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## Ecological succession of pico- and nanophytoplankton in a coastal bay of NW Mediterranean sea

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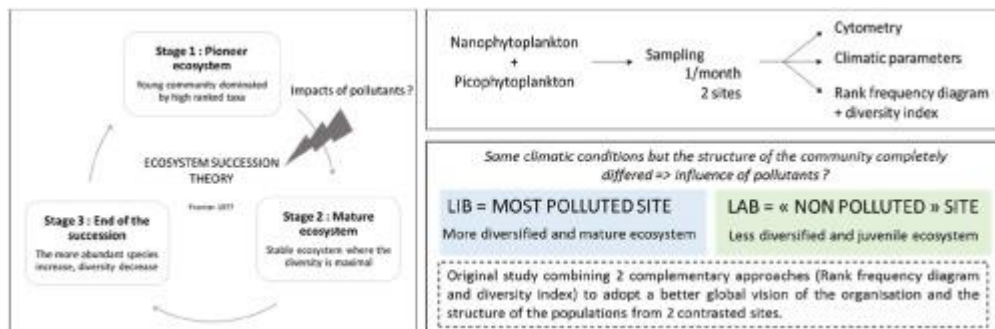
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### Abstract :

The long-term evolution of pico- and nanophytoplanktonic communities in two coastal contrasting sites from the bay of Toulon was studied using monthly flow cytometry analyses during a seven-year period. Results highlighted higher abundance of both pico- and nanophytoplanktonic communities in the more eutrophic site (i. e. the Little Bay). Moreover, even if both sites were widely dominated by *Synechococcus* spp. population, it represented more than 90% of the population during summer in the Large Bay. Using diversity indices and rank frequency diagrams to study ecological successions in both sites, communities from the Little Bay (the most polluted site) appeared, paradoxically, as more diversified and more mature than that from the Large Bay. Communities from the Large Bay appeared as less diversified and juvenile linked to the dominance of *Synechococcus* spp. The complementarity of diversity indices and rank frequency diagram was revealed in this present work.

## Graphical abstract



## Highlights

► Combined use of diversity indices and RFD is relevant to study coastal ecosystems. ► Ecosystems were more diversified and mature in the more polluted site. ► *Synechococcus* spp. population widely dominated coastal areas from the Bay of Toulon.

**Keywords** : ecological succession, diversity, pico- and nanophytoplankton, Mediterranean Sea, Rank Frequency Diagram

## 39 1. Introduction

40 Pico- (size range  $< 2 \mu\text{m}$ ) and nanophytoplankton (size range  $2 - 20 \mu\text{m}$ ) constitute an important  
41 part of the microbial loop by being the base of the trophic web (Chiang et al., 2013) and thus have  
42 been recognized as major contributors to the total primary production in marine ecosystems (Azam  
43 et al., 1983; Platt et al., 1983). Their importance is well established in open oceans, notably for  
44 picophytoplankton, but their role and succession in more coastal waters is not well documented  
45 (Mitbavkar et al., 2012). However, understanding ecosystem structure and functioning is essential to  
46 monitor and predict their evolution. In most cases, community structure and dynamics are shaped by  
47 a wide variety of environmental variables including nutrients availability, salinity or temperature (Bec  
48 et al., 2005; Sommer et al., 1986). Yet coastal waters are subject to nutrient enrichment by  
49 anthropogenic activities and freshwaters discharges (Paerl et al., 2006, 2003). Indeed, freshwater  
50 flow (i) are a main source of nutrients and (ii) cause hydrological perturbations as salinity variation  
51 or water resilience which play a determinant role in nutrients availability (Paerl et al., 2006). These

52 anthropogenic perturbations and freshwater discharges, typical of coastal waters, may play an  
53 important role in shaping community structure and determine ecological succession in these marine  
54 systems. Indeed, according to the model of ecological succession proposed by Margalef (1951),  
55 Reynolds (1984, 1980) or Sommer et al. (1986), succession of species represents a response to  
56 physical, chemical and biological changes. To our knowledge, only few studies focused on ecological  
57 succession in coastal waters and even less on pico- and nanophytoplanktonic succession.

58 This study was realised along a seven-year time series at the Bay of Toulon, at two linked but  
59 contrasted sites, *i. e.* the Little Bay (LiB) and the Large Bay (LaB). They are subjected to the same  
60 climatic conditions but differently affected by anthropogenic activities. The LiB, at the West, is semi  
61 enclosed, characterised by smaller dimensions (9.8 km<sup>2</sup> and 15 m deep – Dang et al., 2014), a longer  
62 residence time of water (3 - 6 days – Jouan et al., 2001), and is strongly affected by human activities  
63 (leisure, commercial and military harbours, shipyards). Whereas the LaB, at the East, is characterised  
64 by bigger dimensions (42 km<sup>2</sup> and 5 to 30 m deep– Coclet et al., 2018) with a shorter residence time  
65 of water (1 - 2 days – Duffa et al., 2011), less impacted by anthropogenic activities and more open on  
66 the Mediterranean Sea. Surface waters and sediments are strongly polluted by metalloids and trace  
67 metals especially in the LiB (Tessier et al., 2011; Cossa et al., 2014; Dang et al., 2014, 2015, Coclet et  
68 al., 2018). Inversely to the LaB, the LiB is considered as eutrophic, with a higher abundance of pico-  
69 and nanophytoplanktonic organisms (Coclet et al., 2018; Delpy et al., 2018). The same pattern has  
70 been observed for the micro phytoplanktonic and meso zooplanktonic communities (Rossi and  
71 Jamet, 2008; Serranito et al., 2016). The diversity observed in the LiB is lower, due to a characteristic  
72 presence of *Oithona nana* GIESBRECHT (1892) (Crustacea: Cyclopoida – Bandeira et al., 2013, Richard  
73 & Jamet, 2001).

74 Regarding the functional importance of pico- and nanoeukaryotes in food webs and in carbon  
75 fluxes in coastal marine waters, this study aimed at (i) investigating composition and abundance of  
76 pico- and nanophytoplanktonic communities between two contrasting sites from the Bay of Toulon,  
77 and (ii) defining the ecological successions of these sites using both diversity indices and Rank

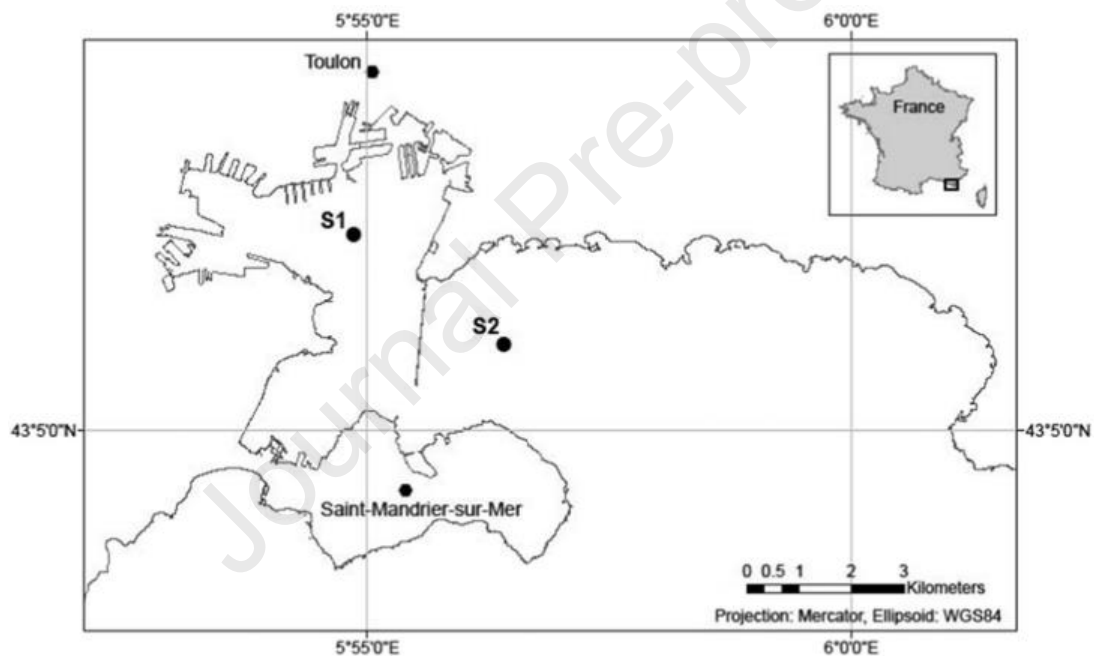
78 Frequency Diagram (RFD). This study will allow us to determine if diversity indices and rank  
79 frequency diagrams are relevant tools to assess the anthropogenic impact on coastal communities.

80

## 81 2. Material and Methods

### 82 2.1. Sampling

83 Samples were collected monthly between October 2013 and August 2020, in the Bay of Toulon,  
84 at two stations: Little Bay (LiB - S1, 43°06'30" N / 05°55'00" E) and Large Bay (LaB - S2, 43°05'45" N /  
85 05°56'30" E). All samples were collected between 08:00 and 11:00 AM at both 3 and 10 m depth  
86 (Figure 1).



87

88 Figure 1: Localization of the two stations in the LiB (S1) and LaB (S2) in the Bay of Toulon (from  
89 Serranito et al., 2016).

90 Environmental variables (temperature, salinity, pH and dissolved oxygen concentration) were  
91 measured *in situ* in LiB and LaB, at 3 and 10 m deep using calibrated sensors: Hydrolab HL7® and  
92 Hydrolab Quanta® multiparametric probes. These variables were used as descriptive factors to  
93 characterize differences among the two contrasted sites.

94 To determine pico- and nanophytoplankton communities' abundance, water samples were  
95 collected using a 10L Niskin bottle at 3 and 10 m depth at both sites (LiB and LaB). Samples were pre-  
96 filtered through a 90  $\mu\text{m}$  mesh to remove grazers and other macroparticules. Subsamples of 4.5 mL  
97 were then immediately transferred into 5 mL cryotubes and fixed with a glutaraldehyde solution  
98 (final concentration 0.25 %) and a pluronic acid solution (final concentration 0.01 %). These  
99 subsamples were kept at 4°C until analysis and then thawed out just prior analysis by flow cytometry.

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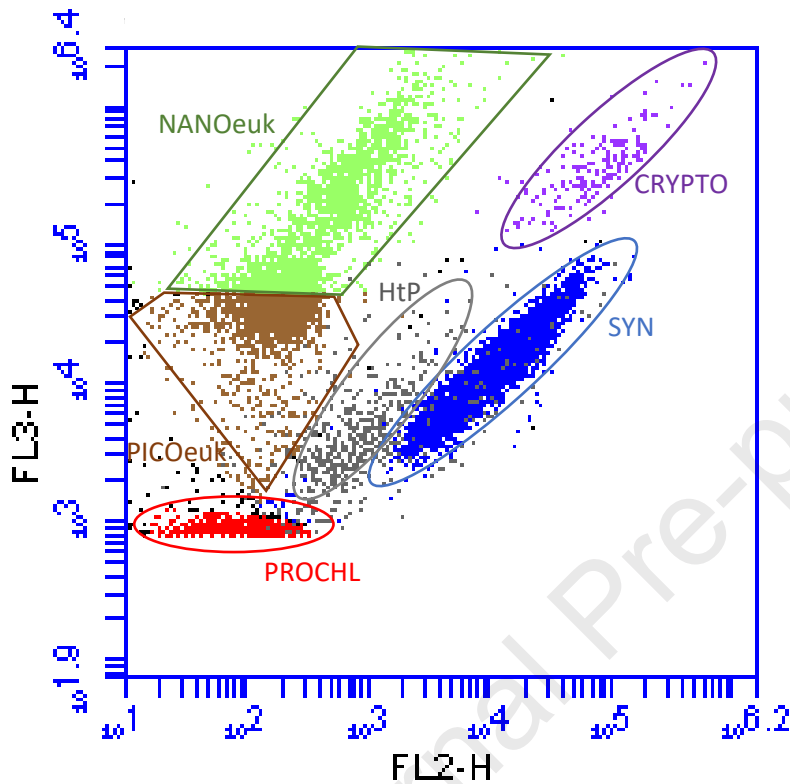
## 101 2.2. Flow cytometry analysis

102 Flow cytometry analyses were performed using an Accuri C6 flow cytometer (BD Biosciences®)  
103 equipped with 488 and 640 nm laser. The sheath fluid used was ultrapure water filtered through  
104 0.2  $\mu\text{m}$ . The 488 nm blue laser induced two natural fluorescence: Chlorophyll a (red fluorescence  
105 FL3 > 670 nm) and Phycoerythrin (orange fluorescence FL2: 585 +/- 20 nm). The FL4 phycocyanin (far  
106 red fluorescence 675 +/- 25 nm) is induced by the 640 red laser. The forward scattered light (FSC)  
107 was related to the size of the cells while Side scattered light (SSC) was related to shape and cell  
108 structure; 500  $\mu\text{l}$  sample were analysed at a flow rate of 60  $\mu\text{l}\cdot\text{min}^{-1}$ . Beads of 2  $\mu\text{m}$  diameter  
109 (Fluoresbrite YG, Polysciences) were periodically added to the samples as internal standards to  
110 differentiate between Picoeukaryotes and Nanoeukaryotes.

111 Thanks to their optical properties, six different clusters of pico- and nanophytoplankton were  
112 discriminated (Figure 2): (i) *Synechococcus* spp. (SYN) characterised by small cells (FSC < 12 000), rich  
113 in phycoerythrin (mean FL2  $\approx$ 14 950) and low in chlorophyll a (mean FL3  $\approx$ 15 950);  
114 (ii) *Prochlorococcus* spp. (PROCHL) which cells are differentiated from SYN cells by lower  
115 fluorescence (mean FL2  $\approx$ 117 and mean FL3  $\approx$  895); (iii) Picoeukaryotes (PICOeuk) characterized by  
116 larger cells than cyanobacteria (FSC  $\approx$  45 000) and higher red FL3 fluorescence (Mean FL3  $\approx$  29 000);  
117 (iv) Nanoeukaryotes (NANOeuk) are separated from PICOeuk by higher FSC (> 200 000) and higher  
118 red FL3 fluorescence (mean FL3  $\approx$  167 592) ; (v) Cryptophyceae (CRYPTO) are discriminated from  
119 NANOeuk thanks to higher orange FL2 fluorescence ; and (vi) Heterotrophic Protist (HtP)

120 characterized by low red and orange fluorescence (respectively < 2 000 and < 1 000) and high FSC  
 121 (> 900 000).

122



123

124 Figure 2 : Resolution by flow cytometry of ultraphytoplankton assemblage composition during the  
 125 study period.

126

### 127 2.3. Statistical analysis

128 Differences of pico- and nanophytoplanktonic abundances, and abiotic factors between sites  
 129 (LiB and LaB) and depth (3 m and 10 m) were assessed by Kruskal-Wallis analysis. As no significant  
 130 difference was observed for abiotic factors and abundances between 3 m and 10 m deep ( $p > 0.05$ ),  
 131 values were averaged.

132 A Principal Components Analysis (PCA) was used to assess the relationships between pico-  
 133 and nanophytoplankton abundances and their hydrological environment. The dataset consisted of  
 134 81-month values, from October 2013 to August 2020, described by 36 biological variables

135 (abundances, FL2, FL3, FL4, SSC and FSC of SYN, PROCHL, PICOeuk, NANOeuk, CRYPTO, HtP) and 4  
 136 hydrological variables (temperature, salinity, pH, dissolved oxygen concentration). The multifactorial  
 137 treatment was made using R software with FactoMineR package. To determine the number of  
 138 principal components used in this analysis, a scree plot was used to represent eigenvalues ordered  
 139 from the largest to the smallest (Peres-Neto et al., 2005). Only the first two components were used  
 140 since more than 64 % of the variances were explained by these components. As a second step,  
 141 correlation of Bravais-Pearson was used to assess the relation between abiotic factors and  
 142 abundances of the communities.

143 Using the classification of diversity indices proposed by Bandeira et al. (2013), 2 types of  
 144 indicators were chosen: (i) Shannon and (ii) Piélou indicators which were monthly calculated to  
 145 investigate the diversity of both sites. Shannon index ( $H'$ ) is one of the most frequently used  
 146 biodiversity indexes. It originates from the information theory as a measure of entropy (Shannon,  
 147 1948) and is used to estimate the specific diversity of planktonic communities and calculated as  
 148 follow:

$$149 \quad H' = -\sum_{i=1}^S p_i \log_2 p_i$$

150 with  $p_i = n_i/N$ ;  $p_i$  is the frequency of each class,  $S$  the total number of classes,  $n_i$  the number of  
 151 individuals from the  $i$  class and  $N$  the total abundance. The minimum value of the index is 0 when all  
 152 the observations belong to a single class. The maximum value equals  $\ln(1/S)$  and it can be reached  
 153 when the observations are equally divided between all the classes.

154 Piélou regularity index ( $J'$ ) (1966) reflect the evenness of a community and is calculated as follow:

$$155 \quad J' = \frac{H'}{H'_{max}} = \frac{H'}{\log_2(S)}$$

156 This index represents a standardization form of the Shannon index, displaying the relations between  
 157 the class frequencies (Piélou, 1966). The evenness equals one when the class frequencies are similar  
 158 and it approaches zero when the majority of observations belong to a single class.

159 Succession of pico- and nanophytoplanktonic communities were assessed using Rank Frequency  
 160 Diagrams (RFD, Frontiers 1976). Status of the community were empirically defined according to the

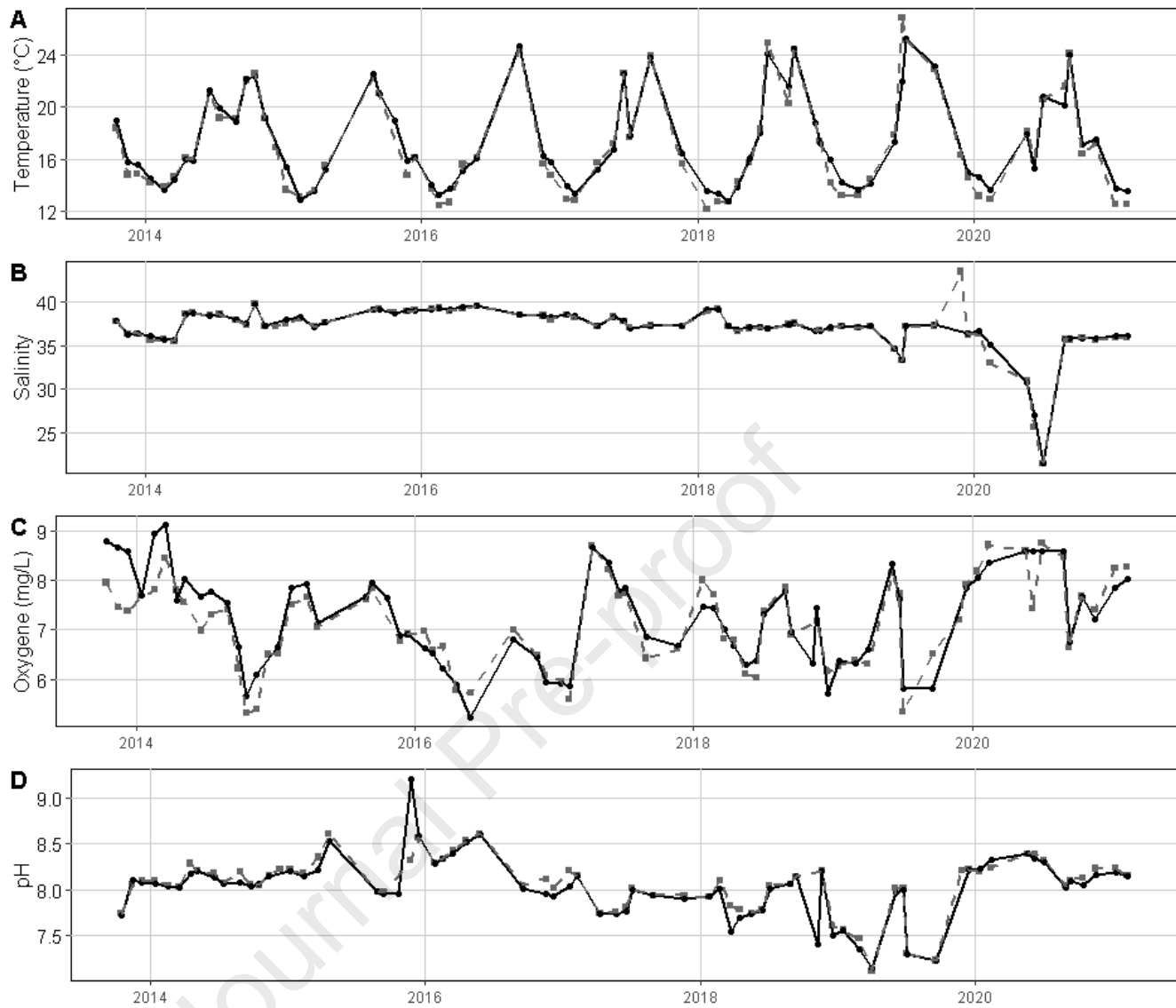


161 trend of the RFD curves; various models of species distributions have been described in the past as  
162 Broken Stick model (MacArthur, 1957), lognormal (Preston, 1948), geometric (Motomura, 1932) or  
163 even Zipf-Mandelbrot (Frontier, 1985). No fitting to any model is intended here since RFD are only  
164 used to describe ecological succession, following Frontier's description for planktonic ecosystem  
165 (Frontier, 1985, 1983, 1976). Depending on the shape of the curve, three main stages have been  
166 described. In stage 1, beginning of the succession, the curve is concave at the top left and become  
167 convex on the right part, typical of a young community with low diversity structure. In stage 2, the  
168 curve is entirely convex and the diversity at its maximum. In stage 3, end of the succession, the curve  
169 becomes straight and diversity is intermediate.-The determination of the stage was done using the  
170 linear trend curve in excel: when the determination coefficients were higher than 0.82 and 0.87 for  
171 LaB and LiB respectively, the curve was determined as convex; for lower determination coefficients,  
172 curve was determined as concave or straight.

173

### 174 3. Results

#### 175 3.1. Temporal variability of abiotic variables, pico- and nanophytoplanktonic abundances



176

177

178 Figure 3: Abiotic variables in the Little Bay (dashed lines) and the Large Bay (solid lines):

179 (A) Temperature ( $^{\circ}\text{C}$ ), (B) Salinity, (C) Dissolved Oxygen ( $\text{mg}\cdot\text{L}^{-1}$ ), (D) pH. Data are acquired as an

180 average of values measured at 3 m and 10 m of depth.

181

182 The annual variations of the hydrological variables (*i. e.* temperature, salinity, dissolved

183 oxygen and pH) were presented in the Figure 3. No significant difference was observed between LiB

184 and LaB, whatever the considered variables (Kruskal-Wallis;  $p > 0.05$ ).

185 Seasonal variations of temperature were observed in LiB and LaB (Figure 3A): maximal values  
 186 ( $> 20^{\circ}\text{C}$ ) were recorded during summers and minimal ones during the winter period ( $< 15^{\circ}\text{C}$ ).  
 187 Maximal temperature was reached in June 2019, in LiB.  
 188 No variation of salinity was observed along the study period (LiB:  $37.07 \pm 2.89$ ; LaB:  $37.11 \pm 2.70$ )  
 189 excepted in July 2020, when salinity reached respectively 21.53 and 21.54 for LiB and LaB (Figure 3B).  
 190 Dissolved oxygen concentrations varied from  $5.23 \text{ mg.L}^{-1}$  (October 2014) and  $9.11 \text{ mg.L}^{-1}$  (March  
 191 2014) without following a seasonal trend (Figure 3C).  
 192 pH values were stable across the study period (LiB:  $8.12 \pm 0.36$ ; LaB:  $8.09 \pm 0.40$ ) and varied between  
 193 7.11 (April 2019 – LiB) and 9.22 (July 2015 – LaB – Figure 3D).

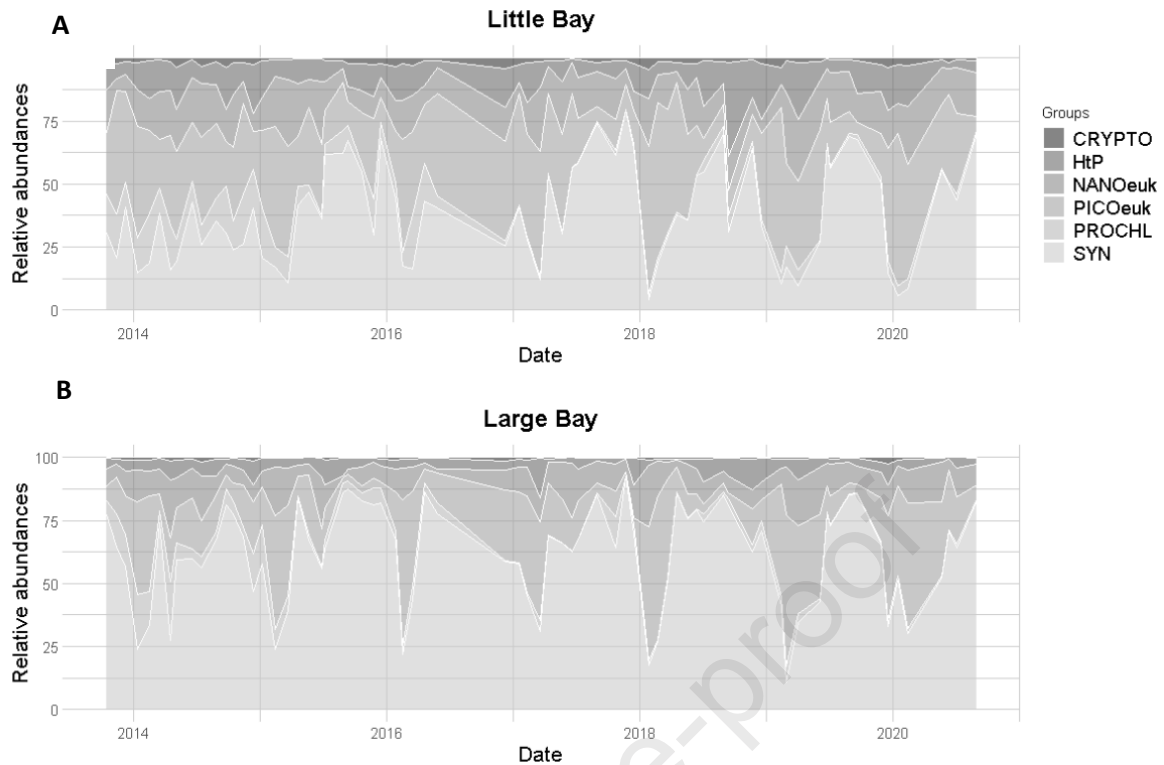
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195 Table 1 : Mean annual abundances of each communities ( $\text{cells.mL}^{-1}$ ) in both sites (LiB and LaB)

Date	Site	CRYPTO	NANOeuk	PICOeuk	HtP	PROCHL	SYN
2013	PR	854	4033	16486	3134	6425	12708
2013	GR	173	2222	4072	1049	3443	21256
2014	PR	725	5438	10284	4260	4865	10094
2014	GR	231	2616	4786	1276	2380	17489
2015	PR	402	4789	9021	2948	2622	15643
2015	GR	110	2283	4316	1129	1427	18045
2016	PR	479	2385	6773	2309	2475	6808
2016	GR	196	1982	7391	1002	1193	21935
2017	PR	338	3345	6412	1569	238	14774
2017	GR	82	1995	2924	611	332	13243
2018	PR	596	4001	10567	4745	888	16952
2018	GR	273	3296	7362	1942	616	31004
2019	PR	469	5294	7934	3123	825	14585
2019	GR	172	2805	5750	910	429	15049
2020	PR	447	4637	9482	1939	579	13606
2020	GR	80	2445	6474	579	278	16490

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197



198

199

200 Figure 4: Repartition of *Synechococcus* spp., *Prochlorococcus* spp., Heterotrophic Protist,  
 201 Picoeukaryotes, Nanoeukaryotes and Cryptophyceae and in LiB (A) and LaB (B).

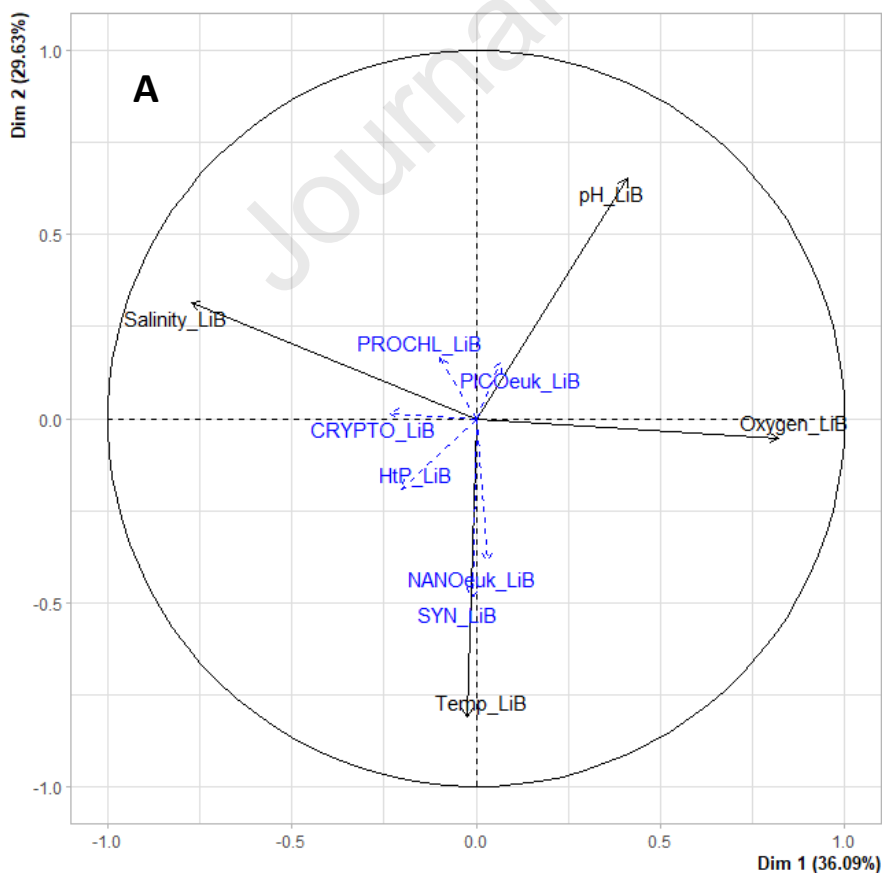
202 The mean annual abundances of the communities identified by flow cytometry are presented  
 203 in the Table 1; the composition of the communities is represented in the Figure 4. Total abundances  
 204 of the 6 communities were significantly different between the 2 studied site (Kruskal-Wallis,  $p < 0.05$ )  
 205 with in average higher values in LiB ( $32\,419\text{ cells.mL}^{-1}$ ) than in LaB ( $28\,747\text{ cells.mL}^{-1}$ ). Both sites are  
 206 largely dominated by SYN: in average, SYN represents respectively 38 % and 61 % of total  
 207 abundances in LiB and LaB.

208 Strong variations of SYN abundances was observed between October 2013 and February  
 209 2015 in LiB, not linked with seasonality: abundances peaked between 35 and 40 % in December  
 210 2013, March, June, August and December 2014. Apart from that, abundance was between 10 and  
 211 26 %. PICOeuk was especially abundant during this period and varied between 17 % in September  
 212 2014 and 48 % in November 2013. PROCHL and HtP abundances varied between 9 to 19 % and 6 to

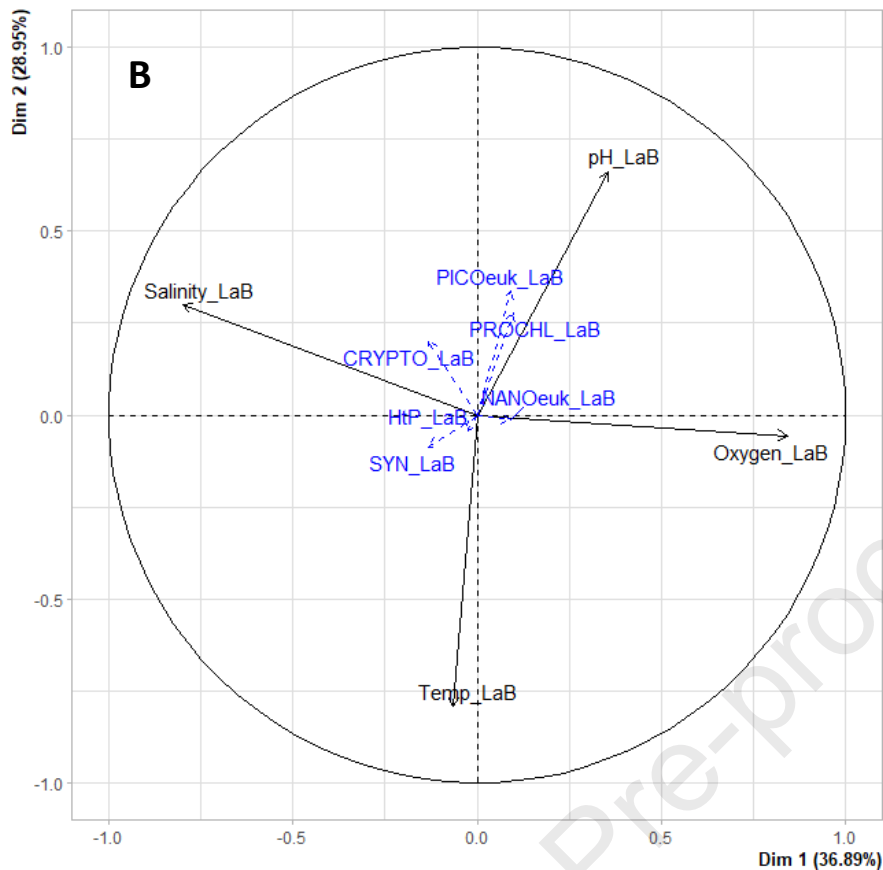
213 12 % respectively. NANO represented less than 20 % of the community. CRYPTO abundance varied  
 214 between 0.3 and 3 %. In 2015, SYN proportion increased and reached 67 % in September 2015; at the  
 215 same date, PICOeuk abundance substantially decreased to 9 %. From 2016, seasonal variation of SYN  
 216 abundances was observed: its proportion reached up to 80 % during spring and decreased to less  
 217 than 15 % in winter (4 % in January 2018). In the meantime, proportion of PICOeuk was minimal  
 218 during summer (5 % in August 2017) and maximal during winter (62 % in February 2018). PROCHL,  
 219 HtP, NANO and CRYPTO represented respectively 3, 10, 14 % and less than 3 % of the community.

220 Communities from LaB were largely dominated by SYN which represented in average 61 % of  
 221 the communities. Seasonal variations of its proportion were observed; it is minimal during winter  
 222 (25 % in February 2015) and maximal during summer (92 % in September 2017). PICOeuk was the  
 223 second more abundant group and represents 7 to 50 % of the community. NANO, PROCHL, CRYPTO  
 224 and HtP represents less than 15 % of the total communities.

225



226



227 Figure 5: Principal Components Analysis (PCA) applied to data from LiB (A) and LaB (B).

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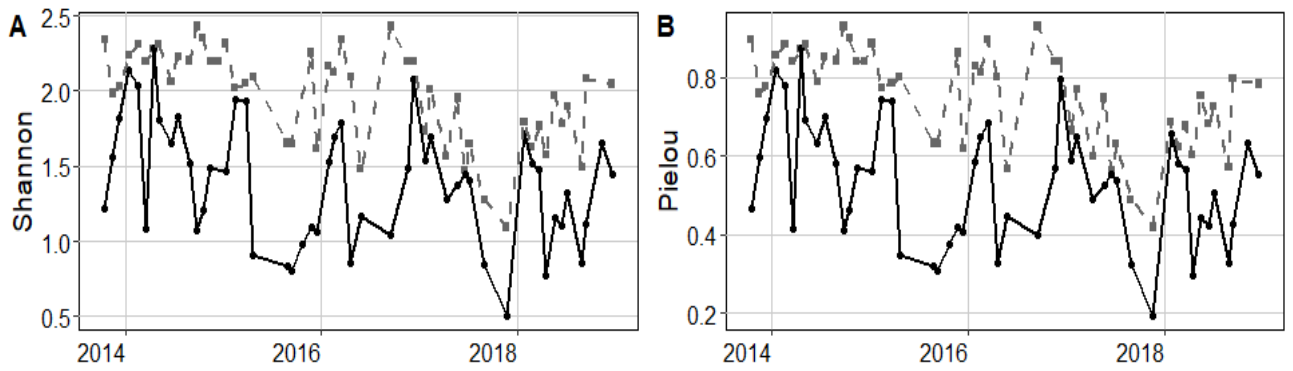
229 The results of the PCA variables representing the 7 years sampling years are shown in Figure 5. The  
 230 first two axes accounted for 36 % and 29 % of the total variance for both LiB and LaB, demonstrating  
 231 that, the major factor affecting our data was temperature. But, most likely due to photosynthesis,  
 232 oxygen was also highlighted by the PCA as a variable affecting the communities' structure.

233 In LiB, abundances of both SYN and NANOeuk were strongly influenced by high temperature,  
 234 explaining SYN peaks during summer (Bravais-Pearson, respectively  $p < 0.00001$  and  $p < 0.01$ ). On the  
 235 contrary, PICOeuk from both LiB and LaB, were influenced by low temperature since it peaked during  
 236 winter.

237 In LaB, no clear relations could be determined; abiotic factors used in this analysis seems  
 238 insufficient to explain the variances of abundances from all studied communities.

239

240 3.2. Diversity indices



241

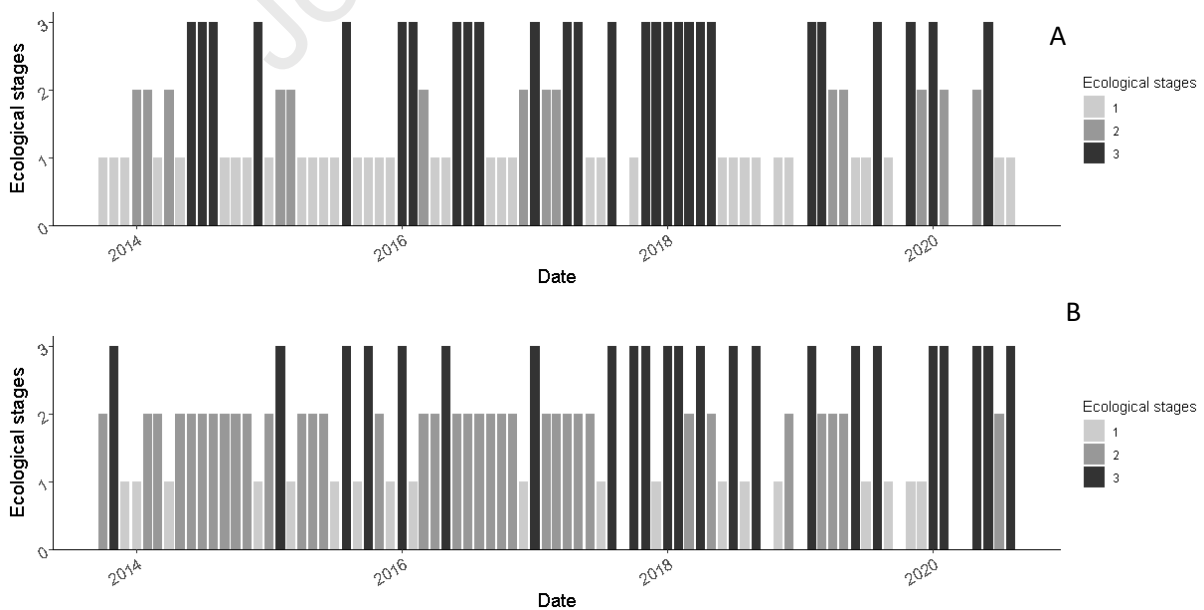
242 Figure 6: Evolution of plankton indices along the study period: Shannon (A) and Pielou indices (B) for  
 243 LiB (dashed grey lines) and LaB (solid black lines).

244 Diversity indices are presented in Figure 6. Shannon index was significantly lower in LaB than in  
 245 LiB (Mann Whitney,  $p < 0.05$ ). In LiB, Shannon index varied from 1.1 (November 2017) to 2.43  
 246 (September 2016) without a seasonal trend; in LaB, this index peaked at the beginning of the year (it  
 247 reached 2.08 in December 2016) and is minimal during summer (0.84 in August 2015).

248 Equitability index or Pielou index has the same trend as Shannon index since its calculation  
 249 derives from Shannon's one.

250

### 251 3.3. Community assemblage



252

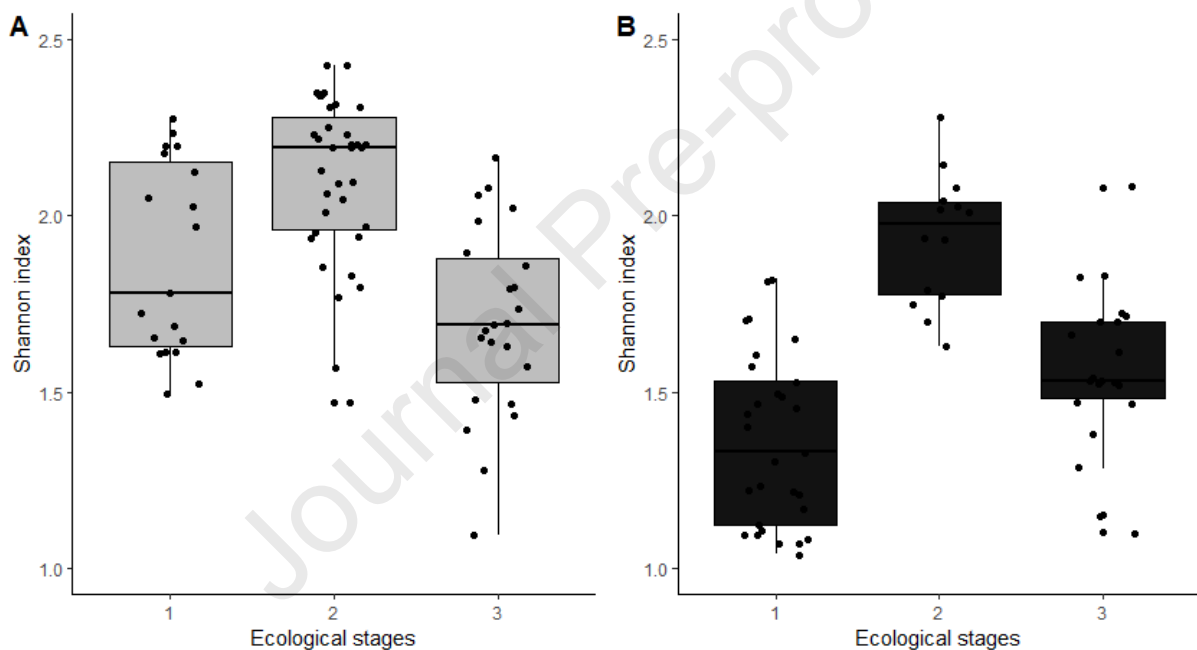
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254 Figure 7: Ecological stages determined for LiB (A) and LaB (B)

255

256 Ecological stages determined following Frontier's descriptions (1985) are presented in the  
 257 Figure 7. In LiB, a total of 19 stages 1 have been determined; 35 months have been determined as  
 258 stage 2 and 23 as stage 3. Three complete successions are observed between December 2014 to  
 259 February 2015, February and May 2016, and November and February 2019. In LaB, 36 months were  
 260 determined as stage 1, 14 as stage 2 and 27 as stage 3. Only one complete succession was  
 261 determined in November 2016 to January 2017.

262



263

264 Figure 8: Evolution of Shannon index and ecological stages for both LiB (A) and Lab (B). Stages were  
 265 determined following Frontier's recommendations (see Frontier 1985, 1983, 1976).

266

267 Shannon indices varied with ecological stages. In LiB, Shannon index varied between 1.50 and  
 268 2.28 in stage 1, between 1.47 and 2.43 in stage 2 and decrease to 1.10 and 2.17 in stage 3. In LaB,  
 269 Shannon indices was between 0.81 and 1.82 for stage 1. They increased at stage 2 to reach the  
 270 minimum of 1.63 and the maximal value of 2.28. At stage 3, this index decreased and was between  
 271 0.51 and 2.08 (Figure 8).



272

## 273 4. Discussion

274 Because of its topography and anthropogenic pressure this site is currently facing, the Bay of  
275 Toulon is relevant to explore annual variability of zooplankton (Jamet et al., 2001; Richard and Jamet,  
276 2001) and microphytoplankton (Bogé et al., 2006; Jamet et al., 2005; Rossi and Jamet, 2009). A  
277 previous study (Delpy et al., 2018) has investigated pico and nanoplanktonic dynamics during a two  
278 year cycle (October 2013 to December 2015). By using a longer time series (2013-2020), our study  
279 explores the evolution of pico- and nanophytoplanktonic communities to determine ecological  
280 successions in the two contrasting sites of the Bay of Toulon.

281

## 282 4.1. Variations of abiotic variables and pico- and nanophytoplanktonic abundances

283 The monitoring of abiotic variables (*i. e.* temperature, salinity, pH and oxygen) did not reveal a  
284 stratification phenomenon in both sites since no significant difference was observed between depths  
285 of 0 and 10 m, probably due to wind which can disturb vertical stratification in coastal waters  
286 (Charles et al., 2005). Moreover, no significant difference in abiotic variables have been revealed  
287 between the both studied sites.

288 Water temperature showed a clear seasonal pattern as usually found in coastal sites from the  
289 Mediterranean sea (Rossi and Jamet, 2009). Salinity and pH were quite stable along the sampling  
290 period excepted in July 2020 when salinity reached 21, related to the summer storms.

291 Pico- and nanophytoplankton abundances are significantly higher in LiB than in LaB, as previously  
292 described by Delpy et al. (2018). It reflected the trends observed for other higher planktonic species  
293 such as mesozooplankton or microphytoplankton (Jamet et al., 2001; Serranito et al., 2016). Such  
294 differences of abundances between sites were attributed to eutrophic conditions characterising LiB  
295 (Rossi and Jamet, 2009). Indeed, mean chlorophyll a concentration in the Mediterranean Sea ranges  
296 between 0,1 to 0,5  $\mu\text{g}\cdot\text{L}^{-1}$  although values measured in LiB are significantly higher (1 to 1,5  $\mu\text{g}\cdot\text{L}^{-1}$  -

297 Jamet et al., 2001) and characterize eutrophic waters according to Jacques and Tréguer (1986) and  
298 Psyllidou-Giouranovits et al. (1997).

299         Seasonal variations of abundances were observed in both sites: abundances of SYN  
300 significantly increased during late spring/summer with maximum abundance of 127 300 cells.mL<sup>-1</sup> (in  
301 2017, at LaB), close to maxima observed for Mediterranean coastal systems (Agawin and Agusti,  
302 1997; Cerino et al., 2012; Ferrier-Pages and Rassoulzadegan, 1994); in winter, PROCHLO became  
303 dominant and represented 50 % of the total communities (34 648 cells.mL<sup>-1</sup>), close to values  
304 observed by Grégori et al. (2001) in Marseille. Such seasonality in pico- and nanophytoplanktonic  
305 communities was in agreement with previous observations in the Gulf of Lion (Lefevre et al., 1997)  
306 and in Marseille (Grégori et al., 2001).

307         Seasonal changes in both abiotic variables and groups abundances were analysed using a  
308 PCA. Paradoxically, a clear relation between temperature and SYN abundances was only determined  
309 in LiB but not on LaB. As previously described by Sohm et al. (2016), two different ecotypes may  
310 occupy these two different bays; SYN occupying LiB could largely be defined by temperature  
311 contrarily to the SYN ecotype from LaB.

312         Moreover, no clear relation was observed for the other communities with abiotic variables  
313 presented here. Previous studies have demonstrated that abundance of these different groups is  
314 driven by nutrient availability and species growth rate. Indeed, LiB is considered as more eutrophic  
315 than LaB due to higher Chlorophyll a and nutrient concentrations (Rossi et al., 2008; Rossi and Jamet,  
316 2009). LaB exhibited lower P-PO<sub>4</sub><sup>3-</sup> concentrations due to (i) a more regularly mixing of waters and  
317 (ii) the predominance of SYN which can contribute up to 35 % of the total Pi uptake (Delpy et al.,  
318 2018). It is important to note that biotic interaction such as grazing pressure or competition for  
319 nutrient and space by larger phytoplankton could also play an important role in shaping community  
320 structure and need to be clarified.

321

322         4.2. Composition of pico- and nanophytoplanktonic community

323 Even if SYN was dominant in both LiB and LaB, this group was largely more abundant in LaB as  
324 previously described by Delpy et al. (2018). It represented between 25 to 92 % of total communities  
325 in LaB, although communities of the LiB were represented by 2 major codominant groups *i. e.* SYN  
326 (representing between 15 to 75 % of the total communities) and PICOeuk (5 to 62 % of the  
327 community). The dominance of SYN in terms of abundance and contribution to primary productivity,  
328 has now been widely recognised (Johnson et al., 1982; Morris and Glover, 1981). Because of their  
329 small size and higher surface to volume ratios, SYN are more competitive than other to acquire  
330 nutrients and explained their dominance in both sites (Bec et al., 2011). The ability of SYN to acquire  
331 soluble reactive phosphorus (notably in P-limited zones) provides an ecological advantage over other  
332 phytoplankton classes (Collos et al., 2009). Differences of abundances between sites could be  
333 explained by higher trace metals' concentration in LiB (Rossi and Jamet, 2008). Indeed, a strong  
334 inhibition of SYN growth rate was observed when exposed to Copper ( $EC_{50} = 4 \mu\text{g}\cdot\text{L}^{-1}$  - Debelius et al.,  
335 2011, 2009). Moreover, pico- and nanophytoplanktonic communities collected in LaB exposed to  
336 trace metal cocktail, revealed a shift from SYN dominated community (typical of LaB) to a less SYN  
337 dominated community (with higher proportion of picoeukaryotes) as observed in LiB (Coclet et al.,  
338 2018). Codominance of PICOeuk and SYN (as observed in LiB) has been previously observed in other  
339 coastal waters (Changjiang Estuary - Pan et al., 2007), where PICOeuk can even surpass  
340 cyanobacteria in terms of biomass. Other groups (NANOeuk, HtP) represented less than 20 % of total  
341 communities. Differences between both cyanobacteria biomass (*i. e.* PROCHL and SYN) was  
342 consistent with previous study since PROCHL abundance vary inversely with SYN ones (Partensky et  
343 al., 1996) and is predominantly abundant in oligotrophic waters (Pan et al., 2007; Partensky et al.,  
344 1999).

345 In previous studies (Agawin et al., 1998; Delpy et al., 2018; Moisan et al., 2010), successions of  
346 pico- and nanophytoplanktonic communities appeared mainly driven by the ability to acquire  
347 nutrients and species growth rate. However, communities of the Bay of Toulon showed SYN  
348 dominated the communities, suggesting bottom up processes as key variables in the structuring of

349 communities. Indeed, the dominance of SYN during the spring/summer period in LaB explained the  
350 seasonal variations of Shannon (and Piélou) index with a clear decrease of this index during summer;  
351 in winter, diversity increased again, notably linked to the fall in SYN proportion. In LiB, no clear  
352 seasonal variation of Shannon index was observed. However, diversity was significantly higher in LiB  
353 than LaB and thus, confirmed by RFD analysis (with more month described as in LiB than in LaB),  
354 contrary to the previous results obtained at the same site for zooplankton and phytoplankton  
355 suggesting that diversity was higher in LaB (Jamet et al., 2005; Rossi and Jamet, 2009; Serranito et al.,  
356 2019). Such contradiction could be induced by the dominance of SYN in the Bay of Toulon: in LaB,  
357 SYN largely drive the community's dynamic; in LiB, SYN was still very abundant but due to high trace  
358 metals concentration, it became less competitive than in LaB, and other groups, as PICOeuk, became  
359 more abundant. Moreover, in LiB, the proportion of HtP is higher than in LaB and could also control  
360 SYN population by grazing pressure (Christaki et al., 1999; Dolan and Šimek, 1999; Guillou et al.,  
361 2001).

362 Interestingly, according to Frontier (1985, 1983), a chronic pollution induces a recover to a stage  
363 1 (typical of a juvenile population) since few species are able to maintain themselves in such  
364 conditions of stress. However, our results indicated more stage 1 in LaB than LiB, and even, more  
365 evolution to a complex ecosystem typical of a stage 2 in LiB than in LaB. The LaB is characterized by  
366 stage 1 corresponding to a little evolution of the population, whose structure and organisation are  
367 deeply affected by disturbances, here the domination of SYN population linked to its ability to better  
368 acquire nutrients. Paradoxically, populations from LiB naturally evolved towards more mature and  
369 diversified ecosystems. This is explained by anthropogenic activities increasing trace metals'  
370 concentrations limiting SYN domination in LiB, by (i) decreasing SYN growth rate (Debelius et al.,  
371 2011, 2009) and (ii) increasing grazing pressure by ciliates and other heterotrophic protists (Christaki  
372 et al., 1999; Dolan and Šimek, 1999; Guillou et al., 2001).

373

374 4.3. The combine use of RFD and diversity index to monitor pico- and nanophytoplanktonic  
375 assemblage

376 To better understand the structure and the functioning of ecosystems, ecologists have worked  
377 on principals establishing theoretical ecology. Among these are the diversity indices used to complete  
378 the description of a community by describing the richness or the evenness of species. The whole idea  
379 was to obtain quantitative information to compare biological entities (Heip et al., 1998). In our study,  
380 we used two different indices based on their mathematical convergence highlighted by the work of  
381 Bandeira et al. (2013). Shannon and Piélou revealed important variations of diversity and evenness in  
382 agreement with our observations.

383 Another method used to determine ecological succession of an ecosystem is to build Rank  
384 Frequency Diagram, establishing “the numerical repartition of individuals by species within a  
385 biocenosis” (Frontier, 1976). Considered as a fuller description of biological communities than  
386 diversity indices (although not independent from them), it introduces maturity degrees of the  
387 populations divided in 3 stages. First, stage 1, the beginning of the succession, is dominated by high  
388 ranked taxa. The community is composed by one or a few small species with predominantly  $r$   
389 selected species described as invasive, fast growing and prolific (Reynolds, 1997). In stage 2, the  
390 abundance of rare species continues to decrease and common species become more numerous. This  
391 community is characterized by  $K$  selected species, with bigger size, lower growth rate, favoured by  
392 physiological capabilities allowing their survival towards the supportive capacity of the resource  
393 available (Reynolds, 2006). According to Khemakhem et al. (2010), this favoured retroactive loops  
394 within the trophic network, allowing a more stable ecosystem where the diversity is maximal. And, in  
395 stage 3, the end of the succession, the numbers of the most abundant species increase whereas the  
396 diversity decreases, characterising the maturity of the community. This tool, notably used in other  
397 field of application as language study (Mandelbrot, 1953), or socio-economy (Petruszewicz, 1972),  
398 were widely used fifty years ago in ecology notably by Margalef (1957) and Frontier (1985, 1983,  
399 1976). According to these authors, ecosystems have a structure in terms that they are composed by

400 different species arranged in a define pattern according to the availability of resources. Stages are  
401 described following different fitting models: broken stick model (MacArthur, 1957), general  
402 lognormal (Preston, 1948), geometric (Motomura, 1947) or Zipf-Mandelbroat (Mandelbrot, 1977).  
403 This method wasn't used much during these past decades, notably linked to the difficulty in  
404 interpreting results. However, the relevance of this method has been well documented in both  
405 marine (Davoult et al., 1993; Fromentin et al., 1997; Frontier, 1985; Jamet et al., 2005; Sanvicente-  
406 Añorve et al., 2002) and terrestrial ecosystems (Kevan et al., 1997; Vahid et al., 2013; Watkins and  
407 Wilson, 1994; Wilson et al., 1998, 1996; Wilson and Gitay, 1995).

408 For terrestrial ecosystems, Grime (1974, 1977) described three primary strategies in plants and  
409 their relevance to ecological and evolutionary theory. The first one, C-selection, were growth is  
410 maximized in productive and relatively undisturbed conditions. The second one, S-selection, is  
411 characterized by environmental stresses and resource depletion. The last one, R-selection, is  
412 associated to organisms with a short lifespan and high reproduction rates in severely perturbed  
413 environments. Following Smayda & Reynolds (2001) adaptations of the Grime's triangle (1974) and  
414 within the framework of this study, Frontier's (1976) stage 1 could be hypothesized as an equivalent  
415 of Grime's (1977) R-selection with fast growing, predominantly *r* selected species. The stage 2  
416 characterized by K selected species could be considered as an equivalent of the S-selection with  
417 stress tolerant species and stage 3 that of the C-selection with highly competitive species. In the  
418 current context of global change, future environmental conditions could largely affect marine coastal  
419 ecosystems, resulting in an increase of their entropy creating stronger perturbations affecting the  
420 structure, diversity and functioning of communities. Subsequently, future monitoring of such  
421 ecosystems could more frequently highlight communities transitioning towards a composition of  
422 fewer smaller, fast growing *r* selected species as observed in Frontiers' stage 1 (1976) and Grime's  
423 (1977) R-selection.

424 Our study combines quantitative information from diversity indices and qualitative information  
425 given by RFD to study two contrasted sites from Toulon. The strong correlations between Shannon

426 indices and ecological stages highlighted the relevance to use both methods in the analysis of  
427 ecosystems structure and ecological stages of pico- and nanophytoplankton in the NW  
428 Mediterranean Sea. Previous studies (Dugenne et al., 20014; Fuchs et al., 2021; Thyssen et al., 2014,  
429 2008) showed that pico- and nanophytoplanktonic communities react rapidly to climatic conditions  
430 variations and wind-driven events. By dint of their short turnover time, pico- and  
431 nanophytoplanktonic communities could be used as a pertinent biological indicator to conduct future  
432 ecosystem monitoring. This study's combined use of RFD and diversity indices to determine the  
433 ecological successions stages in marine coastal ecosystems provides a better understanding of their  
434 functioning. Our contribution through regional databases, French national observation networks  
435 (RESOMAR - Réseau des Stations et Observatoires Marins - IR ILICO) and at European scale (MSFD -  
436 Marine Strategy Framework Directive - European Commission - MSFD, 2008/56/EC) grants more  
437 tools for reflexion on management and conservation of marine coastal ecosystems to decision  
438 makers and public politics administrators.

439

## 440 5. Conclusion

441 Our result showed that, even if the two sites are geographically close and exposed to the same  
442 environmental conditions, the structure of the community completely differed. Paradoxically,  
443 communities from the most polluted site (LiB) were more diversified (in terms of Shannon index) and  
444 more often evolved to mature ecosystem. LaB is characterized by juvenile ecosystems with lower  
445 diversity index. Such differences are attributed to the dominance of *Synechococcus* in the Bay of  
446 Toulon which completely drives community structure: widely dominant in LaB, SYN induced a  
447 decrease of diversity and contributed to stay in a juvenile ecosystem. Regulated by grazing pressure  
448 and trace metal contaminant in LiB, the diversity increased and ecosystem became more mature.  
449 The two methods used in this work to study coastal ecosystems (*i. e.* diversity indices and rank  
450 frequency diagram) were complementary and allowed us to adopt a better global vision of the  
451 organisation and the structure of the populations from the bay of Toulon. Our multiscale

452 contribution through regional, national and European networks grants more tools for reflexion on  
453 management and conservation of marine coastal ecosystems to decision makers and public politics  
454 administrators.

455

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706 Supplementary data

707 Table 1 : Ecological stages of each month for LiB and LaB. Determination coefficient ( $r^2$ ) of the linear  
708 trend line are also represented. When  $r^2$  is higher than 0.82 and 0.87 for respectively LaB and LiB, the  
709 curve is determined as convex; when  $r^2$  is lower, the curve is determined as straight or concave.

710 Complete succession is represented in bold.

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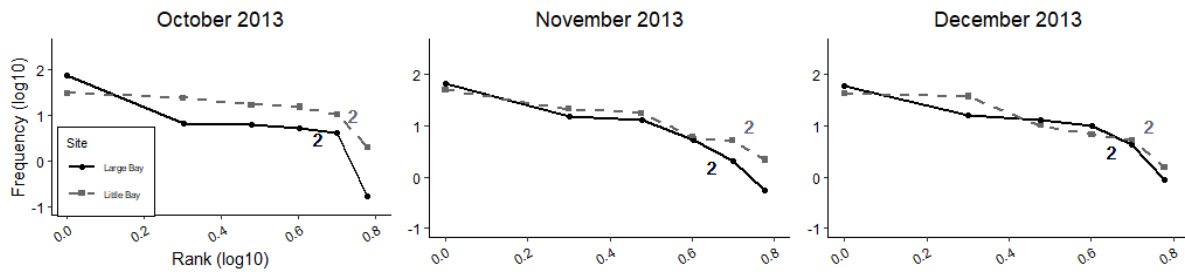
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Date	LiB		LaB		Date	LiB		LaB	
	Stage	r <sup>2</sup>	Stage	r <sup>2</sup>		Stage	r <sup>2</sup>	Stage	r <sup>2</sup>
oct-13	2	0.97	1	0.49	juin-17	2	0.91	1	0.8
nov-13	3	0.8	1	0.65	juil-17	1	0.74	1	0.66
déc-13	1	0.83	1	0.69	août-17	3	0.57	3	0.51
janv-14	1	0.71	2	0.97	oct-17	3	0.68	1	0.62
févr-14	2	0.9	2	0.91	nov-17	3	0.54	3	0.47
mars-14	2	0.88	1	0.54	déc-17	1	0.62	3	0.6
avr-14	1	0.83	2	0.93	janv-18	3	0.69	3	0.81
mai-14	2	0.87	1	0.63	févr-18	3	0.69	3	0.79
juin-14	2	0.9	3	0.72	mars-18	2	0.87	3	0.78
juil-14	2	0.94	3	0.71	avr-18	3	0.76	3	0.52
août-14	2	0.93	3	0.61	mai-18	2	0.95	3	0.58
sept-14	2	0.88	1	0.49	juin-18	1	0.81	1	0.54
oct-14	2	0.97	1	0.51	juil-18	3	0.73	1	0.54
nov-14	2	0.96	1	0.61	août-18	1	0.54	1	0.49
déc-14	1	0.85	3	0.82	sept-18	3	0.73	1	0.5
janv-15	2	0.92	1	0.69	nov-18	1	0.65	1	0.65
févr-15	3	0.82	2	0.91	déc-18	2	0.9	1	0.55
mars-15	1	0.85	2	0.9	févr-19	3	0.54	3	0.77
avr-15	2	0.9	1	0.51	mars-19	2	0.98	3	0.7
mai-15	2	0.87	1	0.67	avr-19	2	0.96	2	0.93
juin-15	2	0.95	1	0.79	mai-19	2	0.93	2	0.91
juil-15	1	0.63	1	0.6	juin-19	3	0.69	1	0.56
août-15	3	0.68	3	0.5	juil-19	1	0.78	1	0.6
sept-15	1	0.53	1	0.46	août-19	3	0.63	3	0.51
oct-15	3	0.73	1	0.49	sept-19	1	0.78	1	0.5
nov-15	2	0.93	1	0.5	nov-19	1	0.75	3	0.65
déc-15	1	0.53	1	0.49	déc-19	1	0.75	2	0.87
janv-16	3	0.8	3	0.62	janv-20	3	0.63	3	0.79
févr-16	1	0.79	3	0.75	févr-20	3	0.85	2	0.87
mars-16	2	0.92	2	0.84	mai-20	3	0.81	2	0.87
avr-16	2	0.88	1	0.48	juin-20	3	0.82	3	0.66
mai-16	3	0.81	1	0.55	juil-20	2	0.91	1	0.7
juin-16	2	0.93	3	0.76	août-20	3	0.62	1	0.52
juil-16	2	0.94	3	0.71					
août-16	2	0.93	3	0.61					
sept-16	2	0.88	1	0.5					
oct-16	2	0.97	1	0.51					
nov-16	2	0.96	1	0.61					
déc-16	1	0.85	2	0.82					
janv-17	3	0.78	3	0.78					
févr-17	2	0.94	2	0.84					
mars-17	2	0.92	2	0.95					
avr-17	2	0.88	3	0.67					
mai-17	2	0.96	3	0.63					

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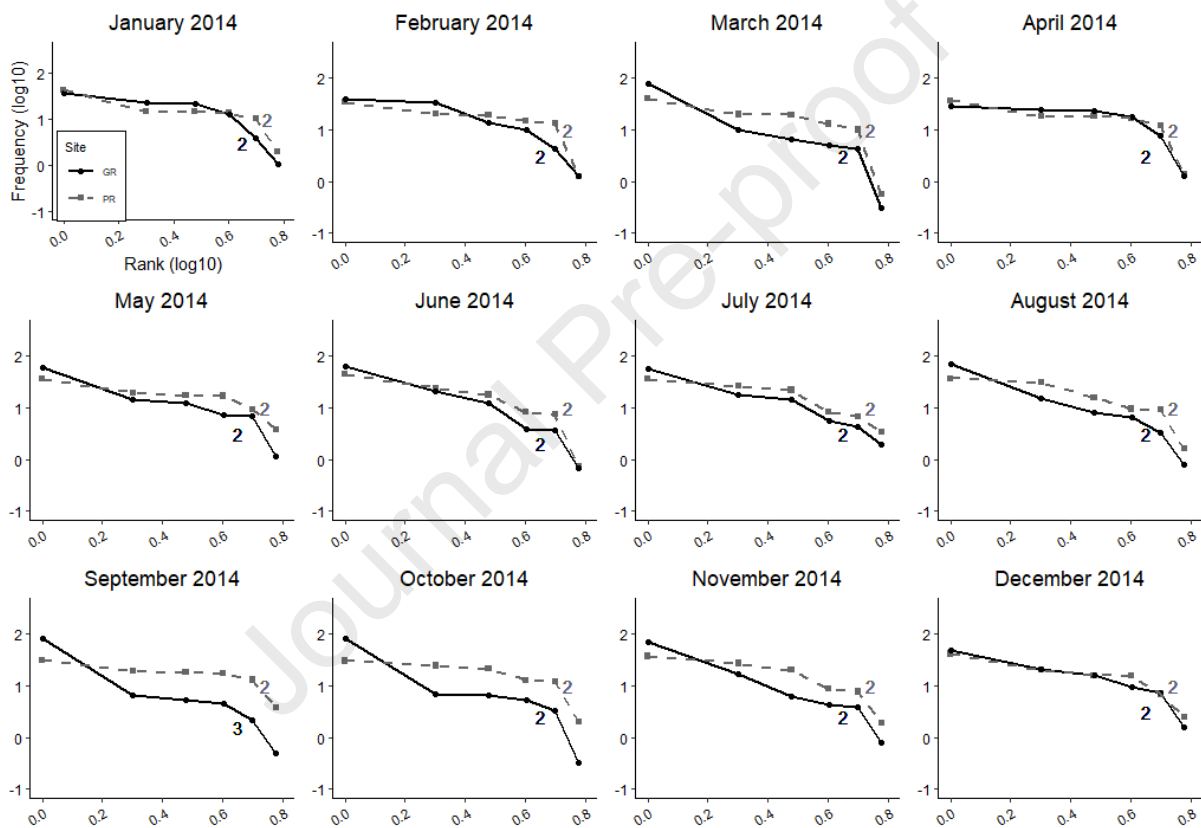
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718 Figure 1: Rank frequency diagrams of pico- and nanoplankton assemblage in the Little Bay (dashed

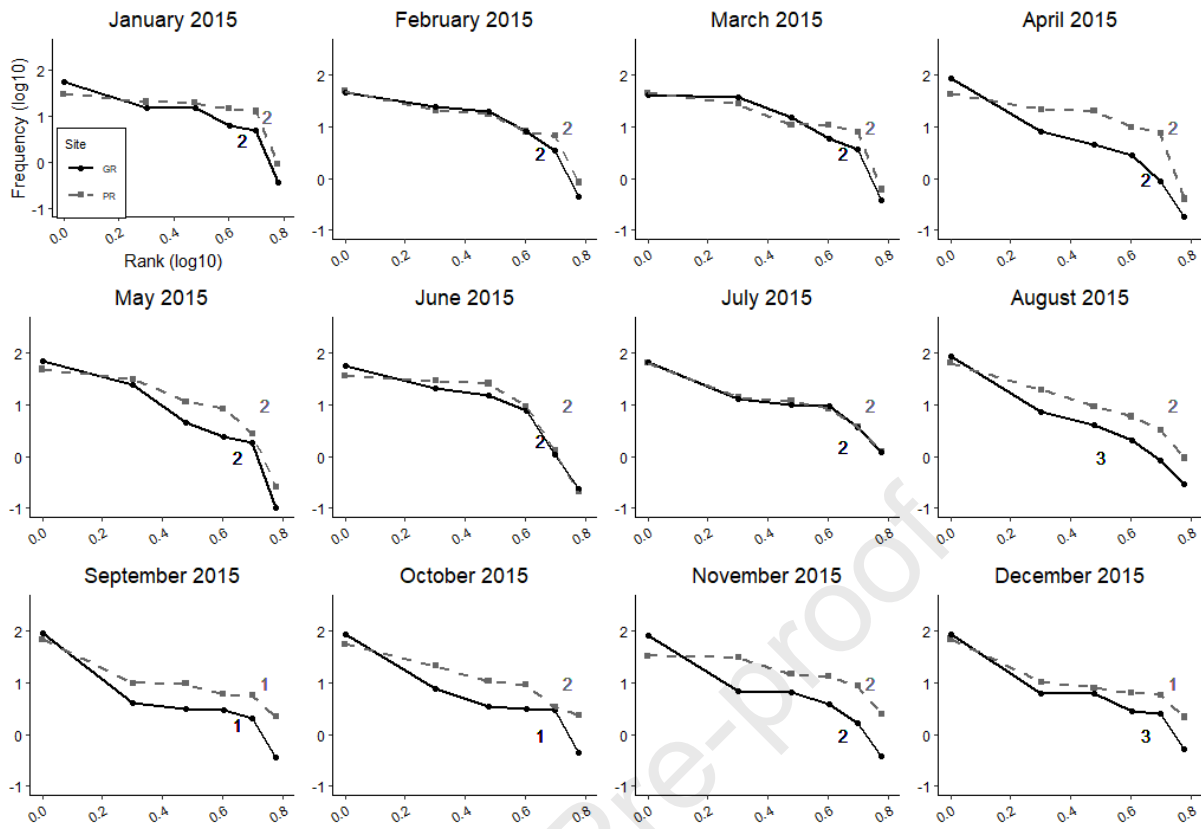
719 grey lines) and the Large Bay (solid black lines) in 2013. Both axes are on a logarithmic scale.



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721 Figure 2: Rank frequency diagrams of pico- and nanoplankton assemblage in the Little Bay (dashed

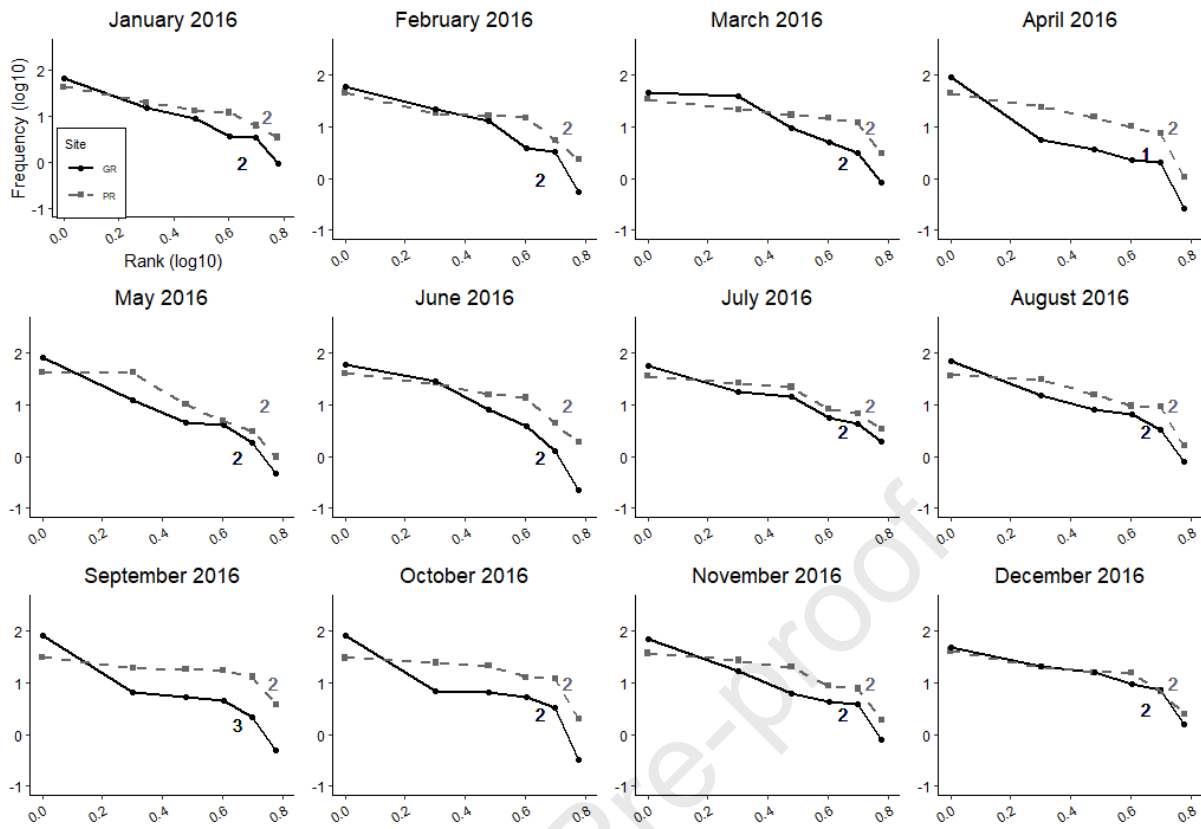
722 grey lines) and the Large Bay (solid black lines) in 2014. Both axes are on a logarithmic scale.



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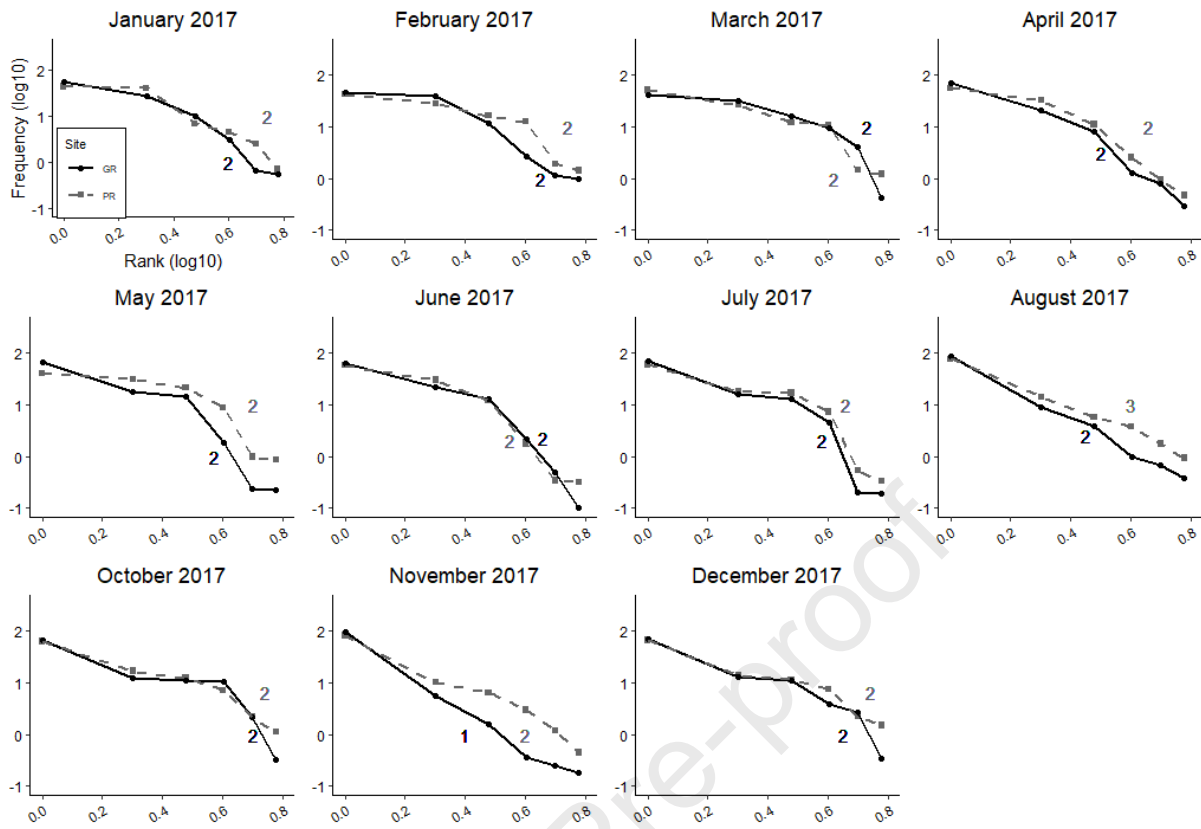
724 Figure 3: Rank frequency diagrams of pico- and nanoplankton assemblage in the Little Bay (dashed

725 grey lines) and the Large Bay (solid black lines) in 2015. Both axes are on a logarithmic scale.



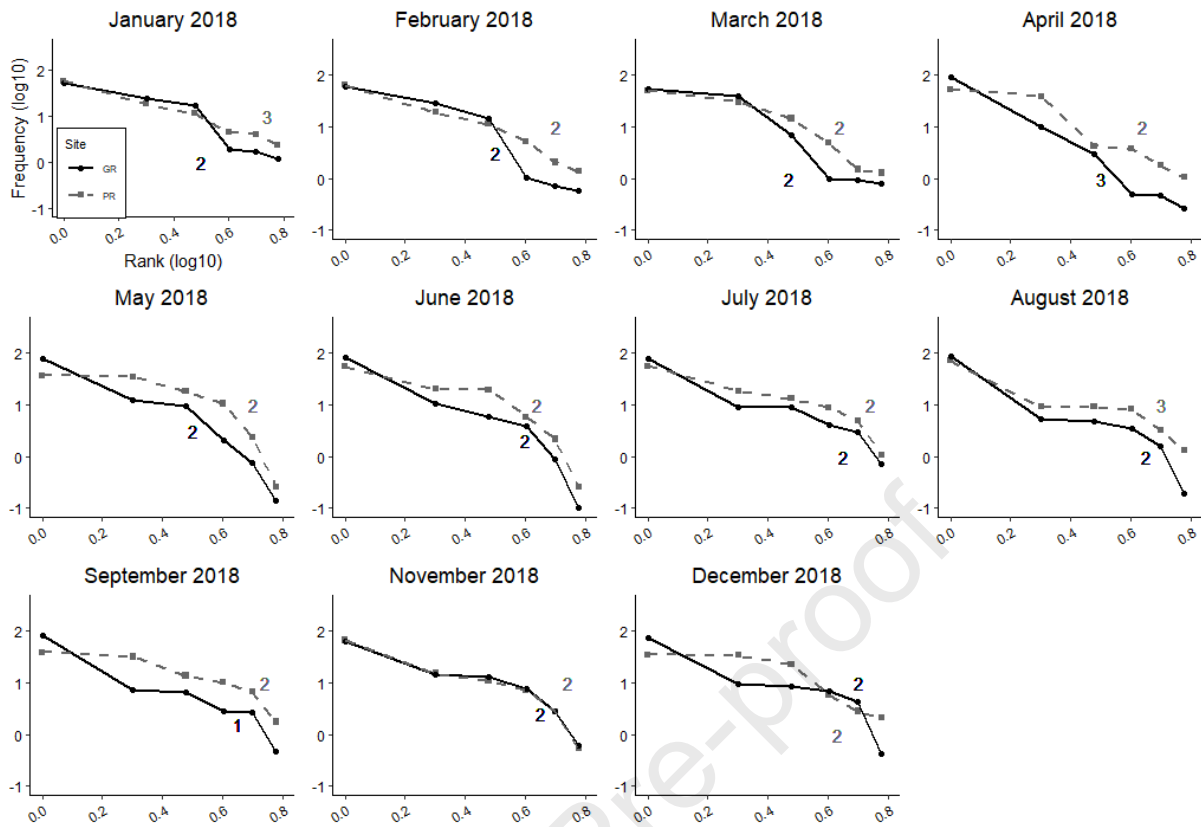
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727 Figure 4: Rank frequency diagrams of pico- and nanoplankton assemblage in the Little Bay (dashed  
 728 grey lines) and the Large Bay (solid black lines) in 2016. Both axes are on a logarithmic scale.



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730 Figure 5: Rank frequency diagrams of pico- and nanoplankton assemblage in the Little Bay (dashed  
 731 grey lines) and the Large Bay (solid black lines) in 2017. Both axes are on a logarithmic scale.

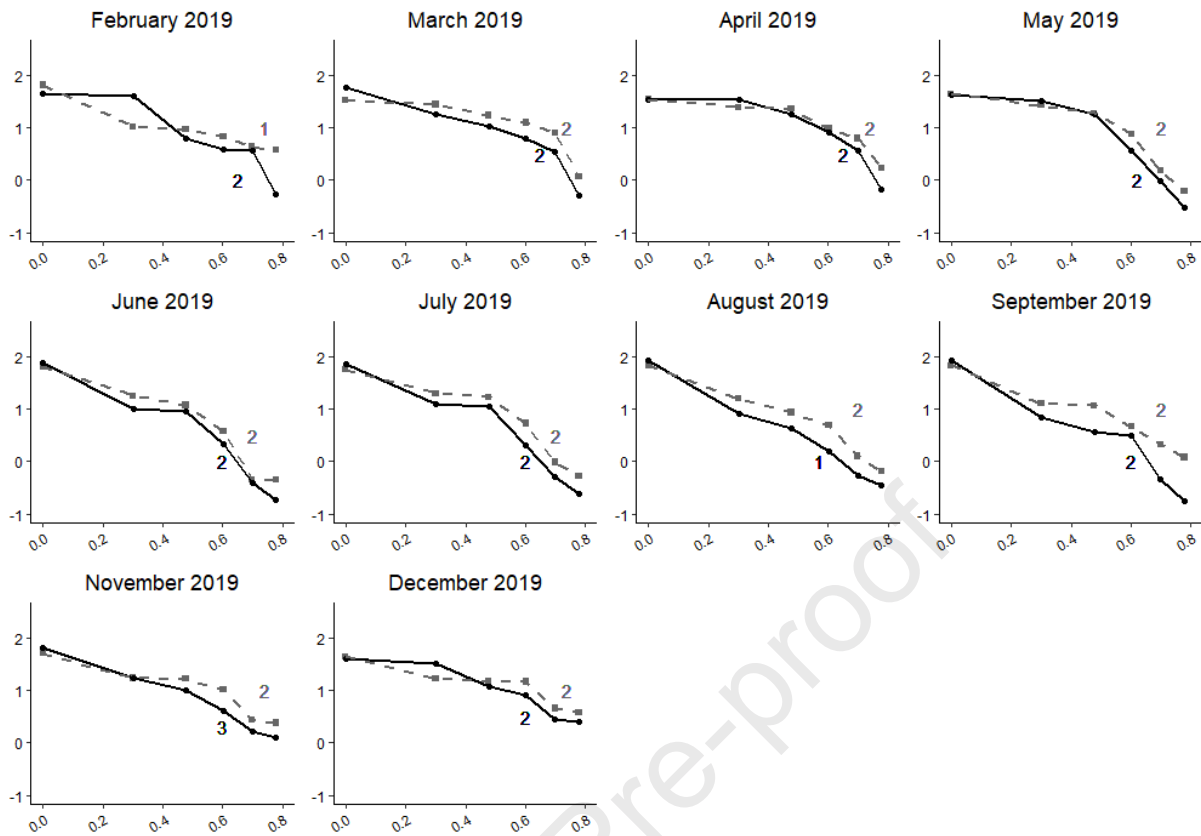


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733 Figure 6: Rank frequency diagrams of pico- and nanoplankton assemblage in the Little Bay (dashed

734 grey lines) and the Large Bay (solid black lines) in 2018. Both axes are on a logarithmic scale.

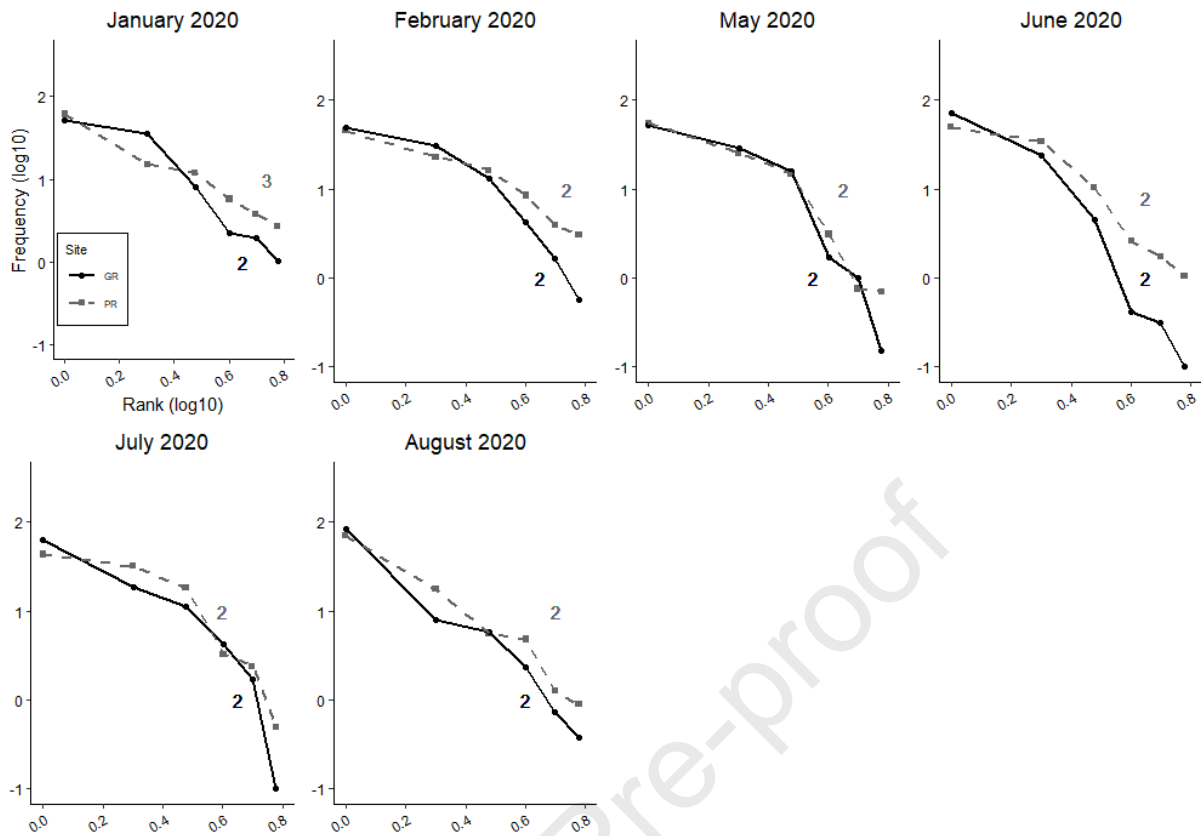




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736 Figure 7: Rank frequency diagrams of pico- and nanoplankton assemblage in the Little Bay (dashed

737 grey lines) and the Large Bay (solid black lines) in 2019. Both axes are on a logarithmic scale.



738

739 Figure 8: Rank frequency diagrams of pico- and nanoplankton assemblage in the Little Bay (dashed  
 740 grey lines) and the Large Bay (solid black lines) in 2020. Both axes are on a logarithmic scale.

741

### Highlights

- Combined use of diversity indices and RFD is relevant to study coastal ecosystems
- Ecosystems were more diversified and mature in the more polluted site
- *Synechococcus* spp. population widely dominated coastal areas from the Bay of Toulon

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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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