOVA). When significant differences for the main effect were observed ($p < 0.05$), a Fisher's pairwise

177 comparison test was also performed. Pearson's correlations were performed on non-transformed

178 environmental data. The statistical analyses were conducted using Statistica 12 (StatSoft Inc., Tulsa, OK, USA)

179 software.

8

180 To identify characterizing phytoplankton species for each month, the Indicator Value (IndVal) was applied,

181 which combines the relative abundance of a species with its relative frequency of occurrence in a given

182 period (Dufrêne and Legendre, 1997). The IndVal analysis was run separately for the 1988-2002 and 2007-

183 2016 periods, to test long-term differences in the structures of phytoplankton communities.

184

185 **3. Results**

186 *3.1. Meteorological conditions*

187 Meteorological parameters are shown in Figg. 2-5 (seasonal trends) and in Figg. S1-S4 (annual trends). On

188 an annual basis, temperature showed a significant increasing trend (Fig. S1). In the ~~2002~~1988-2016,

189 temperature anomalies were mainly positive in all seasons except than in autumn, and the trend of T

190 anomalies along the entire study period showed significant positive tendencies in spring and summer (Fig.

191 2). The year 2014 was noteworthy due to the unusually warm winter and autumn seasons and the

192 unusually cool summer.

193 The annual trend of sea surface pressure anomaly showed a slight but significant decreasing trend ($p <$

194 0.05, Fig. S2). On a seasonal basis, the trend of anomalies across the entire study period showed a

195 significantly decreasing trend during winter (Fig. 3): while in the 1988-2002 period mainly positive

196 anomalies were recorded, in the 2007-2016 negative anomalies were more frequent. The widest

197 fluctuations in the winter and autumn seasons.

198 The seasonal trend of 99th percentile of rainy-days (i.e. days with cumulative precipitation ≥ 1 mm) is shown

199 in Fig. 4. Although significant only in winter, a positive trend was observed in all seasons, and even in the

200 mean annual trend (Fig. S3) indicating an increase in the occurrence of heavy rainfall. Regarding the Dry

201 Spell Length that indicates the length (in days) of dry periods, no significant variations were observed for

202 this parameter, both on a seasonal (Fig. 5) and on an annual basis (Fig. S4).

203

204 *3.2. Physical and chemical parameters*

205 The water temperature anomaly along the entire study period (Fig. 6A) showed a slight but significant

206 increasing trend (n=180, r= 0.19. p<0.05). The average seasonal values for both periods are reported in

207 Table 1: in 2007-2016, only a slight increase was observed in winter (8.6 ± 0.4 and 9.4 ± 0.4 °C for the 1988-

208 2002 and 2007-2016 period respectively), and in summer (24.4 ± 0.3 and 25.1 ± 0.3 °C for the 1988-2002

209 and 2007-2016 period respectively), while in autumn values were higher in the 1988-2002 than in 2007-

210 2016 period (15.1 ± 1.0 and 14.5 ± 1.0 °C, respectively) (Table 1).

211 Salinity anomaly exhibited a significant decreasing trend throughout the study period (Fig. 6B) (n=181, r= -

212 0.15, p < 0.05). However, comparing the average seasonal values of the two study periods, such decrease

213 was evident in winter (35.0 ± 0.3 vs 33.6 ± 0.5, 1988-2002 and 2007-2016 period respectively), spring (34.5

214 ± 0.5 vs 33.4 ± 0.7, 1988-2002 and 2007-2016 period respectively) and autumn (33.1 ± 0.8 vs 32.8 ± 0.8,

215 1988-2002 and 2007-2016 period respectively) although such differences were not significant (Table 1).

216 Nutrient concentration anomaly showed a significant increasing trend along the study period for both DIN

217 (n=123, r= 0.37, p< 0.001) and PO_4 (n=125, r=0.38, p< 0.001), (Fig. 6 C and D), while the trend of $Si(OH)_4$

218 concentrations showed a slight but significant increase throughout the study period (n=124, r=0.18, p<0.05)

219 (Fig. 6E).

220 The comparison of mean seasonal values between the two periods showed significantly higher values of

221 DIN in the 2007-2016 winter and autumn (both p < 0.001) and spring periods (Table 1). In 2007-2016 a wide

222 variability of phosphate concentrations was observed. Comparing mean values of PO_4 concentrations

223 between each season of both periods, a significant increase was recorded in the last decade for spring (p

224 <0.05), summer (p <0.01) and autumn (p <0.01) (Table 1).

225 Comparing orthosilicate seasonal mean values, higher values were observed in the 2007-2016 in all seasons

226 than in the 1988-2002 period, although such difference was not significant (Table 1).

227

228 *3.3. Po River outflow*

229 The Po river outflow measured at Pontelagoscuro is shown in Fig. 7. In winter, the mean outflow was

230 significantly higher in the 2007-2016 period than in the 1988-2002 one (p<0.001).

10

231   In autumn, the outflow was higher in the 1988-2002 than in the 2007-2016 period ($p < 0.001$). In all years of

232   the 1988-2002 period, autumn months were characterized by high outflow. On the contrary, the 2007-2016

233   period was characterized by longer droughts in summer until the end of October, when high autumnal

234   outflow started abruptly. The lowest discharge of the Po River was recorded in the period 2002-2007.

235

236   *3.4. Phytoplankton communities*

237   3.4.1. 1988-2002 period

238   In the 1988-2002 period, the number of identified taxa was 247 (118 diatoms, 83 dinoflagellates, 18

239   coccolithophores, 4 cryptophytes, 7 prasinophytes, 5 euglenophytes, 7 chrysophytes, 1 raphidophyte, 4

240   silicoflagellates, 1 cyanophyte). Total phytoplankton abundance ranged between 241,070 and 36,332,400

241   cells l^{-1} (in September 1997 and January 1998 respectively). Diatoms and phytoflagellates were the main

242   components of the coastal phytoplankton assemblages, accounting from 10 to 74% (average 44%) and from

243   32 to 78% (average 51%) of the total abundance, respectively. Dinoflagellates (2 to 6%) and

244   coccolithophores (0 to 2%) represented minor components.

245   The mean annual cycle of phytoplankton group abundances in the period 1988-2002 period is shown in Fig.

246   8A, C, E, G and I, while the mean abundance percent composition of the phytoplankton community is

247   shown in Fig. 9A. The annual maximum of phytoplankton abundance (on average 11,967,891 ± 3,857,705

248   cells l^{-1}) was observed in early winter (January), because of the typical winter bloom of diatoms accounting

249   for the 74% of the total abundance. Winter communities were dominated by diatoms (*Skeletonema*

250   *marinoi*) showing their maximum abundance in January. Winters were also characterized also by the annual

251   maximum of coccolithophores (mainly *Emiliania huxleyi*). In spring, a lower peak (5,544,446 ± 1,797,029

252   cells l^{-1}) was recorded in April, again due to the diatoms (multispecific bloom) that represented the 63% of

253   the total abundance. In summer, the minimum values of phytoplankton abundances were observed, with

254   the lowest values in August (2,243,704 ± 437,683); the phytoplankton assemblages were dominated by

255   phytoflagellates that accounted up to 78% of the total abundance with 1,748,239 ± 375,019 cells l^{-1}. In

256   autumn, an abundance peak was recorded in October (5,015,654 ± 1,406,303 cells l^{-1}), mainly due to

257   phytoflagellates (60%) followed by diatoms (35%).

258   The community structure in terms of significant taxa as revealed by Ind-Val analysis in 1988-2002 period is

259   shown in Table 2: in winter the highest IndVal values were observed for *Skeletonema marinoi* (77.50,

260   p<0.001), *Pseudo-nitzschia delicatissima* complex (29.95, p<0.05) and *Emiliania huxleyi* (28.10, p<0.01).

261   In spring, the most characterizing taxa were *Cyclotella* spp. (35.20, p<0.001), und. cryptophytes (33.48,

262   p<0.01), *Syracosphaera pulchra* (31.72, p<0.05), *Nitzschia longissima* (21.34, p<0.001) and *Dinophysis*

263   *caudata* (11.46, p<0.05).

264   In summer, the highest values were observed in *Cerataulina pelagica* (35.39, p< 0.01), *Proboscia alata*

265   (34.01, p<0.05), *Guinardia flaccida* (31.51, p<0.01) and *Prorocentrum compressum* (17.54, p<0.01).

266   In autumn the most significant species were *Lioloma pacificum* (36.70, p<0.001 *Asterionellopsis glacialis*

267   (21.63, p<0.05), *Thalassionema frauenfeldii* (14.25, p<0.05) and several *Chaetoceros* species.

268   The mean annual cycle of phytoplankton biomass is shown in Fig. S5 (A,C,E,G,I), while the percent

269   composition of phytoplankton community in terms of biomass is shown in Fig. S6A. In 1988-2002, biomass

270   trend fitted with that of abundance with peaks in January and February (258.06 ± 83.51 and 289.90 ±

271   153.55 µC l^{-1} in respectively). Diatoms accounted for the 92 and 82 % of total biomass in January and

272   February respectively. In June a biomass peak was observed (141.93 ± 82.57 µC l^{-1}), due to a high

273   contribution of dinoflagellates that were represented by large-sized taxa (47%) and diatoms (45%).

274

275   3.4.2. 2007-2016 period

276   In the 2007-2016 period, the number of identified taxa was 262 (125 diatoms, 96 dinoflagellates, 19

277   coccolithophores, 1 cryptophyte, 1 prasinophyte, 2 euglenophytes, 9 chrysophytes, 2 raphidophytes, 5

278   silicoflagellates, 3 cyanophytes). Phytoplankton abundance ranged from 448,191 to 66,015,480 cells l^{-1}, in

279   July 2008 and March 2010 respectively. Again, diatoms and phytoflagellates were the main component of

280   the coastal phytoplankton assemblages, accounting from 10 to 83% (average 54%) and from 16 to 88%

281 (average 45%) of the total abundance, respectively (Fig. 9B). Dinoflagellates (0 to 4%) and coccolithophores

282 (0 to 1%) represented minor components.

283 The mean annual cycle of phytoplankton group abundance in 2007-2016 is shown in Figg. 8B, D, F, H and J,

284 while the mean percent composition of phytoplankton community in terms of abundance is shown in Fig.

285 9B. Two peaks were observed: March (15,515,233 ± 8,764,850 cells l^{-1}) and May (14,913,738 ± 6,452,528

286 cells l^{-1}), both due to diatom blooms that accounted for the 83 and 71% of the total abundance

287 respectively. While the March bloom was almost monospecific (*Skeletonema marinoi*), the May bloom was

288 due to several diatom taxa. In summer and autumn, no peaks were observed, and the abundance values

289 never exceeded the $3.5 * 10^6$ cells l^{-1}. The annual peak of coccolithophores was recorded in late spring.

290 The community structure in terms of significant species as revealed by Ind-Val analysis in 2007-2016 period

291 is shown in Table 3. In winter, the highest IndVal values were observed for *S. marinoi* (92.43, p<0.001),

292 *Thalassiosira* spp. (60.74, p<0.001), *Dytilum brightwellii* (52.10, p<0.001), *Thalassiosira rotula* (46.43,

293 p<0.001) and *Lauderia annulata* (32.49, p<0.01).

294 In spring, the most significant IndVal values were found for *Prorocentrum cordatum* (58.39, p<0.001), *P.*

295 *micans* (57.3, p<0.001), *Dactyliosolen fragilissimus* (56.60, p<0.05), *Noctiluca scintillans* (51.99, p<0.001),

296 *Euglena* sp. (46.53, p<0.01), *Nitzschia longissima* (43.64, p<0.001), *Prorocentrum triestinum* (36.70,

297 p<0.001), *Alexandrium* spp. (34.88, p<0.01), *Protoperidinium steinii* (31.10, p<0.01) and *Dinophysis sacculus*

298 (25.70, p<0.01).

299 In summer, the highest Ind-Val were observed for *Proboscia alata* (87.83, p<0.001), *Rhizosolenia* spp.

300 (43.39, p<0.001), *Pseudo-nitzschia pseudodelicatissima* complex (40.31, p<0.01), *Pleurosigma* sp. (38.75,

301 p<0.01), *Pseudo-nitzschia galaxiae* (34.10, p<0.01), *Prorocentrum compressum* (33.67, p<0.001),

302 *Rhabdolithes claviger* (26.81, p<0.01) and *Guinardia flaccida* (25.38, p<0.05).

303 In autumn, the highest indVal were found for *Lioloma pacificum* (51.84. p<0.001), *Cylindrotheca closterium*

304 (46.87, p<0.01), *Asterionellopsis glacialis* (42.42, p<0.001), *Dictyocha fibula* (37.92, p<0.001), *Eucampia*

305 *cornuta* (36.05, p<0.001), *Chaetoceros rostratus* (29.94, p<0.001), *C. costatus* (27.31, p<0.01), *C. tortissimus*

13

306 (27.25, p<0.01), *C. brevis* (26.26, p<0.01), *C. lauderi* (24.73, p<0.01), *Calciosolenia murrayi* (27.00, p<0.01)

307 and *Pseudo-nitzschia multistriata* (22.05, p<0.01).

308 The mean annual cycle of phytoplankton biomass is shown in Fig. S5 (B,D,F,H,J), while the percent

309 composition of phytoplankton community in terms of biomass is shown in Fig. S6B. In 2007-2016, biomass

310 trend showed peaks in March (600.51 ± 347.81 µg C l^{-1}) due mainly to diatom contribution (81%), May

311 (691.99 ± 227.70 µg C l^{-1}) largely attributed to dinoflagellates (68%), followed by diatoms (28%) and June

312 (824.95 ± 418.89 µg C l^{-1}) due to large-sized dinoflagellates (92%). A weaker peak was observed in October

313 (174.22 ± 90.09 µg C l^{-1}) attributed to the diatoms (87%). Biomass trend showed also peaks in March

314 (600.51 ± 347.81 µg C l^{-1}), May (691.99 ± 227.70 µg C l^{-1}) and June (824.95 ± 418.89 µg C l^{-1}).

315

316 3.4.3 Comparison between the 1988-2002 and 2007-2016 periods

317 Considering the general trend during the whole study period, a significantly decreasing trend of both

318 dinoflagellates (r=-0.23; n=181; p < 0.01) and coccolithophores (r=-0.15; n= 183; p < 0.05) abundances was

319 recorded. When comparing the phytoplankton abundances between the two study periods on a seasonal

320 basis (Table 4), higher values of total phytoplankton and diatom abundance were observed in the second

321 period for all seasons except than in autumn, although such differences did not result significant.

322 Dinoflagellates abundances were lower in the 2007-2016 than in the 1988-2002 period in all seasons except

323 in spring, although these differences were not significant. Coccolithophore abundances were lower in 2007-

324 2016 than in 1988-2002, although such difference was significant only in winter in terms of abundance (p <

325 0.05, Table 4), and in autumn in terms of biomass (p < 0.05, Table 5). Phytoflagellate abundances did not

326 show differences among seasons of the two periods.

327 Comparing the phytoplankton biomass between the two study periods on a seasonal basis (Table 5), higher

328 values were observed in all seasons of the 2007-2016 period, whereas difference was significant only for

329 spring (p < 0.001). Diatom biomass values were higher in all seasons of the 2007-2016, and particularly in

330 spring, although such differences were not significant. During spring, mean seasonal biomass of

331 dinoflagellates showed significantly higher values in the 2007-2016 than in 1988-2002 period (p < 0.001),

14

332 due the blooms of large sized species. Regarding coccolithophores, any significant difference was

333 highlighted between the two periods. In the 2007-2016 period, phytoflagellate biomass showed a

334 significant decrease in winter ($p < 0.01$) and autumn ($p < 0.05$), while an increase was observed in spring (p

335 < 0.05).

336

337   **4. Discussion**

338 In the last decade, a number of long-term studies on phytoplankton communities in the Adriatic Sea have

339 been published, highlighting major interdecadal changes occurred in phytoplankton communities related to

340 climate and meteorological changes (Bernardi Aubry et al., 2012; Cabrini et al., 2012; Maric et al., 2012).

341 Results of this study showed that in the coastal station of the LTER Senigallia-Susak transect, phytoplankton

342 abundance and biomass as well as inorganic nutrient concentrations, significantly increased in the period

343 2007-2016 compared to the period 1988-2002. This increase seems related to the increase of the Po River

344 flow observed since 2008. Our results seem indicate that the tendency to oligotrophication outlined in

345 previous studies, mainly driven by the marked drop of Po River outflow occurred in period 2002-2007

346 (Giani et al., 2012; Mozetič et al., 2010) was reversed.

347 Unexpectedly, the increase in ~~nutrient~~ phosphorous levels ~~(particularly phosphorus),~~ highlighted in our

348 study is not explained by the ~~nutrient~~ PO4 loads measured in the Po River waters (the main nutrient source

349 in the Adriatic Sea) which are decreasing (Giani et al., 2018). We hypothesized that this P enrichment could

350 be explained considering the contributions of minor torrential rivers close to the study site (e.g. Cesano and

351 Misa rivers), coupled with the increase of anomalous and intense meteorological events observed in this

352 area, that may have affected the pathways and fate of N and P, and their ratio (Viaroli et al., 2018; Zoboli et

353 al., 2015). Unfortunately, nutrient loads of these regional rivers are not available for the entire study

354 period, and such hypothesis should be carefully verified. Moreover, also other possible nutrient sources,

355 i.e. the regenerative processes in the water column (Tedesco et al., 2007) and the resuspension of bottom

356 sediments (Boldrin et al., 2009) may have played a role.

357 This study highlighted that in the last decade some major changes occurred in the phytoplankton

358 community structure, in the timing of the seasonal cycle, and in the period of bloom peak occurrences.

359 Although the phytoplankton trend in the northern Adriatic Sea is typically characterized by a marked

360 interannual variability (Bernardi Aubry et al., 2012), some events occur with a remarkably regular

361 frequency. The most regular event in the phytoplankton annual cycle of the Adriatic Sea is represented by

362 the diatom winter bloom of *Skeletonema marinoi* which causes the annual maximum of phytoplankton

363 abundance and biomass every year. While in the period 1988-2002 the *S. marinoi* maximum peak was

364 recorded in early winter (January), in the last decade this bloom extended its temporal span from January

365 to April-May, with its peak in late winter (March), often overlapping to the multispecific diatom spring

366 bloom. A phytoplankton bloom occurring in the earlier months of the year is a common feature of several

367 Mediterranean (Bernardi Aubry et al., 2006; Cabrini et al., 2012; Duarte et al., 1999; Goffart et al., 2002;

368 Morán and Estrada, 2005; Piredda et al., 2017; Totti et al., 2005; Zingone et al., 2010) and extra

369 Mediterranean (Álvarez et al., 2009; Backhaus et al., 2003; Glé et al., 2007) areas. Such bloom has been

370 indicated as winter, late winter, winter-spring, late winter-early spring, irrespectively to the actual month of

371 appearance. Differently from what occurs in other areas where the winter blooms are short-lasting

372 (Zingone et al., 2010), at present the winter bloom in the Adriatic Sea lasts 2-3 months and supports the

373 highest biomass values in the year. In the northern Adriatic Sea, this bloom represents the most remarkable

374 event among the phytoplankton annual cycle and it is expected that the high productivity of the northern

375 Adriatic is supported by the regularity of such bloom, as outlined for other Mediterranean areas (Duarte et

376 al., 1999). The most common explanation of the winter bloom, that appears to contradict the classical

377 Sverdrup's hypothesis, is a transitory limitation of the depth of mixed layer induced by conditions of sunny

378 weather or by the formation of thin freshwater surface layer (Fischer et al., 2014; Townsend et al., 1994).

379 The relevance of both these effects in the coastal areas of the Adriatic Sea is supposed not to be so strong,

380 considering the shallowness of the water column (12 m) and the homogeneity of the vertical distribution of

381 phytoplankton throughout the year (data not shown). Nevertheless, our results suggest that the timing (in

382 terms of onset and duration) of this bloom would be somehow affected by the conditions of high

16

383   atmospheric pressure, affecting both the irradiance and the stability of water column. This hypothesis is

384   supported by the observation that the significantly higher atmospheric pressures recorded in winter during

385   the period 1988-2002 compared to the previous one (1977-1987) allowed an earlier onset of the winter

386   bloom that peaked in January (Totti et al., 2002), as already observed in northernmost areas (Regione

387   Emilia Romagna, n.d.). On the contrary, in the 1977-1987, as well as in the 2007-2016, the atmospheric

388   pressure recorded in winter showed mainly negative anomalies which could have delayed the diatom

389   winter bloom peak.

390   The analysis of indicator species revealed that, although in the second period the winter bloom shifted

391   onward, *Skeletonema marinoi* represented the main indicator species in the winter phytoplankton in both

392   periods. On the other hand, a change in the winter community structure was highlighted: from a *P.*

393   *delicatissima* complex- and *Emiliania huxleyi*- characterized community in the 1988-2002 period to a

394   *Thalassiosira* spp., *Dytilum brightwellii* and *Lauderia annulata* in the 2007-2016 one. Such increase in large

395   diatoms could be explained considering the increase in nutrient availability observed right in this period.

396   The spring bloom (April-May) has a completely different physiognomy, being typically characterized by a

397   diatom multispecific association. Such bloom is typically affected by the rainfall regime and the related river

398   runoff, and showed both an increase and a shift onwards, with the maximum occurring in April in the 1988-

399   2002 period and in May in the 2007-2016, respectively. The spring community of 2007-2016 markedly

400   differed from that of the 1988-2002 for the more relevant contribution of dinoflagellates, in that (i) the

401   presence of *Noctiluca scintillans* was highly significant (see below), and (ii) *Prorocentrum cordatum* and *P.*

402   *triestinum* regularly occurred.

403   In both periods, phytoplankton showed the minimum annual abundances in summer. However, the

404   composition of the community differed between the two periods: in the 1988-2002 period, when in

405   summer the northern Adriatic regularly experienced the persistent and regular appearance of large mucus

406   aggregates (Giani et al., 2005), phytoplankton communities were typically characterized by mucilage-

407   inhabiting large diatom species (e.g., *Cerataulina pelagica*, *Proboscia alata* and *Guinardia flaccida*) (Totti et

408   al., 2005). In 2007-2016, when the mucilage appearance was only sporadic, the indicator diatom taxa in the

409   summer season were more diverse: *Proboscia alata*, *Rhizosolenia* spp*.*, *Pseudo-nitzschia*

410   *pseudodelicatissima* complex, *Pleurosigma* sp. and *Pseudo-nitzschia galaxiae*.

411   In autumn the significantly higher nutrient concentrations observed in the 2007-2016 period did not result

412   in a significant increase in phytoplankton abundances and biomass, but an increase of larger diatoms was

413   observed among the indicator taxa. The variability of phytoplankton blooms in spring and autumn could

414   mainly be related to the intensity of river runoff, in terms of both occurrence and magnitude of peaks. After

415   the input of low salinity- nutrient-rich waters coming from the Po River outflow and carried southward by

416   the WAC, a sudden response of phytoplankton is always observed, with mixed diatom blooms

417   characterized by several species of the genus *Chaetoceros*. It is noteworthy that in the 2007-2016 period,

418   an alien species, i.e. the diatom *Pseudo-nitzschia multistriata* (Corriero et al., 2016), became a regular

419   inhabitant of the phytoplankton communities of the NW Adriatic Sea representing a significant indicator

420   species for the autumn season. The placement of *P. multistriata* inside the native communities has already

421   been reported in other Mediterranean areas (Zenetos et al., 2010).

422   In both study periods, diatoms were the most relevant group in the phytoplankton community, being

423   responsible of the bulk of abundance and biomass values. However, their occurrence become more

424   irregular in the 2007-2016, with unexpected proliferations of heterogeneous groups of species occurring

425   throughout the year. The patterns of variation in atmospheric pressure probably have important effects for

426   the marine climate. Extended periods of high atmospheric pressure are associated with weak horizontal

427   pressure gradients with weak winds and low wave heights (Duarte et al., 1999). The irregularity of intense

428   meteorological events caused the appearance of anomalous blooms and altered the seasonal trends.

429   In both periods, dinoflagellates showed their maximum in spring-summer, as expected in temperate areas

430   (Smayda and Trainer, 2010). However, in the 2007-2016 period a decrease of dinoflagellates in terms of

431   abundances has been observed in this study, in combination with an increase in terms of biomass, because

432   of the occurrence with a regular frequency of *Noctiluca scintillans* red tides, that particularly affected the

433   biomass values. Considering the phagotrophical behavior of *N. scintillans*, its blooms could be indirectly

18

434 associated to the increase of phytoplankton biomass and nutrients observed in the 2007-2016 period, even

435 if in the past such relationship was not proved (Fonda Umani et al., 2004).

436 One of the most surprising result of this study is that coccolithophores showed a significantly decreasing

437 trend in the 2007-2016 period, in terms of abundance and a shift onward of their annual peak which was

438 observed in June, i.e. 2-4 months later than in the 1988-2002 period. Some species that were among the

439 most relevant seasonal indicator species in the 1988-2002 period, such as *Emiliania huxleyi* in winter, and

440 *Syracosphaera pulchra* in spring, lose this role in the 2007-2016 period. The maximum values of *E. huxleyi* in

441 winter were already reported in other Mediterranean (Dimiza et al., 2008) and extra Mediterranean (Chen

442 et al., 2007) areas. On the other hand, in 2007-2016 *Rhabdolithes claviger* was highly relevant in summer,

443 in agreement with reports for other areas where that species is considered indicator of the oligotrophic

444 conditions typical of subtropical waters (Narciso et al., 2016). The coccolithophore decrease observed in

445 the last decade could be tentatively explained considering the highest performance of diatoms in nutrient-

446 rich conditions (Litchman et al., 2006; Xiao et al., 2018). In the North Sea, blooms of *E. huxleyi* typically

447 follow those of diatoms in waters that have been recently depleted of inorganic nutrients and are

448 becoming more stable in terms of vertical mixing (Holligan et al., 1993). In winters of the 2007-2016 period,

449 the anomalous rainfall, combined with freshwater input and nutrient enrichment, altered the diatom cycle,

450 and may have affected the coccolithophore proliferation. Neither temperature nor salinity are likely to be

451 significant causal factors, as *E. huxleyi* is known to be one of the most eurythermal and euryhaline

452 phytoplankton species (Tyrrell and Merico, 2004). Regarding the pH, although an increasing water

453 acidification has been documented in the N Adriatic Sea dense deep waters (Luchetta et al., 2010), such

454 increase is too low and still not significant for lowering the saturation state of carbonates in seawater at

455 levels which could significantly affect the calcification processes, according to the scientific knowledge

456 available. Moreover, unfortunately pH measurements were not available for our dataset. Finally, as this

457 study was carried out using traditional microscopy method, it is not possible to exclude that *E. huxleyi* could

458 have occurred as a non-calcified life stage (Frada et al., 2012), and therefore counted within the

459 'undetermined phytoflagellates' group.

5. Conclusive remarks

In this study, the main changes occurred in phytoplankton community of the northwestern Adriatic basin were documented. In the year 80s, the annual trend was characterized by frequent eutrophication-related phenomena, such as spring-summer dinoflagellate blooms often causing red or even green tides (Artegiani et al., 1985; Boni, 1983; Honsell et al., 1992), and the *Skeletonema marinoi* winter bloom peaking in March (Totti et al., 2002).

In the 1988-2002, dinoflagellate red tides almost disappeared in summer, while the northern Adriatic Sea experienced the appearance of large mucilage aggregates, with diatoms often being the main component of summer communities (Del Negro et al., 2005)05; (Totti et al., 2005) together with some dinoflagellates that were suspected to play a key role in mucilage production (Pistocchi et al., 2005). Both these phenomena have been related to tThe phosphorus depletion that occurred in the years after 1986 (Cozzi and Giani, 2011; Giani et al., 2012), after Italy implemented a new legislation for the control of nutrient loads, . This P depletion induced a severe P limitation in the N Adriatic Sea, which has been invoked as one of the main reasons affecting the mucilage aggregate formation, which regularly took place in the period 1988-2002, as it enhances both the extracellular release by phytoplankton and the bacterial activity (Giani et al., 2005). In the same period, the diatom winter bloom peaked in January, probably favored by long periods (from 10 to 60 days) of high and steady values of barometric pressure (>1020 mbar) preceding the winter blooms (Totti et al., 2002).

The 2002-2007 period was characterized by a marked decrease in the discharge of the Po River (although limited to 5 years only), that determined the decline of phytoplankton biomass which has been already intercepted by a number of interannual analyses (see Mozetič et al., 2010).

For the last decade, new tendencies in phytoplankton communities structure and annual trend have been highlighted. A significant increase in DIN and PO_4 was observed. The P limiting conditions typical of the northern Adriatic Sea (Giani et al., 2012) seem to be attenuated in the study area, and the observed P levels were not explained by the P concentrations measured in the Po River waters, suggesting the occurrence of

Codice campo modificato

486   other P sources, possibly related to the intensity of heavy rainfall. Beside a restoration of diatom

487   abundance and biomass values that merely followed the nutrient enrichment, the timing of the seasonal

488   cycle of the main groups changed. The *Skeletonema marinoi* winter bloom shifted onwards, and other

489   diatom blooms occurred irregularly throughout the year reflecting the irregularity of meteorological events.

490   Dinoflagellate abundances decreased, but the irregular proliferation of large sized species caused biomass

491   peaks. Coccolithophores showed a remarkable decline particularly in winter months. The causes of this

492   decline are not clear and need to be investigated in depth.

493   The main forcing factors that shape the community structure and seasonal trend of phytoplankton in the

494   Senigallia-Susak coastal station crossed by the WAC are both climatic (intensity and trend of rainfall) and

495   related to anthropogenic pressure (nutrient ~~charge~~load). However, other important factors unfortunately

496   not available in this study, such wind speed and direction, affecting turbulence and mixing with potential

497   direct effects on nutrients and the phytoplankton dynamics, may play a key role. This study confirms that

498   the analysis of long-time series of plankton represents a powerful tool to estimate the effect of climate in

499   the pelagic ecosystems. These changes should be followed with a great attention considering the effects

500   that they can have on the trophic chain.

501

522

523   **References**

524   Accoroni, S., Colombo, F., Pichierri, S., Romagnoli, T., Marini, M., Battocchi, C., Penna, A., Totti, C., 2012.

525   Ecology of *Ostreopsis* cf . *ovata* blooms in the northwestern Adriatic Sea. Cryptogam. Algol. 33, 191–

526   198. https://doi.org/10.7872/crya.v33.iss2.2011.191

527   Álvarez, E., Nogueira, E., Acuñ, J.L., Lopez-Álvarez, M., Sostres, J.A., 2009. Short-term dynamics of late-

528   winter phytoplankton blooms in a temperate ecosystem (Central Cantabrian Sea, Southern Bay of

529   Biscay). J. Plankton Res. 31, 601–617. https://doi.org/10.1093/plankt/fbp012

530   Artegiani, A., Azzolini, R., Marzocchi, M., Morbidoni, M., Solazzi, A., Cavolo, F., 1985. Prime osservazioni su

531   un bloom fitoplanctonico lungo la costa marchigiana nell'anno 1984. Nov. Thalass. 7, 137–142.

532   Artegiani, A., Bregant, D., Paschini, E., Pinardi, N., Raicich, F., Russo, A., 1997a. The Adriatic Sea general

533   circulation. Part I: air-sea interactions and water mass structure. J. Phys. Oceanogr. 27, 1492–1514.

534   https://doi.org/10.1175/1520-0485(1997)027<1492:TASGCP>2.0.CO;2

535   Artegiani, A., Bregant, D., Paschini, E., Pinardi, N., Raicich, F., Russo, A., 1997b. The Adriatic Sea general

536   circulation. Part II: Baroclinic circulation structure. J. Phys. Oceanogr. 27, 1515–1532.

537   https://doi.org/10.1175/1520-0485(1997)027<1515:TASGCP>2.0.CO;2

538 Artegiani, A., Pariante, R., Paschini, E., Russo, A., Totti, C., 1999. Seasonal evolution of the physical water

539   properties observed from the Senigallia transect, in: Hopkins, T.S., Artegiani, A., Cauwet, G., Degobbis,

540   D., Malej, A. (Eds.), Ecosystems Research Report, No.32. The Adriatic Sea. (EUR 18834). pp. 59–70.

541 Backhaus, J.O., Hegseth, E.N., Wehde, H., Irigoien, X., Hatten, K., Logemann, K., 2003. Convection and

542   primary production in winter. Mar. Ecol. Prog. Ser. 251, 1–14. https://doi.org/10.3354/meps251001

543 Bernardi Aubry, F., Berton, A., Bertaggia, R., Bianchi, F., Socal, G., 1999. Popolamenti fitoplanctonici in

544   masse d'acqua ipossiche nell'area costiera della regione Veneto. Biol. Mar. Mediterr. 6, 648–651.

545 Bernardi Aubry, F., Berton, A., Bastianini, M., Socal, G., Acri, F., 2004. Phytoplankton succession in a coastal

546   area of the NW Adriatic, over a 10-year sampling period (1990–1999). Cont. Shelf Res. 24, 97–115.

547   https://doi.org/10.1016/j.csr.2003.09.007

548 Bernardi Aubry, F., Acri, F., Bastianini, M., Bianchi, F., Cassin, D., Pugnetti, A., Socal, G., 2006. Seasonal and

549   interannual variations of phytoplankton in the Gulf of Venice (Northern Adriatic Sea). Chem. Ecol. 22,

550   71–91. https://doi.org/10.1080/02757540600687962

551 Bernardi Aubry, F., Cossarini, G., Acri, F., Bastianini, M., Bianchi, F., Camatti, E., De Lazzari, A., Pugnetti, A.,

552   Solidoro, C., Socal, G., De Lazzari, A., Pugnetti, A., Solidoro, C., Socal, G., 2012. Plankton communities

553   in the northern Adriatic Sea : Patterns and changes over the last 30 years. Estuar. Coast. Shelf Sci. 115,

554   125–137. https://doi.org/10.1016/j.ecss.2012.03.011

555 Boldrin, A., Carniel, S., Giani, M., Marini, M., Bernardi Aubry, F., Campanelli, A., Grilli, F., Russo, A., 2009.

556   Effects of bora wind on physical and biogeochemical properties of stratified waters in the northern

557   Adriatic. J. Geophys. Res. Ocean. 114, 1–19. https://doi.org/10.1029/2008JC004837.

558 Boni, L., 1983. Red tides of the coast of Emilia Romagna (north-western Adriatic sea) from 1975 to 1982.

559   Inf. Bot. Ital. 15, 18–24.

560 Boni, L., Pompei, M., Reti, M., 1983. The occurence of Gonyaulax tamarensis Lebour bloom in the Adriatic

561   sea along the coast of Emilia-Romagna. G. Bot. Ital. 117, 115–120.

562 Cabrini, M., Fornasaro, D., Cossarini, G., Lipizer, M., Virgilio, D., 2012. Phytoplankton temporal changes in a

563   coastal northern Adriatic site during the last 25 years. Estuar. Coast. Shelf Sci. 115, 113–124.

564   https://doi.org/10.1016/j.ecss.2012.07.007

565   Campanelli, A., Grilli, F., Paschini, E., Marini, M., 2011. The influence of an exceptional Po River flood on the

566   physical and chemical oceanographic properties of the Adriatic Sea. Dyn. Atmos. Ocean. 52, 284–297.

567   https://doi.org/10.1016/j.dynatmoce.2011.05.004

568   Chen, Y.L., Chen, H.-Y., Chung, C.-W., 2007. Seasonal variability of coccolithophore abundance and

569   assemblage in the northern South China Sea. Deep. Res. Part II Top. Stud. Oceanogr. 54, 1617–1633.

570   https://doi.org/10.1016/j.dsr2.2007.05.005

571   Corriero, G., Pierri, C., Accoroni, S., Alabiso, G., Bavestrello, G., Barbone, E., Bastianini, M., Bazzoni, A.M.,

572   Bernardi Aubry, F., Boero, F., Buia, M.C., Cabrini, M., Camatti, E., Cardone, F., Cataletto, B., Cattaneo

573   Vietti, R., Cecere, E., Cibic, T., Colangelo, P., De Olazabal, A., D'onghia, G., Finotto, S., Fiore, N.,

574   Fornasaro, D., Fraschetti, S., Gambi, M.C., Giangrande, A., Gravili, C., Guglielmo, R., Longo, C., Lorenti,

575   M., Lugli??, A., Maiorano, P., Mazzocchi, M.G., Mercurio, M., Mastrototaro, F., Mistri, M., Monti, M.,

576   Munari, C., Musco, L., Nonnis-Marzano, C., Padedda, B.M., Patti, F.P., Petrocelli, A., Piraino, S.,

577   Portacci, G., Pugnetti, A., Pulina, S., Romagnoli, T., Rosati, I., Sarno, D., Satta, C.T., Sechi, N.,

578   Schiaparelli, S., Scipione, B., Sion, L., Terlizzi, A., Tirelli, V., Totti, C., Tursi, A., Ungaro, N., Zingone, A.,

579   Zupo, V., Basset, A., 2016. Ecosystem vulnerability to alien and invasive species: A case study on

580   marine habitats along the Italian coast. Aquat. Conserv. Mar. Freshw. Ecosyst. 26, 392–409.

581   https://doi.org/10.1002/aqc.2550

582   Cozzi, S., Giani, M., 2011. River water and nutrient discharges in the Northern Adriatic Sea: Current

583   importance and long term changes. Cont. Shelf Res. 31, 1881–1893.

584   https://doi.org/10.1016/j.csr.2011.08.010

585   Cucchiari, E.M., Guerrini, F., Penna, A., Totti, C., Pistocchi, R., 2008. Effect of salinity, temperature, organic

586   and inorganic nutrients on growth of cultured Fibrocapsa japonica (Raphidophyceae) from the

587   northern Adriatic Sea. Harmful Algae 7, 405–414. https://doi.org/10.1016/j.hal.2007.09.002

588   D'Ortenzio, F., Ribera d'Alcalà, M., 2009. On the trophic regimes of the Mediterranean Sea: a satellite

589   analysis. Biogeosciences 5, 139–148. https://doi.org/10.5194/bgd-5-2959-2008

590   Dee, D.P., Uppala, S.M., Simmons, A.J., Berrisford, P., Poli, P., Kobayashi, S., Andrae, U., Balmaseda, M.A.,

591   Balsamo, G., Bauer, P., Bechtold, P., Beljaars, A.C., van de Berg, L., Bidlot, J., Bormann, N., Delsol, C.,

592   Dragani, R., Fuentes, M., Geer, A.J., Haimberger, L., Healy, S.B., Hersbach, H., Hólm, E. V., Isaksen, L.,

593   Kållberg, P., Köhler, M., Matricardi, M., McNally, A.P., Monge-Sanz, B.M., Morcrette, J., Park, B.,

594   Peubey, C., de Rosnay, P., Tavolato, C., Thépaut, J., Vitart, F., 2011. The ERA-Interim reanalysis:

595   configuration and performance of the data assimilation system. Q. J. R. Meteorol. Soc. 137, 553–597.

596   https://doi.org/10.1002/qj.828

597   Degobbis, D., Precali, R., Ivancic, I., Smodlaka, N., Fuks, D., Kveder, S., 2000. Long-term changes in the

598   northern Adriatic ecosystem related to anthropogenic eutrophication. Int. J. Environ. Pollut. 13, 495–

599   533. https://doi.org/10.1504/IJEP.2000.002332

600   Degobbis, D., Smodlaka, N., Pojed, I., Skrivanic, A., Precali, R., 1979. Increased eutrophication of the

601   northern Adriatic sea. Mar. Pollut. Bull. 10, 298–301. https://doi.org/10.1016/0025-326X(89)90066-0

602   Del Negro, P., Crevatin, E., Larato, C., Ferrari, C., Totti, C., Pompei, M., Giani, M., Berto, D., Fonda Umani, S.,

603   2005. Mucilage microcosms. Sci. Total Environ. 353, 258–69.

604   https://doi.org/10.1016/j.scitotenv.2005.09.018

605   Dimiza, M.D., Triantaphyllou, M. V., Dermitzakis, M.D., 2008. Seasonality and ecology of living

606   coccolithophores in Eastern Mediterranean coastal environments (Andros Island, Middle Aegean Sea).

607   Micropaleontology 54, 159–175.

608   Djakovac, T., Degobbis, D., Supic, N., Precali, R., 2012. Marked reduction of eutrophication pressure in the

609   northeastern Adriatic in the period 2000-2009. Estuar. Coast. Shelf Sci. 115, 25–32.

610   https://doi.org/10.1016/j.ecss.2012.03.029

611   Djakovac, T., Supić, N., Bernardi Aubry, F., Degobbis, D., Giani, M., 2015. Mechanisms of hypoxia frequency

612   changes in the northern Adriatic Sea during the period 1972-2012. J. Mar. Syst. 141, 179–189.

613   https://doi.org/10.1016/j.jmarsys.2014.08.001

614   Duarte, C.M., Agustí, S., Kennedy, H., Vaqué, D., 1999. The Mediterranean climate as a template for

615   Mediterranean marine ecosystems: the example of the northeast Spanish littoral [WWW Document].

616   Prog. Oceanogr.

617 Dufrêne, M., Legendre, P., 1997. Species assemblages and indicator species: The need for a flexible

618   asymmetrical approach. Ecol. Monogr. 67, 345–366. https://doi.org/10.2307/2963459

619 Facca, C., Bilaničovà, D., Pojana, G., Sfriso, A., Marcomini, A., 2014. Harmful algae records in Venice lagoon

620   and in Po River Delta (northern Adriatic Sea, Italy). Sci. World J. 806032.

621   https://doi.org/10.1155/2014/806032

622 Fischer, A.D., Moberg, E.A., Alexander, H., Brownlee, E.F., Hunter-Cevera, K., Pitz, K.J., Rosengard, S.Z.,

623   Sosik, H.M., 2014. Sixty years of Sverdrup. Oceanography 27, 222–235.

624 Flander-Putrle, V., Malej, A., 2008. The evolution and phytoplankton composition of mucilaginous

625   aggregates in the northern Adriatic Sea. Harmful Algae 7, 752–761.

626   https://doi.org/10.1016/j.hal.2008.02.009

627 Fonda Umani, S., 1996. Pelagic production and biomass in the Adriatic Sea. Sci. Mar. 60, 65–77.

628 Fonda Umani, S., Beran, A., Parlato, S., Virgilio, D., Zollet, T., Olazabal, A.D.E., Lazzarini, B., Cabrini, M., De

629   Olazabal, A., Lazzarini, B., Cabrini, M., 2004. *Noctiluca scintillans* McCartney in the Northern Adriatic

630   Sea: Long-term dynamics, relationships with temperature and eutrophication, and role in the food

631   web. J. Plankton Res. 26, 545–561. https://doi.org/10.1093/plankt/fbh045

632 Frada, M.J., Bidle, K.D., Probert, I., de Vargas, C., 2012. In situ survey of life cycle phases of the

633   coccolithophore Emiliania huxleyi (Haptophyta). Environ. Microbiol. 14, 1558–1569.

634   https://doi.org/10.1111/j.1462-2920.2012.02745.x

635 Fuks, D., Ivan, I., Najdek, M., Lu, D., Njire, J., Godrijan, J., Mari, D., Tina, Š., Paliaga, P., Bla, M., Precali, R.,

636   Orli, S., 2012. Changes in the planktonic community structure related to trophic conditions : The case

637   study of the northern Adriatic Sea. J. Mar. Syst. 96–97, 95–102.

638   https://doi.org/10.1016/j.jmarsys.2012.02.006

639 Giacobbe, M.G., Bianchi, F., Maimone, G., Puglisi, A., Socal, G., 1998. Diel observations on populations of

640   *Dinophysis* and *Alexandrium* spp. (Dinophyceae) from the Adriatic Sea. Acta Bot. Croat. 57, 19–28.

641 Giani, M., Rinaldi, A., Degobbis, D., Vollenweider, R., 2005. Mucilages in the Adriatic and Tyrrhenian Seas:

642   An introduction. Sci. Total Environ. 353, 3–9. https://doi.org/10.1016/j.scitotenv.2005.09.007

643 Giani, M., Djakovac, T., Degobbis, D., Cozzi, S., Solidoro, C., Fonda Umani, S., 2012. Recent changes in the

644   marine ecosystems of the northern Adriatic Sea. Estuar. Coast. Shelf Sci. 115, 1–13.

645   https://doi.org/10.1016/j.ecss.2012.08.023

646 Giani, M., Cozzi, S., Tartari, G., 2018. Trends of nutrient loads discharged from the Po River into the

647   northern Adriatic, in: 49° Congresso Della Società Italiana Di Biologia Marina. Cesenatico, 4-8 giugno

648   2018.

649 Glé, C., Del Amo, Y., Bec, B., Sautour, B., Froidefond, J.M., Gohin, F., Maurer, D., Plus, M., Laborde, P.,

650   Chardy, P., 2007. Typology of environmental conditions at the onset of winter phytoplankton blooms

651   in a shallow macrotidal coastal ecosystem, Arcachon Bay (France). J. Plankton Res. 29, 999–1014.

652   https://doi.org/10.1093/plankt/fbm074

653 Goffart, A., Hecq, J.-H., Legendre, L., 2002. Changes in the development of the winter-spring phytoplankton

654   bloom in the Bay of Calvi (NW Mediterranean) over the last two decades : a response to changing

655   climate ? Mar. Ecol. Prog. Ser. 236, 45–60.

656 Grilli, F., Paschini, E., Precali, R., Russo, A., Supić, N., 2005. Circulation and horizontal fluxes in the northern

657   Adriatic Sea in the period June 1999-July 2002. Part I: Geostrophic circulation and current

658   measurement. Sci. Total Environ. 353, 57–67. https://doi.org/10.1016/j.scitotenv.2005.09.008

659 Hasle, G.R., 1978. The inverted microscope method, in: Sournia, A. (Ed.), Phytoplankton Manual.

660   Monographs on Oceanographic Methodology. UNESCO, Paris, pp. 88–96.

661 Hillebrand, H., Durselen, C.-D., Kirschtel, D., Pollingher, U., Zohary, T., 1999. Biovolume calculation for

662   pelagic amd benthic microalgae. J. Phycol. 35, 403–424.

663 Holligan, P.M., Groom, S.B., Harbour, D.S., 1993. What controls the distribution of the coccolithophore,

664   Emiliania huxleyi, in the North Sea? Fish. Oceanogr. 2, 175–183.

665 Honsell, G., Boni, L., Cabrini, M., Pompei, M., 1992. Toxic or potentially toxic dinoflagellates from the

666   northern Adriatic Sea. Sci. Total Environ. 107–114.

667 Jeffiries, M.A., Lee, C.M., 2007. A climatology of the northern Adriatic Sea's response to bora and river

27

668   forcing. J. Geophys. Res. Ocean. 112, 1–18. https://doi.org/10.1029/2006JC003664

669   Litchman, E., Klausmeier, C.A., Miller, J.R., Schofield, O.M., Falkowski, P.G., 2006. Multi-nutrient, multi-

670   group model of present and future oceanic phytoplankton communities. Biogeosciences Discuss. 3,

671   607–663. https://doi.org/10.5194/bgd-3-607-2006

672   Luchetta, A., Cantoni, C., Catalano, G., 2010. New observations of CO_2-induced acidification in the northern

673   Adriatic Sea over the last quarter century. Chem. Ecol. 26, 1–17.

674   https://doi.org/10.1080/02757541003627688

675   Maric, D., Kraus, R., Godrijan, J., Supi, N., Djakovac, T., Precali, R., 2012. Phytoplankton response to climatic

676   and anthropogenic influences in the north-eastern Adriatic during the last four decades. Estuar. Coast.

677   Shelf Sci. 115, 98–112. https://doi.org/10.1016/j.ecss.2012.02.003

678   Marini, M., Jones, B.H., Campanelli, A., Grilli, F., Lee, C.M., 2008. Seasonal variability and Po River plume

679   influence on biochemical properties along western Adriatic coast. J. Geophys. Res. Ocean. 113, 1–18.

680   https://doi.org/10.1029/2007JC004370

681   Menden-Deuer, S., Lessard, E.J., 2000. Carbon to volume relationships for dinoflagellates, diatoms, and

682   other protist plankton. Limnol. Oceanogr. 45, 569–579. https://doi.org/10.4319/lo.2000.45.3.0569

683   Monti, M., Minocci, M., Beran, A., Ivesa, L., 2007. First record of *Ostreopsis* cfr. *ovata* on macroalgae in the

684   Northern Adriatic Sea. Mar. Pollut. Bull. 54, 598–601.

685   https://doi.org/10.1016/j.marpolbul.2007.01.013

686   Morán, X.A.G., Estrada, M., 2005. Winter pelagic photosynthesis in the NW Mediterranean. Deep Sea Res.

687   Part I Oceanogr. Res. Pap. 52, 1806–1822. https://doi.org/10.1016/j.dsr.2005.05.009

688   Mozetič, P., Solidoro, C., Cossarini, G., Socal, G., Fonda Umani, S., 2010. Recent trends towards

689   oligotrophication of the northern Adriatic: evidence from chlorophyll a time series. Estuaries and

690   Coasts 33, 362–375. https://doi.org/10.1007/s12237-009-9191-7

691   Mozetič, P., Cangini, M., Francé, J., Bastianini, M., Bernardi Aubry, F., Bužančić, M., Cabrini, M., Cerino, F.,

692   Čalić, M., D'Adamo, R., Drakulović, D., Finotto, S., Fornasaro, D., Grilli, F., Kraus, R., Marić

693   Pfannkuchen, D., Ninčević Gladan, Ž., Pompei, M., Rotter, A., Servadei, I., Skejić, S., 2017.

694   Phytoplankton diversity in Adriatic ports : Lessons from the port baseline survey for the management

695   of harmful algal species. Mar. Pollut. Bull. https://doi.org/10.1016/j.marpolbul.2017.12.029

696   Narciso, Á., Gallo, F., Valente, A., Cachão, M., Cros, L., Azevedo, E.B., Barcelos e Ramos, J., 2016. Seasonal

697   and interannual variations in coccolithophore abundance off Terceira Island, Azores (Central North

698   Atlantic). Cont. Shelf Res. 117, 43–56. https://doi.org/10.1016/j.csr.2016.01.019

699   Penna, A., Ingarao, C., Ercolessi, M., Rocchi, M., Penna, N., 2006. Potentially harmful microalgal distribution

700   in an area of the NW Adriatic coastline: Sampling procedure and correlations with environmental

701   factors. Estuar. Coast. Shelf Sci. 70, 307–316. https://doi.org/10.1016/j.ecss.2006.06.023

702   Pfannkuchen, M., Godrijan, J., Marić Pfannkuchen, D., Iveša, L., Kružić, P., Ciminiello, P., Dell'Aversano, C.,

703   Dello Iacovo, E., Fattorusso, E., Forino, M., Tartaglione, L., Godrijan, M., 2012. Toxin-producing

704   Ostreopsis cf. ovata are likely to bloom undetected along coastal areas. Environ. Sci. Technol. 46,

705   5574–5582. https://doi.org/10.1021/es300189h

706   Piraino, S., Aglieri, G., Martell, L., Mazzoldi, C., Melli, V., Milisenda, G., Scorrano, S., Boero, F., 2014. Pelagia

707   benovici sp. nov. (Cnidaria, Scyphozoa): a new jellyfish in the Mediterranean Sea. Zootaxa 3794, 155–

708   468. https://doi.org/10.11646/zootaxa.3794.3.7

709   Piredda, R., Tomasino, M.P., D'Erchia, A.M., Manzari, C., Pesole, G., Montresor, M., Kooistra, W.H.C.F.,

710   Sarno, D., Zingone, A., 2017. Diversity and temporal patterns of planktonic protist assemblages at a

711   Mediterranean LTER site. FEMS Microbiol. Ecol. 93, fiw200. https://doi.org/10.1093/femsec/fiw200

712   Pistocchi, R., Cangini, M., Totti, C., Urbani, R., Guerrini, F., Romagnoli, T., Sist, P., Palamidesi, S., Boni, L.,

713   Pompei, M., 2005. Relevance of the dinoflagellate Gonyaulax fragilis in mucilage formations of the

714   Adriatic Sea. Sci. Total Environ. 353, 307–16. https://doi.org/10.1016/j.scitotenv.2005.09.087

715   Pugnetti, A., Acri, F., Alberighi, L., Barletta, D., Bastianini, M., Bernardi Aubry, F., Berton, A., Bianchi, F.,

716   Socal, G., Totti, C., 2004. Phytoplankton photosynthetic activity and growth rates in the NW Adriatic

717   Sea. Chem. Ecol. 20, 399–409. https://doi.org/10.1080/02757540412331294902

718   Raicich, F., 1996. On the fresh water balance of the Adriatic Sea. J. Mar. Syst. 9, 305–319.

719   https://doi.org/10.1016/S0924-7963(96)00042-5

29

720   Regione Emilia Romagna, 1978-2016. Qualità ambientale delle acque marine in Emilia-Romagna. Rapporti

721   annuali.

722   Revelante, N., Gilmartin, M., 1976. The effects of Po river discharge on phytoplankton dynamics in the

723   northern Adriatic Sea. Mar. Biol. 34, 259–271.

724   Russo, A., Artegiani, A., 1996. Adriatic Sea hydrography. Sci. Mar. 60, 33–43.

725   Smayda, T.J., Trainer, V.L., 2010. Dinoflagellate blooms in upwelling systems: Seeding, variability, and

726   contrasts with diatom bloom behaviour. Prog. Oceanogr. 85, 92–107.

727   https://doi.org/10.1016/j.pocean.2010.02.006

728   Solic, M., Krstulovic, N., Marasovic, I., Baranovic, A., Pucher-Petkovic, T., Vucetic, T., 1997. Analysis of time

729   series of planktonic communities in the Adriatic Sea: distinguish between natural and man-induced

730   changes. Oceanol. Acta 20, 131–143.

731   Tedesco, L., Socal, G., Bianchi, F., Acri, F., Veneri, D., Vichi, M., 2007. NW Adriatic Sea biogeochemical

732   variability in the last 20 years (1986-2005). Biogeosciences 4, 673–687.

733   Throndsen, J., 1978. Preservation and storage, in: Sournia, A. (Ed.), Phytoplankton Manual. Monographs on

734   Oceanographic Methodology. UNESCO, Paris, pp. 69–74.

735   Totti, C., Artegiani, A., 2001. Phytoplankton time series in the Northern Adriatic Sea: the Senigallia transect

736   (1988-1994). Arch. Oceanogr. Limnol. 22, 107–111.

737   Totti, C., Marzocchi, M., Cucchiari, E.M., Romagnoli, T., Barletta, D., Artegiani, A., Solazzi, A., 2002. The

738   winter diatom bloom of the northern Adriatic Sea: long-term recurrence (1979-2001) and possible

739   relationships with climate, in: 2nd National Conference SINAPSI, Climate Variability and Effects on the

740   Mediterranean Marine Ecosystems. Portonovo (Ancona) 13–15 May 2002.

741   Totti, C., Cangini, M., Ferrari, C., Kraus, R., Pompei, M., Pugnetti, A., Romagnoli, T., Vanucci, S., Socal, G.,

742   2005. Phytoplankton size-distribution and community structure in relation to mucilage occurrence in

743   the northern Adriatic Sea. Sci. Total Environ. 353, 204–217.

744   https://doi.org/10.1016/j.scitotenv.2005.09.028

745   Townsend, D.W., Cammen, L.M., Holligan, P.M., Campbell, D.A., 1994. Causes and consequences of

746   variability in the timing of spring phytoplankton blooms. Deep. Res. I 41, 747–765.

747   Turk, V., Hagström, Å., Kovač, N., Faganeli, J., 2010. Composition and function of mucilage macroaggregates

748   in the northern Adriatic. Aquat. Microb. Ecol. 61, 279–289. https://doi.org/10.3354/ame01447

749   Tyrrell, T., Merico, A., 2004. Emiliania huxleyi: bloom observation and the conditions that induce them, in:

750   Thierstein, H.T., Young, J.R. (Eds.), Coccolithophores. From Molecular Processes to Global Impact.

751   Springer, pp. 75–97.

752   Viaroli, P., Soana, E., Pecora, S., Laini, A., Naldi, M., Fano, E.A., Nizzoli, D., 2018. Space and time variations of

753   watershed N and P budgets and their relationships with reactive N and P loadings in a heavily

754   impacted river basin (Po river, Northern Italy). Sci. Total Environ. 639, 1574–1587.

755   https://doi.org/10.1016/j.scitotenv.2018.05.233

756   Xiao, W., Liu, X., Irwin, A.J., Laws, E.A., Wang, L., Chen, B., Zeng, Y., Huang, B., 2018. Warming and

757   eutrophication combine to restructure diatoms and dinoflagellates. Water Res. 128, 206–216.

758   https://doi.org/10.1016/j.watres.2017.10.051

759   Zenetos, A., Gofas, S., Verlaque, M., Inar, M.E., Garci, J.E., Bianchi, C.N., Morri, C., Azzurro, E., Bilecenoglu,

760   M., Froglia, C., Siokou, I., Violanti, D., Sfriso, A., Giangrande, A., Mastrototaro, F., An, T.K., Ballesteros,

761   E., Zingone, A., Gambi, M.C., Streftaris, N., 2010. Alien species in the Mediterranean Sea by 2010. A

762   contribution to the application of European Union's Marine Strategy Framework Directive (MSFD).

763   Part I . Spatial distribution. Mediterr. Mar. Sci. 12, 381–493. https://doi.org/10.12681/mms.87

764   Zingone, A., Dubroca, L., Iudicone, D., Margiotta, F., Corato, F., Ribera d'Alcalà, M., Saggiomo, V., Sarno, D.,

765   2010. Coastal phytoplankton do not rest in winter. Estuaries and Coasts 33, 342–361.

766   https://doi.org/10.1007/s12237-009-9157-9

767   Zoboli, O., Viglione, A., Rechberger, H., Zessner, M., 2015. Impact of reduced anthropogenic emissions and

768   century flood on the phosphorus stock , concentrations and loads in the Upper Danube. Sci. Total

769   Environ. 518–519, 117–129. https://doi.org/10.1016/j.scitotenv.2015.02.087

770

Legends of figures

Figure 1

The LTER Senigallia transect in the northern Adriatic Sea. The study station is highlighted by the red circle.

Figure 2

Seasonal anomalies of air temperature [°C] at the meteorological station of Torrette (Ancona) from 1988 to 2016. The red segment represents the linear regression, calculated by means of the ordinary least square method. The slope represents the annual rate [°C/year].

Figure 3

Seasonal anomalies of atmospheric pressure (~~mbar~~hPa) from 1988 to 2016 calculated by ECMWF ERA-Interim model. The red segment represents the linear regression, calculated by means of the ordinary least square method. The slope represents the annual rate (~~[mbar~~hPa/year~~]~~).

Figure 4

Seasonal 99th percentile of rainy-day precipitation [mm] from 1988 to 2016 at Senigallia meteorological station. The red segment represents the linear regression, calculated by means of the ordinary least square method. The slope represents the annual rate [mm/year].

Figure 5

Seasonal mean of Dry Spell Length from 1988 to 2016 at Senigallia meteorological station. Vertical bars represent the range between the seasonal minimum and maximum values. The red segment represents the linear regression of maximum values, calculated by means of the ordinary least square method. The slope represents the annual rate [days/year].

796   Figure 6

797   Trends of anomalies of physical and chemical parameters from 1988 to 2016 of (A) surface seawater

798   temperature (°C), (B) salinity, (C) dissolved inorganic nitrogen (DIN) concentration (µmol l^{-1}), (D) phosphate

799   concentration, (µmol l^{-1}), (E) silicate concentration (µmol l^{-1}).

800

801   Figure 7

802   Po River discharge [m^{3}s^{-1}] daily (grey line) and annual averages (triangles). The dashed red line represents

803   the average over the whole period, equal to 1502 m^{3}s^{-1}. Source: Annali Idrologici, Servizio Idro-Meteo-

804   Clima, ARPA Emilia-Romagna, 1979-2016.

805

806   Figure 8

807   Mean annual cycle of phytoplankton abundance (cells l^{-1}) in 1988-2002 (A,C,E,G,I) and 2007-2016

808   (B,D,F,H,Jb,d,f,h,j) periods: (A,Ba,b) total phytoplankton, (C,Dc,d) diatoms, (E,Fe,f) dinoflagellates, (G,Hg,h)

809   coccolithophores, (I,Ji,j) phytoflagellates. Box plot report the data distribution with the mean ($+$) median

810   ($\square$)), the interquartile range (black box), the non-outlier range (vertical bars), the outliers ($\bigcirc$) and the

811   extremes ($\ast$).

812

813   Figure 9

814   Mean percent composition of phytoplankton community in terms of abundance during the study period on

815   a monthly basis: (aA) 1988-2002, (bB) 2007-2016.

816

817 Table 1. Results of ANOVA and Fisher's tests about the physical and chemical parameters (surface seawater

818 temperature (°C), salinity, dissolved inorganic nitrogen (DIN)(µmol l^{-1}), phosphate (µmol l^{-1}), silicate (µmol l^{-}

819 ^{1}) in 1988-2002 and 2007-2016. Mean values ± standard error (SE). ns, not significant; * p<0.05; ** p<0.01;

820 *** p<0.001.

821

Parameter	Season	1988-2002 Avg ± SE	2007-2017 Avg ± SE	p-level	Fisher test
Temperature (°C)	Winter	8.55 ± 0.43	9.35 ± 0.42	ns	
	Spring	18.23 ± 0.86	18.32 ± 0.89	ns	
	Summer	24.44 ± 0.28	25.12 ± 0.33	ns	
	Autumn	15.09 ± 0.95	14.52 ± 0.98	ns	
Salinity	Winter	35.0 ± 0.3	33.6 ± 0.5	ns	
	Spring	34.5 ± 0.5	33.4 ± 0.7	ns	
	Summer	35.1 ± 0.4	35.0 ± 0.4	ns	
	Autumn	33.1 ± 0.8	32.8 ± 0.8	ns	
DIN (µmol l^{-1})	Winter	9.71 ± 1.19	23.98 ± 5.04	***	88-02 < 07-16
	Spring	6.42 ± 1.89	10.96 ± 2.65	ns	
	Summer	1.60 ± 0.27	1.98 ± 0.54	ns	
	Autumn	10.05 ± 2.24	28.65 ± 5.63	***	88-02 < 07-16
PO$_4$ (µmol l^{-1})	Winter	0.11 ± 0.03	0.12 ± 0.03	ns	
	Spring	0.04 ± 0.01	0.20 ± 0.13	*	88-02 < 07-16
	Summer	0.08 ± 0.01	0.26 ± 0.10	**	88-02 < 07-16
	Autumn	0.11 ± 0.03	0.26 ± 0.07	**	88-02 < 07-16
Si(OH)$_4$ (µmol l^{-1})	Winter	3.83 ± 0.90	6.36 ± 1.96	ns	
	Spring	2.75 ± 0.54	3.55 ± 1.35	ns	
	Summer	2.28 ± 0.44	3.57 ± 0.98	ns	
	Autumn	6.61 ± 1.40	9.15 ± 2.90	ns	

822

823

824

825   Table 2. List of phytoplankton taxa characterized by the highest IndVal for each season in the 1988-2002

826   period. IndVal values indicated in italic are significant at p <0.05, those in bold italic are significant at p

827   <0.01, those in bold italic and underlined are significant at p <0.001.

Taxa	INDval Winter	INDval Spring	INDval Summer	INDval Autumn
Skeletonema marinoi	***77.50***	3.40	0.00	0.35
Pseudo-nitzschia delicatissima complex	*29.95*	13.81	1.81	1.60
Emiliania huxleyi	***28.10***	13.94	1.95	10.61
Protoperidinium spp.	15.81	6.54	4.14	0.72
Chaetoceros curvisetus	13.73	2.25	0.00	4.09
Und. prasinophytes	13.07	6.79	0.88	13.04
Cyclotella spp.	1.65	***35.20***	4.34	1.95
Und. cryptophytes	9.61	***33.48***	10.67	14.84
Syracosphaera pulchra	0.28	*31.72*	3.15	3.53
Dactyliosolen fragilissimus	1.73	27.82	5.26	11.34
Prorocentrum micans	0.36	25.49	6.39	15.46
Chaetoceros tenuissimus	1.16	*22.51*	1.53	0.07
Tripos fusus	1.59	22.40	1.04	7.47
Nitzschia longissima	0.26	***21.34***	0.04	0.74
Cylindrotheca closterium	4.39	19.79	12.32	22.71
Tripos furca	0.01	16.53	6.69	7.64
Pseudokephyrion spp.	0.81	14.96	2.30	0.64
Thalassiosira spp.	10.03	12.46	1.59	5.75
Dinophysis caudata	0.00	*11.46*	2.38	1.41
Gymnodinium spp.	0.56	10.71	8.06	8.00
Gyrodinium spp.	2.53	10.47	0.01	0.55
Cerataulina pelagica	6.74	14.82	***35.39***	3.53
Proboscia alata	0.53	3.07	***34.01***	9.46
Guinardia flaccida	1.46	0.29	***31.51***	2.66
Pseudo-nitzschia pseudodelicatissima complex	2.53	13.78	20.90	15.61
Hemiaulus hauckii	0.12	0.30	19.95	0.62
Thalassionema nitzschioides	1.36	1.48	19.56	20.71
Anoplosolenia brasiliensis	0.09	6.36	*17.70*	1.68
Prorocentrum compressum	0.00	1.42	***17.54***	0.02
Pseudoscourfieldia marina	0.00	12.45	*17.36*	0.64
Calciosolenia murrayi	0.00	0.00	*17.32*	2.53
Guinardia striata	0.00	0.00	15.77	4.69
Calycomonas spp.	0.74	5.28	12.07	0.94
Lioloma pacificum	0.01	0.00	2.06	***36.70***
Chaetoceros spp.	1.37	7.49	1.90	29.36
Asterionellopsis glacialis	8.07	1.16	0.01	*21.63*
Leptocylindrus danicus	0.16	1.58	12.65	19.76
Chaetoceros affinis	0.00	0.29	0.87	*17.33*
Chaetoceros socialis	6.26	0.18	0.00	14.73
Thalassionema frauenfeldii	1.10	3.93	0.03	*14.25*
Pleurosigma spp.	2.00	2.32	12.01	13.26
Und. pennate diatoms	8.34	3.84	7.30	11.84
Chaetoceros lorenzianus	0.06	0.83	10.59	11.45

828

829

830   Table 3. List of phytoplankton taxa characterized by the highest IndVal for each season in the 2007-2016

831   period. IndVal values indicated in italic are significant at p <0.05, those in bold italic are significant at p

832   <0.01, those in bold italic and underlined are significant at p <0.001.

Taxa	INDval Winter	INDval Spring	INDval Summer	INDval Autumn
Skeletonema marinoi	***92.43***	0.72	0.01	1.03
Thalassiosira spp.	***60.74***	0.17	0.00	7.04
Ditylum brightwellii	***52.10***	0.00	0.00	1.44
Thalassiosira rotula	***46.43***	0.08	0.00	16.55
Lauderia annulata	***32.49***	0.00	0.00	18.04
Chaetoceros danicus	29.27	0.20	1.83	19.24
Chaetoceros curvisetus	23.34	11.20	9.72	7.78
Dactyliosolen phuketensis	***23.00***	0.11	0.00	1.20
Prorocentrum cordatum	2.17	***58.39***	0.60	0.17
Prorocentrum micans	3.37	***57.30***	3.83	15.63
Dactyliosolen fragilissimus	2.51	***56.60***	8.46	15.54
Noctiluca scintillans	0.48	***51.99***	0.00	0.34
Thalassionema nitzschioides	0.97	47.37	26.75	6.21
Euglena spp.	5.65	***46.53***	2.24	15.48
Chaetoceros spp.	16.31	44.71	6.05	8.00
Nitzschia longissima	1.17	***43.64***	5.57	0.05
Cyclotella spp.	1.07	38.28	14.42	14.46
Prorocentrum triestinum	0.00	***36.70***	2.04	0.03
Alexandrium spp.	1.36	***34.88***	9.16	6.53
Chaetoceros tenuissimus	4.38	34.36	1.90	8.59
Pseudo-nitzschia delicatissima complex	2.48	33.22	7.28	5.55
Protoperidinium cf. *steinii*	1.35	***31.10***	2.93	4.17
Dinophysis sacculus	0.00	***25.70***	12.97	0.06
Diplopsalis lenticula	19.95	24.14	1.44	2.60
Und. prasinophytes	8.39	23.65	15.55	17.56
Meringosphaera mediterranea	0.00	*22.03*	0.06	0.02
Protoperidinium divergens	0.14	19.74	1.18	3.86
Proboscia alata	0.13	0.99	***87.83***	5.29
Rhizosolenia spp.	1.12	0.31	***43.39***	4.31
Pseudo-nitzschia pseudodelicatissima complex	1.19	0.35	***40.31***	12.88
Pleurosigma sp.	0.95	1.84	***38.75***	15.59
Leptocylindrus danicus	8.52	4.93	35.27	24.40
Pseudo-nitzschia galaxiae	0.01	0.36	***34.10***	16.69
Prorocentrum compressum	0.78	8.19	***33.67***	1.57
Chaetoceros lorenzianus	2.99	8.80	28.95	14.96
Rhabdolithes claviger	0.00	0.00	***26.81***	1.25
Guinardia flaccida	0.94	2.59	25.38	7.91
Bacteriastrum spp.	0.00	10.06	21.25	0.72
Anoplosolenia brasiliensis	0.00	13.12	21.22	10.31
Pseliodinium vaubanii	0.00	0.03	*19.84*	2.46
Calycomonas sp.	0.00	13.90	18.33	0.80
Chaetoceros affinis	0.25	0.10	18.24	17.66
Guinardia striata	0.04	0.00	17.18	16.23
Asteromphalus sp.	0.68	0.00	*16.66*	5.30
Ceratium trichoceros	0.00	0.00	*16.69*	5.05
Lioloma pacificum	0.01	0.00	6.90	***51.84***
Cylindrotheca closterium	1.13	18.29	6.20	***46.87***
Asterionellopsis glacialis	0.70	0.03	0.21	***42.42***
Emiliania huxleyi	0.31	9.69	0.21	39.82
Cerataulina pelagica	1.41	15.90	23.48	38.98
Dictyocha fibula	17.24	0.17	0.00	***37.92***
Eucampia cornuta	0.13	0.00	0.41	***36.05***
Chaetoceros rostratus	0.00	0.00	0.19	***29.94***
Chaetoceros costatus	0.08	0.00	0.00	***27.31***
Chaetoceros tortissimus	0.00	0.00	0.18	***27.25***
Calciosolenia murrayi	0.00	0.02	0.26	***27.00***
Chaetoceros brevis	1.23	0.00	1.39	***26.26***
Chaetoceros lauderi	0.15	0.00	1.77	***24.73***

36

Syracosphaera pulchra	13.39	1.05	17.71	23.91
Pseudo-nitzschia multistriata	0.18	0.00	0.21	**22.05**
Protoperidinium spp.	0.22	6.57	7.18	19.97
Hemiaulus sinensis	0.79	0.00	0.41	*19.76*
Chaetoceros diversus	0.00	0.00	9.45	19.33
Tripos fusus	16.04	13.23	2.23	17.81
Distephanopsis staurodon	3.50	0.00	0.00	*16.82*

833

834   Table 4. Results of ANOVA and Fisher's tests about the phytoplankton abundances (total phytoplankton,

835   diatoms, dinoflagellates, coccolithophores, phytoflagellates) in 1988-2002 and 2007-2016. Mean values

836   (cells l^{-1}) ± standard error (SE). ns, not significant; * p<0.05.

837

Parameter	Season	1988-2002 Avg ± SE	2007-2016 Avg ± SE	p-level	Fisher test
Total phyto	Winter	8,621,429 ± 1,760,650	9,287,326 ± 3,367,632	ns	
(cells l^{-1})	Spring	4,404,514 ± 793,052	8,674,748 ± 2,747,719	ns	
	Summer	2,391,304 ± 253,979	3,186,198 ± 485,135	ns	
	Autumn	4,122,920 ± 575,328	2,557,843 ± 511,565	ns	
Diatoms	Winter	4,918,631 ± 1,408,129	6,821,744 ± 3,307,979	ns	
(cells l^{-1})	Spring	1,959,299 ± 725,205	4,997,232 ± 2,215,694	ns	
	Summer	544,510 ± 164,527	543,914 ± 161,908	ns	
	Autumn	1,138,577 ± 396,971	731,001 ± 305,514	ns	
Dinoflagellates	Winter	75,367 ± 13,451	39,868 ± 10,153	ns	
(cells l^{-1})	Spring	164,206 ± 26,521	195,865 ± 57,969	ns	
	Summer	102,044 ± 22,519	76,114 ± 19,164	ns	
	Autumn	127,427 ± 39,372	46,761 ± 8,929	ns	
Coccolithophores	Winter	40,488 ± 10,498	7,731 ± 3,542	*	88-02 > 07-16
(cells l^{-1})	Spring	41,460 ± 13,498	37,198 ± 12,917	ns	
	Summer	14,571 ± 3,478	8,401 ± 2,774	ns	
	Autumn	21,988 ± 5,282	19,171 ± 5,206	ns	
Phytoflagellates	Winter	3,578,648 ± 994,727	2,417,982 ± 290,289	ns	
(cells l^{-1})	Spring	2,048,230 ± 201,226	3,436,440 ± 688,167	ns	
	Summer	1,508,917 ± 178,144	2,557,685 ± 424,376	ns	
	Autumn	2,810,677 ± 426,364	1,760,593 ± 270,294	ns	

838

839

840 Table 5. Results of ANOVA and Fisher's tests about the phytoplankton biomass (total phytoplankton,

841 diatoms, dinoflagellates, coccolithophores, phytoflagellates) in 1988-2002 and 2007-2016. Mean values (µg

842 C l^{-1}) ± standard error (SE). ns, not significant; * $p<0.05$, ** $p<0.01$, ***$p<0.001$.

Parameter	Season	1988-2002 Avg ± SE	2007-2016 Avg ± SE	p-level	Fisher test
Total phyto	Winter	205.44 ± 59.79	406.56 ± 134.65	ns	
(µgC l^{-1})	Spring	107.50 ± 38.24	624.28 ± 167.73	***	88-02 < 07-16
	Summer	45.41 ± 9.52	63.51 ± 14.49	ns	
	Autumn	103.40 ± 34.01	119.36 ± 33.88	ns	
Diatoms	Winter	174.95 ± 57.46	292.03 ± 165.64	ns	
(µgC l^{-1})	Spring	56.84 ± 25.77	102.07 ± 42.97	ns	
	Summer	20.93 ± 5.92	34.17 ± 12.30	ns	
	Autumn	64.92 ± 30.85	79.20 ± 33.80	ns	
Dinoflagellates	Winter	5.33 ± 1.11	102.43 ± 53.36	ns	
(µgC l^{-1})	Spring	39.03 ± 14.71	500.19 ± 157.70	***	88-02 < 07-16
	Summer	16.01 ± 5.10	17.43 ± 2.85	ns	
	Autumn	16.26 ± 3.77	30.07 ± 6.81	ns	
Coccolithophores	Winter	0.97 ± 0.39	1.52 ± 0.92	ns	
(µgC l^{-1})	Spring	1.14 ± 0.46	2.07 ± 0.61	ns	
	Summer	0.80 ± 0.21	0.74 ± 0.27	ns	
	Autumn	2.16 ± 1.06	1.54 ± 0.43	ns	
Phytoflagellates	Winter	24.02 ± 6.96	10.58 ± 1.30	**	88-02 > 07-16
(µgC l^{-1})	Spring	10.15 ± 1.54	19.94 ± 3.97	*	88-02 < 07-16
	Summer	5.98 ± 1.00	11.17 ± 2.57	ns	
	Autumn	17.84 ± 3.52	8.55 ± 1.35	*	88-02 > 07-16

843