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

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1 **Ecological succession of pico- and nanophytoplankton in a coastal bay of NW**

2 **Mediterranean Sea**

3

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14

15 Highlights (3 à 5 bullets points – 85 characters each with spaces):

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

19

20 Abstract (100-150 words):

21 The long-term evolution of pico- and nanophytoplanktonic communities in two coastal  
22 contrasting sites from the bay of Toulon was studied using monthly flow cytometry analyses during a  
23 seven-year period. Results highlighted higher abundance of both pico- and nanophytoplanktonic  
24 communities in the more eutrophic site (*i. e.* the Little Bay). Moreover, even if both sites were widely  
25 dominated by *Synechococcus* spp. population, it represented more than 90 % of the population

26 during summer in the Large Bay. Using diversity indices and rank frequency diagrams to study  
27 ecological successions in both sites, communities from the Little Bay (the most polluted site)  
28 appeared, paradoxically, as more diversified and more mature than that from the Large Bay.  
29 Communities from the Large Bay appeared as less diversified and juvenile linked to the dominance of  
30 *Synechococcus* spp. The complementarity of diversity indices and rank frequency diagram was  
31 revealed in this present work.

32

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

35

#### 36 1. Introduction

37 Pico- (size range < 2  $\mu\text{m}$ ) and nanophytoplankton (size range 2 - 20  $\mu\text{m}$ ) constitute an important  
38 part of the microbial loop by being the base of the trophic web (Chiang et al., 2013) and thus have  
39 been recognized as major contributors to the total primary production in marine ecosystems (Azam  
40 et al., 1983; Platt et al., 1983). Their importance is well established in open oceans, notably for  
41 picophytoplankton, but their role and succession in more coastal waters is not well documented  
42 (Mitbavkar et al., 2012). However, understanding ecosystem structure and functioning is essential to  
43 monitor and predict their evolution. In most cases, community structure and dynamics are shaped by  
44 a wide variety of environmental variables including nutrients availability, salinity or temperature (Bec  
45 et al., 2005; Sommer et al., 1986). Yet coastal waters are subject to nutrient enrichment by  
46 anthropogenic activities and freshwaters discharges (Paerl et al., 2006, 2003). Indeed, freshwater  
47 flow (i) are a main source of nutrients and (ii) cause hydrological perturbations as salinity variation  
48 or water resilience which play a determinant role in nutrients availability (Paerl et al., 2006). These  
49 anthropogenic perturbations and freshwater discharges, typical of coastal waters, may play an  
50 important role in shaping community structure and determine ecological succession in these marine  
51 systems. Indeed, according to the model of ecological succession proposed by Margalef (1951),

52 Reynolds (1984, 1980) or Sommer et al. (1986), succession of species represents a response to  
53 physical, chemical and biological changes. To our knowledge, only few studies focused on ecological  
54 succession in coastal waters and even less on pico- and nanophytoplanktonic succession.

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

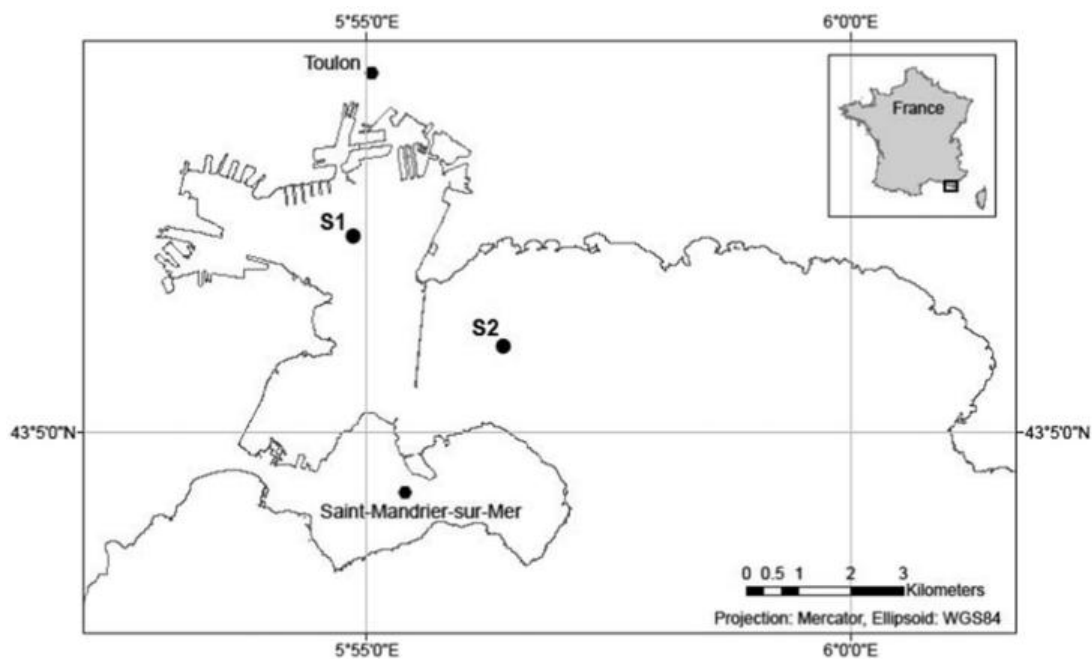
71 Regarding the functional importance of pico- and nanoeukaryotes in food webs and in carbon  
72 fluxes in coastal marine waters, this study aimed at (i) investigating composition and abundance of  
73 pico- and nanophytoplanktonic communities between two contrasting sites from the Bay of Toulon,  
74 and (ii) defining the ecological successions of these sites using both diversity indices and Rank  
75 Frequency Diagram (RFD). This study will allow us to determine if diversity indices and rank  
76 frequency diagrams are relevant tools to assess the anthropogenic impact on coastal communities.

77

78 2. Material and Methods

79 2.1. Sampling

80 Samples were collected monthly between October 2013 and August 2020, in the Bay of Toulon,  
81 at two stations: Little Bay (LiB - S1, 43°06'30" N / 05°55'00" E) and Large Bay (LaB - S2, 43°05'45" N /  
82 05°56'30" E). All samples were collected between 08:00 and 11:00 AM at both 3 and 10 m depth  
83 (Figure 1).



84

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

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

91 To determine pico- and nanophytoplankton communities' abundance, water samples were  
92 collected using a 10L Niskin bottle at 3 and 10 m depth at both sites (LiB and LaB). Samples were pre-  
93 filtered through a 90 µm mesh to remove grazers and other macroparticules. Subsamples of 4.5 mL  
94 were then immediately transferred into 5 mL cryotubes and fixed with a glutaraldehyde solution

95 (final concentration 0.25 %) and a pluronic acid solution (final concentration 0.01 %). These  
96 subsamples were kept at 4°C until analysis and then thawed out just prior analysis by flow cytometry.

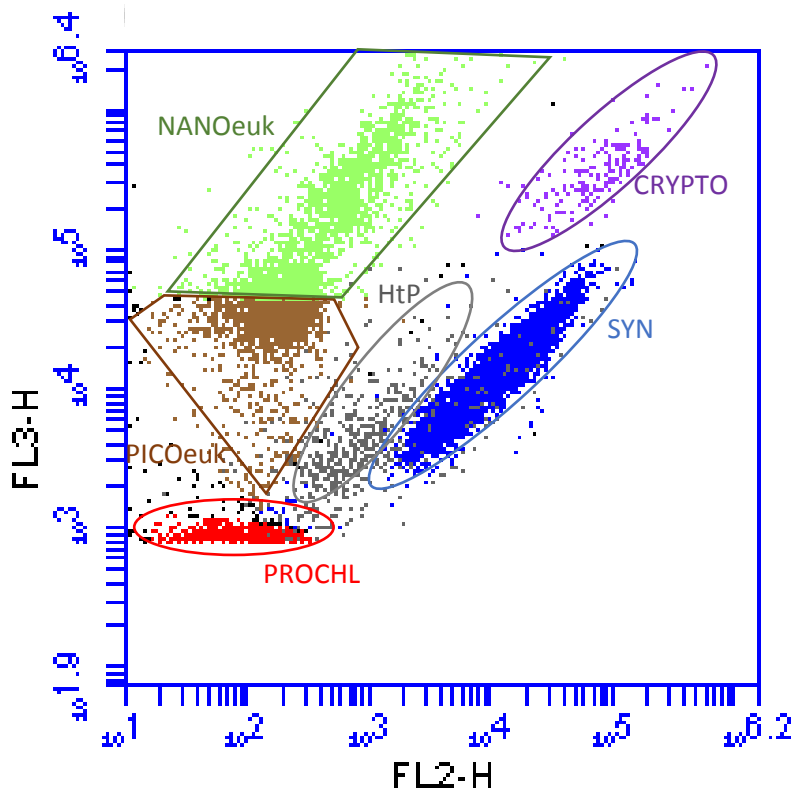
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## 98 2.2. Flow cytometry analysis

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

108 Thanks to their optical properties, six different clusters of pico- and nanophytoplankton were  
109 discriminated (Figure 2): (i) *Synechococcus* spp. (SYN) characterised by small cells (FSC < 12 000), rich  
110 in phycoerythrin (mean FL2 ≈ 14 950) and low in chlorophyll a (mean FL3 ≈ 15 950); (ii)  
111 *Prochlorococcus* spp. (PROCHL) which cells are differentiated from SYN cells by lower fluorescence  
112 (mean FL2 ≈ 117 and mean FL3 ≈ 895); (iii) Picoeukaryotes (PICOeuk) characterized by larger cells  
113 than cyanobacteria (FSC ≈ 45 000) and higher red FL3 fluorescence (Mean FL3 ≈ 29 000); (iv)  
114 Nanoeukaryotes (NANOeuk) are separated from PICOeuk by higher FSC (> 200 000) and higher red  
115 FL3 fluorescence (mean FL3 ≈ 167 592) ; (v) Cryptophyceae (CRYPTO) are discriminated from  
116 NANOeuk thanks to higher orange FL2 fluorescence ; and (vi) Heterotrophic Protist (HtP)  
117 characterized by low red and orange fluorescence (respectively < 2 000 and < 1 000) and high FSC  
118 (> 900 000).

119



120

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

123

124 2.3. Statistical analysis

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

129 A Principal Components Analysis (PCA) was used to assess the relationships between pico-  
 130 and nanophytoplankton abundances and their hydrological environment. The dataset consisted of  
 131 81-month values, from October 2013 to August 2020, described by 36 biological variables  
 132 (abundances, FL2, FL3, FL4, SSC and FSC of SYN, PROCHL, PICOeuk, NANOeuk, CRYPTO, HtP) and 4  
 133 hydrological variables (temperature, salinity, pH, dissolved oxygen concentration). The multifactorial  
 134 treatment was made using R software with FactoMineR package. To determine the number of

135 principal components used in this analysis, a scree plot was used to represent eigenvalues ordered  
136 from the largest to the smallest (Peres-Neto et al., 2005). Only the first two components were used  
137 since more than 64 % of the variances were explained by these components. As a second step,  
138 correlation of Bravais-Pearson was used to assess the relation between abiotic factors and  
139 abundances of the communities.

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

$$146 \quad H' = -\sum_{i=1}^S p_i \log_2 p_i$$

147 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  
148 individuals from the  $i$  class and  $N$  the total abundance. The minimum value of the index is 0 when all  
149 the observations belong to a single class. The maximum value equals  $\ln(1/S)$  and it can be reached  
150 when the observations are equally divided between all the classes.

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

$$152 \quad J' = \frac{H'}{H'_{max}} = \frac{H'}{\log_2(S)}$$

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

156 Succession of pico- and nanophytoplanktonic communities were assessed using Rank Frequency  
157 Diagrams (RFD, Frontiers 1976). Status of the community were empirically defined according to the  
158 trend of the RFD curves; various models of species distributions have been described in the past as  
159 Broken Stick model (MacArthur, 1957), lognormal (Preston, 1948), geometric (Motomura, 1932) or  
160 even Zipf-Mandelbrot (Frontier, 1985). No fitting to any model is intended here since RFD are only

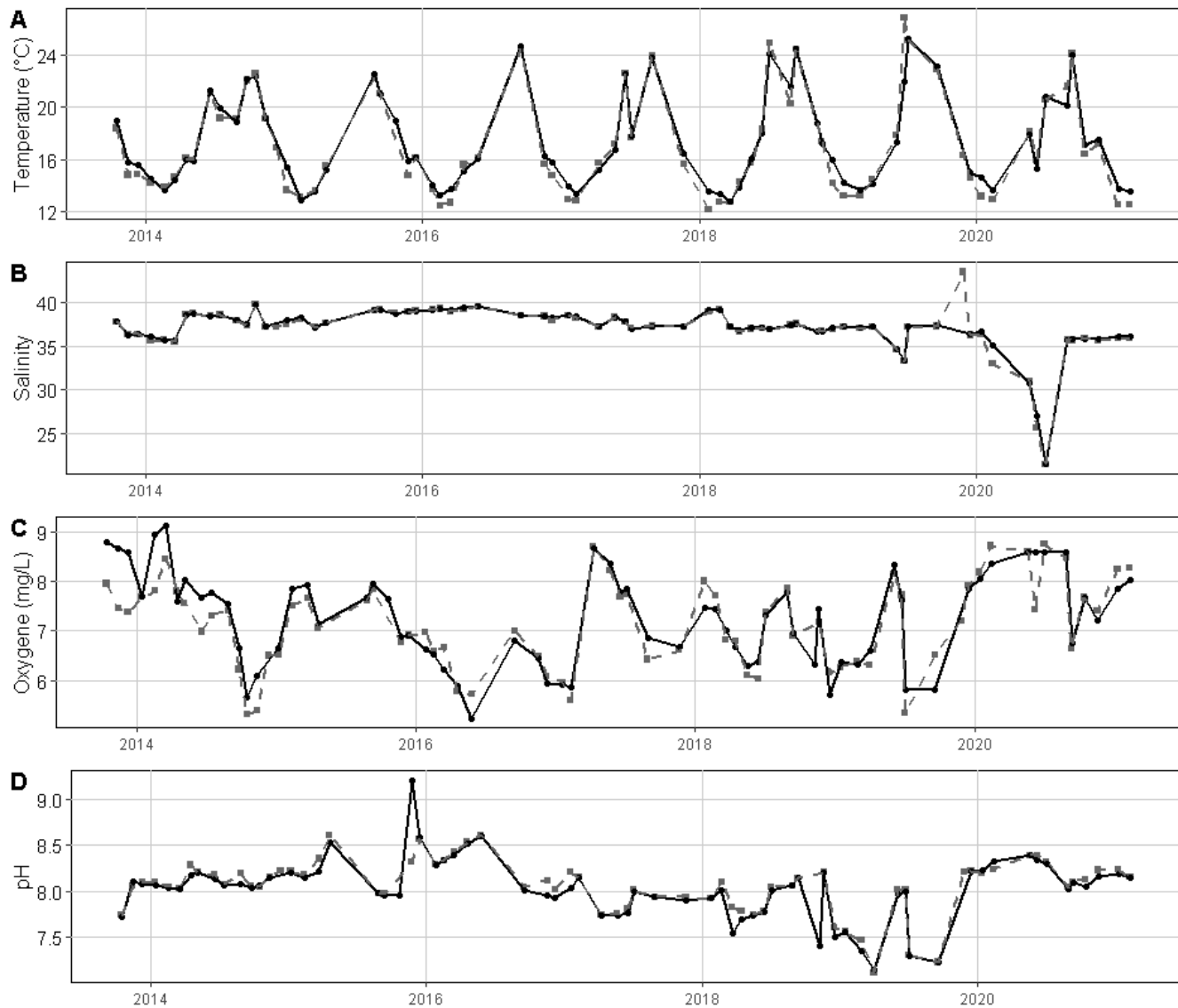


161 used to describe ecological succession, following Frontier's description for planktonic ecosystem  
162 (Frontier, 1985, 1983, 1976). Depending on the shape of the curve, three main stages have been  
163 described. In stage 1, beginning of the succession, the curve is concave at the top left and become  
164 convex on the right part, typical of a young community with low diversity structure. In stage 2, the  
165 curve is entirely convex and the diversity at its maximum. In stage 3, end of the succession, the curve  
166 becomes straight and diversity is intermediate.-The determination of the stage was done using the  
167 linear trend curve in excel: when the determination coefficients were higher than 0.82 and 0.87 for  
168 LaB and LiB respectively, the curve was determined as convex; for lower determination coefficients,  
169 curve was determined as concave or straight.

170

### 171 3. Results

#### 172 3.1. Temporal variability of abiotic variables, pico- and nanophytoplanktonic abundances



173

174

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

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

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

178

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

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

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

182 Seasonal variations of temperature were observed in LiB and LaB (Figure 3A): maximal values  
 183 ( $> 20^{\circ}\text{C}$ ) were recorded during summers and minimal ones during the winter period ( $< 15^{\circ}\text{C}$ ).  
 184 Maximal temperature was reached in June 2019, in LiB.  
 185 No variation of salinity was observed along the study period (LiB:  $37.07 \pm 2.89$ ; LaB:  $37.11 \pm 2.70$ )  
 186 excepted in July 2020, when salinity reached respectively 21.53 and 21.54 for LiB and LaB (Figure 3B).  
 187 Dissolved oxygen concentrations varied from  $5.23 \text{ mg.L}^{-1}$  (October 2014) and  $9.11 \text{ mg.L}^{-1}$  (March  
 188 2014) without following a seasonal trend (Figure 3C).  
 189 pH values were stable across the study period (LiB:  $8.12 \pm 0.36$ ; LaB:  $8.09 \pm 0.40$ ) and varied between  
 190 7.11 (April 2019 – LiB) and 9.22 (July 2015 – LaB – Figure 3D).

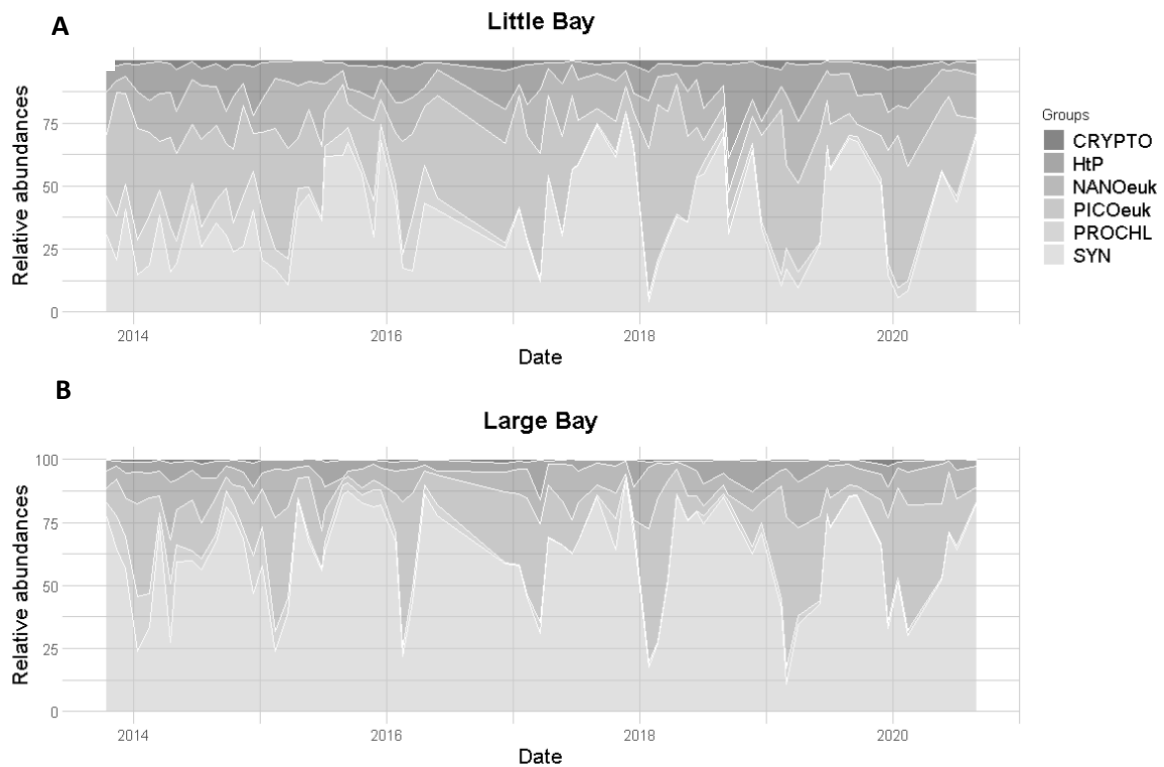
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192 Table 1 : Mean annual abundances of each communities ( $\text{cells.mL}^{-1}$ ) in both sites (LiB and LaB)

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

193

194



195

196

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

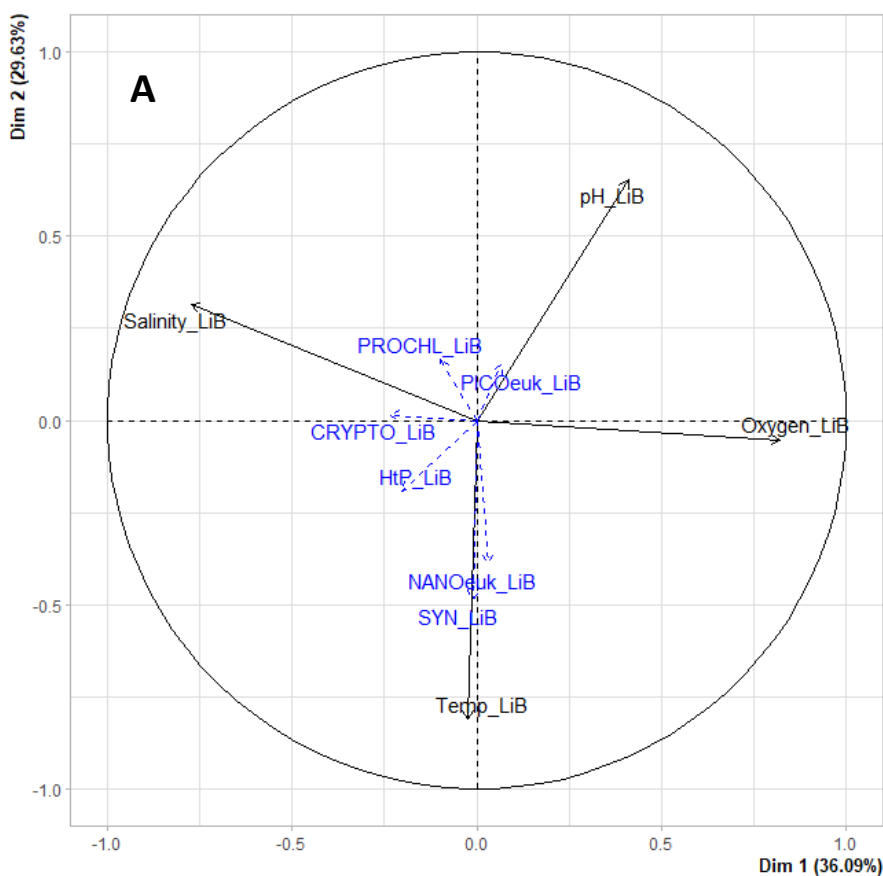
199 The mean annual abundances of the communities identified by flow cytometry are presented  
 200 in the Table 1; the composition of the communities is represented in the Figure 4. Total abundances  
 201 of the 6 communities were significantly different between the 2 studied site (Kruskal-Wallis,  $p < 0.05$ )  
 202 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  
 203 largely dominated by SYN: in average, SYN represents respectively 38 % and 61 % of total  
 204 abundances in LiB and LaB.

205 Strong variations of SYN abundances was observed between October 2013 and February  
 206 2015 in LiB, not linked with seasonality: abundances peaked between 35 and 40 % in December  
 207 2013, March, June, August and December 2014. Apart from that, abundance was between 10 and  
 208 26 %. PICOeuk was especially abundant during this period and varied between 17 % in September  
 209 2014 and 48 % in November 2013. PROCHL and HtP abundances varied between 9 to 19 % and 6 to

210 12 % respectively. NANO represented less than 20 % of the community. CRYPTO abundance varied  
 211 between 0.3 and 3 %. In 2015, SYN proportion increased and reached 67 % in September 2015; at the  
 212 same date, PICOeuk abundance substantially decreased to 9 %. From 2016, seasonal variation of SYN  
 213 abundances was observed: its proportion reached up to 80 % during spring and decreased to less  
 214 than 15 % in winter (4 % in January 2018). In the meantime, proportion of PICOeuk was minimal  
 215 during summer (5 % in August 2017) and maximal during winter (62 % in February 2018). PROCHL,  
 216 HtP, NANO and CRYPTO represented respectively 3, 10, 14 % and less than 3 % of the community.

217 Communities from LaB were largely dominated by SYN which represented in average 61 % of  
 218 the communities. Seasonal variations of its proportion were observed; it is minimal during winter  
 219 (25 % in February 2015) and maximal during summer (92 % in September 2017). PICOeuk was the  
 220 second more abundant group and represents 7 to 50 % of the community. NANO, PROCHL, CRYPTO  
 221 and HtP represents less than 15 % of the total communities.

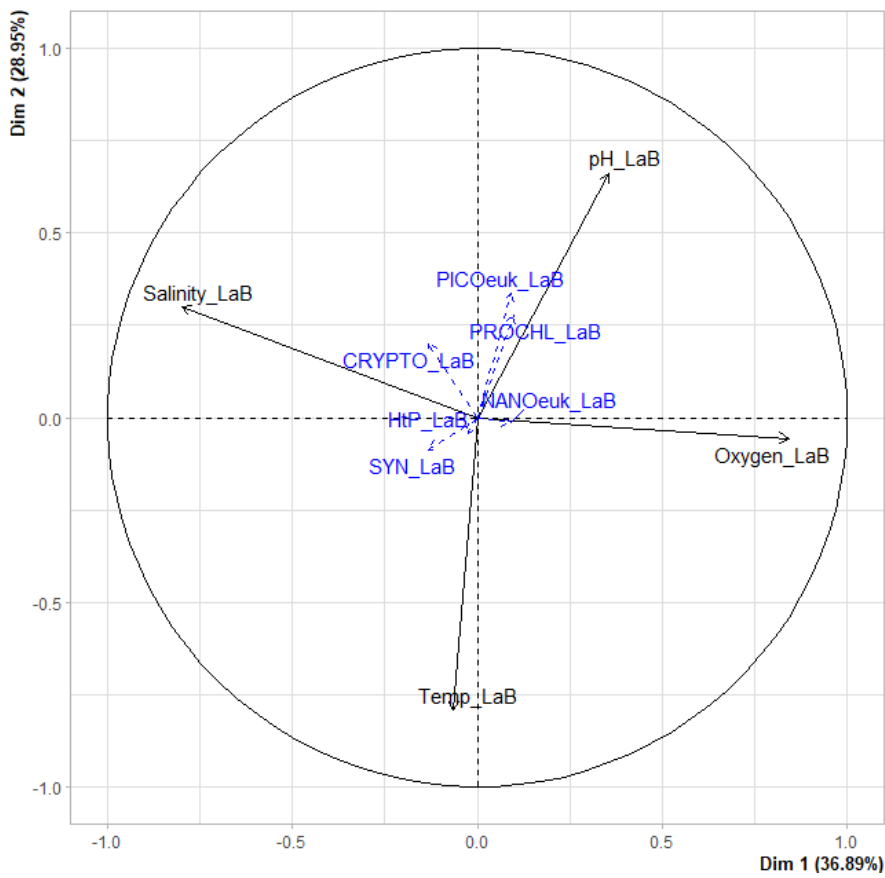
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223

**B**

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



224

225

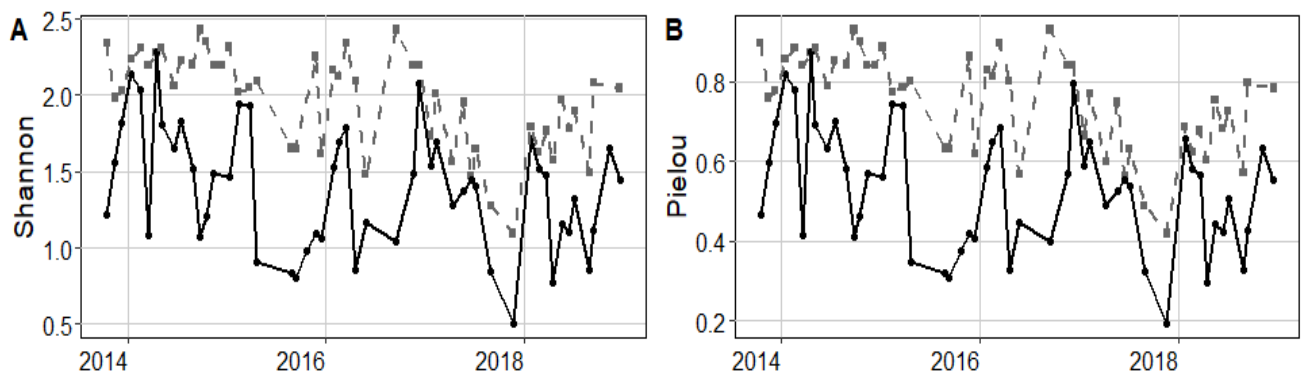
226 The results of the PCA variables representing the 7 years sampling years are shown in Figure 5. The  
227 first two axes accounted for 36 % and 29 % of the total variance for both LiB and LaB, demonstrating  
228 that, the major factor affecting our data was temperature. But, most likely due to photosynthesis,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

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

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

236

### 237 3.2. Diversity indices



238

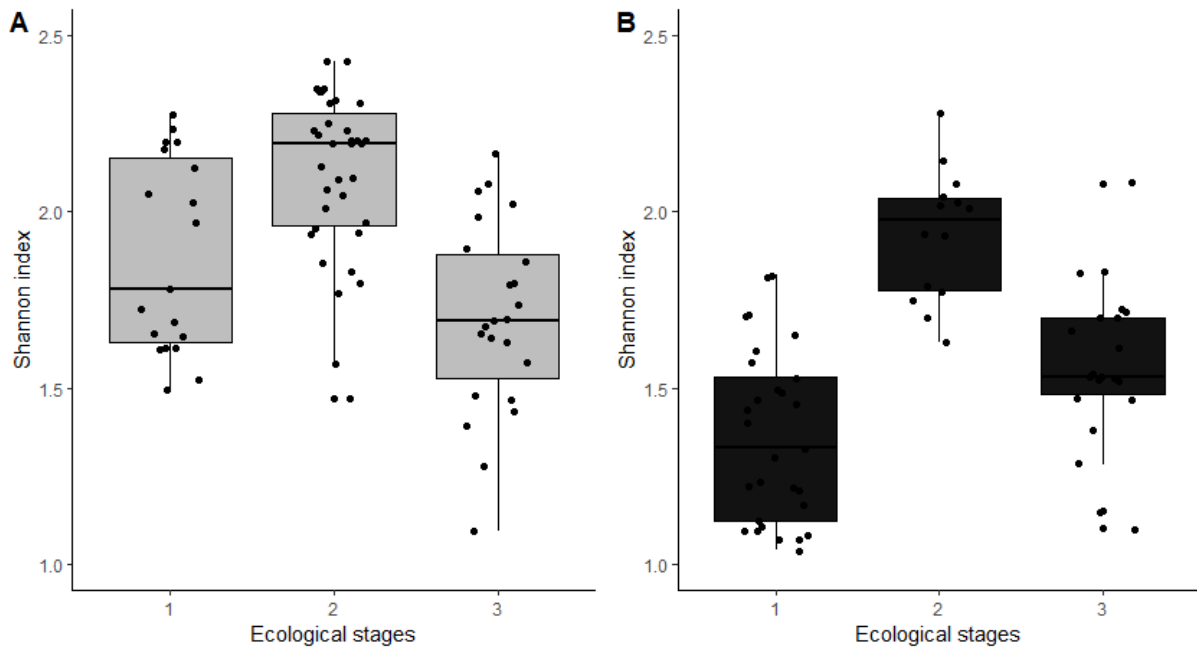
239 Figure 6: Evolution of plankton indices along the study period: Shannon (A) and Piélou indices (B) for  
240 LiB (dashed grey lines) and LaB (solid black lines).

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

245 Equitability index or Piélou index has the same trend as Shannon index since its calculation  
246 derives from Shannon's one.







260

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

263

264 Shannon indices varied with ecological stages. In LiB, Shannon index varied between 1.50 and  
 265 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,  
 266 Shannon indices was between 0.81 and 1.82 for stage 1. They increased at stage 2 to reach the  
 267 minimum of 1.63 and the maximal value of 2.28. At stage 3, this index decreased and was between  
 268 0.51 and 2.08 (Figure 8).

269

270 4. Discussion

271 Because of its topography and anthropogenic pressure this site is currently facing, the Bay of  
 272 Toulon is relevant to explore annual variability of zooplankton (Jamet et al., 2001; Richard and Jamet,  
 273 2001) and microphytoplankton (Bogé et al., 2006; Jamet et al., 2005; Rossi and Jamet, 2009). A  
 274 previous study (Delpy et al., 2018) has investigated pico and nanoplanktonic dynamics during a two  
 275 year cycle (October 2013 to December 2015). By using a longer time series (2013-2020), our study

276 explores the evolution of pico- and nanophytoplanktonic communities to determine ecological  
277 successions in the two contrasting sites of the Bay of Toulon.

278

#### 279 4.1. Variations of abiotic variables and pico- and nanophytoplanktonic abundances

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

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

288 Pico- and nanophytoplankton abundances are significantly higher in LiB than in LaB, as previously  
289 described by Delpy et al. (2018). It reflected the trends observed for other higher planktonic species  
290 such as mesozooplankton or microphytoplankton (Jamet et al., 2001; Serranito et al., 2016). Such  
291 differences of abundances between sites were attributed to eutrophic conditions characterising LiB  
292 (Rossi and Jamet, 2009). Indeed, mean chlorophyll a concentration in the Mediterranean Sea ranges  
293 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  $\mu\text{g}\cdot\text{L}^{-1}$  -  
294 Jamet et al., 2001) and characterize eutrophic waters according to Jacques and Tréguer (1986) and  
295 Psyllidou-Giouranovits et al. (1997).

296 Seasonal variations of abundances were observed in both sites: abundances of SYN  
297 significantly increased during late spring/summer with maximum abundance of 127 300 cells.mL<sup>-1</sup> (in  
298 2017, at LaB), close to maxima observed for Mediterranean coastal systems (Agawin and Agusti,  
299 1997; Cerino et al., 2012; Ferrier-Pages and Rassoulzadegan, 1994); in winter, PROCHLO became  
300 dominant and represented 50 % of the total communities (34 648 cells.mL<sup>-1</sup>), close to values  
301 observed by Grégori et al. (2001) in Marseille. Such seasonality in pico- and nanophytoplanktonic

302 communities was in agreement with previous observations in the Gulf of Lion (Lefevre et al., 1997)  
303 and in Marseille (Grégori et al., 2001).

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

309           Moreover, no clear relation was observed for the other communities with abiotic variables  
310 presented here. Previous studies have demonstrated that abundance of these different groups is  
311 driven by nutrient availability and species growth rate. Indeed, LiB is considered as more eutrophic  
312 than LaB due to higher Chlorophyll a and nutrient concentrations (Rossi et al., 2008; Rossi and Jamet,  
313 2009). LaB exhibited lower  $P-PO_4^{3-}$  concentrations due to (i) a more regularly mixing of waters and  
314 (ii) the predominance of SYN which can contribute up to 35 % of the total Pi uptake (Delpy et al.,  
315 2018). It is important to note that biotic interaction such as grazing pressure or competition for  
316 nutrient and space by larger phytoplankton could also play an important role in shaping community  
317 structure and need to be clarified.

318

#### 319           4.2. Composition of pico- and nanophytoplanktonic community

320           Even if SYN was dominant in both LiB and LaB, this group was largely more abundant in LaB as  
321 previously described by Delpy et al. (2018). It represented between 25 to 92 % of total communities  
322 in LaB, although communities of the LiB were represented by 2 major codominant groups *i. e.* SYN  
323 (representing between 15 to 75 % of the total communities) and PICOeuk (5 to 62 % of the  
324 community). The dominance of SYN in terms of abundance and contribution to primary productivity,  
325 has now been widely recognised (Johnson et al., 1982; Morris and Glover, 1981). Because of their  
326 small size and higher surface to volume ratios, SYN are more competitive than other to acquire  
327 nutrients and explained their dominance in both sites (Bec et al., 2011). The ability of SYN to acquire

328 soluble reactive phosphorus (notably in P-limited zones) provides an ecological advantage over other  
329 phytoplankton classes (Collos et al., 2009). Differences of abundances between sites could be  
330 explained by higher trace metals' concentration in LiB (Rossi and Jamet, 2008). Indeed, a strong  
331 inhibition of SYN growth rate was observed when exposed to Copper ( $EC_{50} = 4 \mu\text{g}\cdot\text{L}^{-1}$  - Debelius et al.,  
332 2011, 2009). Moreover, pico- and nanophytoplanktonic communities collected in LaB exposed to  
333 trace metal cocktail, revealed a shift from SYN dominated community (typical of LaB) to a less SYN  
334 dominated community (with higher proportion of picoeukaryotes) as observed in LiB (Coclet et al.,  
335 2018). Codominance of PICOeuk and SYN (as observed in LiB) has been previously observed in other  
336 coastal waters (Changjiang Estuary - Pan et al., 2007), where PICOeuk can even surpass  
337 cyanobacteria in terms of biomass. Other groups (NANOeuk, HtP) represented less than 20 % of total  
338 communities. Differences between both cyanobacteria biomass (*i. e.* PROCHL and SYN) was  
339 consistent with previous study since PROCHL abundance vary inversely with SYN ones (Partensky et  
340 al., 1996) and is predominantly abundant in oligotrophic waters (Pan et al., 2007; Partensky et al.,  
341 1999).

342 In previous studies (Agawin et al., 1998; Delpy et al., 2018; Moisan et al., 2010), successions of  
343 pico- and nanophytoplanktonic communities appeared mainly driven by the ability to acquire  
344 nutrients and species growth rate. However, communities of the Bay of Toulon showed SYN  
345 dominated the communities, suggesting bottom up processes as key variables in the structuring of  
346 communities. Indeed, the dominance of SYN during the spring/summer period in LaB explained the  
347 seasonal variations of Shannon (and Piélou) index with a clear decrease of this index during summer;  
348 in winter, diversity increased again, notably linked to the fall in SYN proportion. In LiB, no clear  
349 seasonal variation of Shannon index was observed. However, diversity was significantly higher in LiB  
350 than LaB and thus, confirmed by RFD analysis (with more month described as in LiB than in LaB),  
351 contrary to the previous results obtained at the same site for zooplankton and phytoplankton  
352 suggesting that diversity was higher in LaB (Jamet et al., 2005; Rossi and Jamet, 2009; Serranito et al.,  
353 2019). Such contradiction could be induced by the dominance of SYN in the Bay of Toulon: in LaB,

354 SYN largely drive the community's dynamic; in LiB, SYN was still very abundant but due to high trace  
355 metals concentration, it became less competitive than in LaB, and other groups, as PICOeuk, became  
356 more abundant. Moreover, in LiB, the proportion of HtP is higher than in LaB and could also control  
357 SYN population by grazing pressure (Christaki et al., 1999; Dolan and Šimek, 1999; Guillou et al.,  
358 2001).

359 Interestingly, according to Frontier (1985, 1983), a chronic pollution induces a recover to a stage  
360 1 (typical of a juvenile population) since few species are able to maintain themselves in such  
361 conditions of stress. However, our results indicated more stage 1 in LaB than LiB, and even, more  
362 evolution to a complex ecosystem typical of a stage 2 in LiB than in LaB. The LaB is characterized by  
363 stage 1 corresponding to a little evolution of the population, whose structure and organisation are  
364 deeply affected by disturbances, here the domination of SYN population linked to its ability to better  
365 acquire nutrients. Paradoxically, populations from LiB naturally evolved towards more mature and  
366 diversified ecosystems. This is explained by anthropogenic activities increasing trace metals'  
367 concentrations limiting SYN domination in LiB, by (i) decreasing SYN growth rate (Debelius et al.,  
368 2011, 2009) and (ii) increasing grazing pressure by ciliates and other heterotrophic protists (Christaki  
369 et al., 1999; Dolan and Šimek, 1999; Guillou et al., 2001).

370

#### 371 4.3. The combine use of RFD and diversity index to monitor pico- and nanophytoplanktonic 372 assemblage

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

380 Another method used to determine ecological succession of an ecosystem is to build Rank  
381 Frequency Diagram, establishing “the numerical repartition of individuals by species within a  
382 biocenosis” (Frontier, 1976). Considered as a fuller description of biological communities than  
383 diversity indices (although not independent from them), it introduces maturity degrees of the  
384 populations divided in 3 stages. First, stage 1, the beginning of the succession, is dominated by high  
385 ranked taxa. The community is composed by one or a few small species with predominantly *r*  
386 selected species described as invasive, fast growing and prolific (Reynolds, 1997). In stage 2, the  
387 abundance of rare species continues to decrease and common species become more numerous. This  
388 community is characterized by *K* selected species, with bigger size, lower growth rate, favoured by  
389 physiological capabilities allowing their survival towards the supportive capacity of the resource  
390 available (Reynolds, 2006). According to Khemakhem et al. (2010), this favoured retroactive loops  
391 within the trophic network, allowing a more stable ecosystem where the diversity is maximal. And, in  
392 stage 3, the end of the succession, the numbers of the most abundant species increase whereas the  
393 diversity decreases, characterising the maturity of the community. This tool, notably used in other  
394 field of application as language study (Mandelbrot, 1953), or socio-economy (Petruszewicz, 1972),  
395 were widely used fifty years ago in ecology notably by Margalef (1957) and Frontier (1985, 1983,  
396 1976). According to these authors, ecosystems have a structure in terms that they are composed by  
397 different species arranged in a define pattern according to the availability of resources. Stages are  
398 described following different fitting models: broken stick model (MacArthur, 1957), general  
399 lognormal (Preston, 1948), geometric (Motomura, 1947) or Zipf-Mandelbroat (Mandelbrot, 1977).  
400 This method wasn’t used much during these past decades, notably linked to the difficulty in  
401 interpreting results. However, the relevance of this method has been well documented in both  
402 marine (Davoult et al., 1993; Fromentin et al., 1997; Frontier, 1985; Jamet et al., 2005; Sanvicente-  
403 Añorve et al., 2002) and terrestrial ecosystems (Kevan et al., 1997; Vahid et al., 2013; Watkins and  
404 Wilson, 1994; Wilson et al., 1998, 1996; Wilson and Gitay, 1995).

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

421 Our study combines quantitative information from diversity indices and qualitative information  
422 given by RFD to study two contrasted sites from Toulon. The strong correlations between Shannon  
423 indices and ecological stages highlighted the relevance to use both methods in the analysis of  
424 ecosystems structure and ecological stages of pico- and nanophytoplankton in the NW  
425 Mediterranean Sea. Previous studies (Dugenne et al., 2014; Fuchs et al., 2021; Thyssen et al., 2014,  
426 2008) showed that pico- and nanophytoplanktonic communities react rapidly to climatic conditions  
427 variations and wind-driven events. By dint of their short turnover time, pico- and  
428 nanophytoplanktonic communities could be used as a pertinent biological indicator to conduct future  
429 ecosystem monitoring. This study's combined use of RFD and diversity indices to determine the  
430 ecological successions stages in marine coastal ecosystems provides a better understanding of their

431 functioning. Our contribution through regional databases, French national observation networks  
432 (RESOMAR - Réseau des Stations et Observatoires Marins - IR ILICO) and at European scale (MSFD -  
433 Marine Strategy Framework Directive - European Commission - MSFD, 2008/56/EC) grants more  
434 tools for reflexion on management and conservation of marine coastal ecosystems to decision  
435 makers and public politics administrators.

436

## 437 5. Conclusion

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

452

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694 Supplementary data

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

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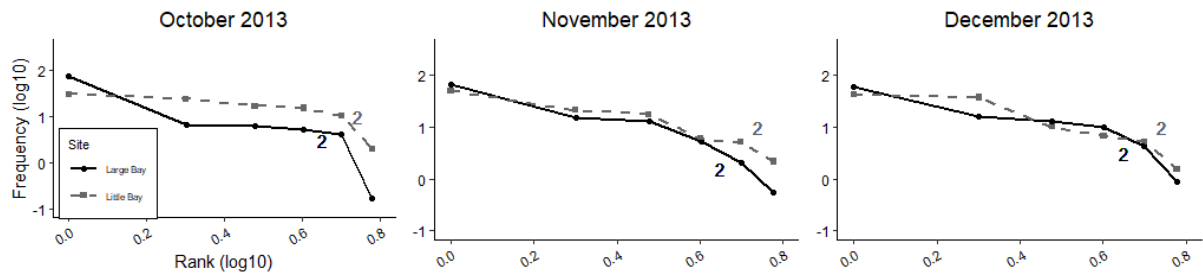
| Date    | LiB   |                | LaB   |                |
|---------|-------|----------------|-------|----------------|
|         | Stage | r <sup>2</sup> | Stage | r <sup>2</sup> |
| oct-13  | 2     | 0.97           | 1     | 0.49           |
| nov-13  | 3     | 0.8            | 1     | 0.65   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

| Date    | LiB   |                | LaB   |                |
|---------|-------|----------------|-------|----------------|
|         | Stage | r <sup>2</sup> | Stage | r <sup>2</sup> |
| juin-17 | 2     | 0.91           | 1     | 0.8            |
| juil-17 | 1     | 0.74           | 1     | 0.66           |
| août-17 | 3     | 0.57           | 3     | 0.51           |
| oct-17  | 3     | 0.68           | 1     | 0.62           |
| nov-17  | 3     | 0.54           | 3     | 0.47           |
| déc-17  | 1     | 0.62           | 3     | 0.6            |
| janv-18 | 3     | 0.69           | 3     | 0.81           |
| févr-18 | 3     | 0.69           | 3     | 0.79           |
| mars-18 | 2     | 0.87           | 3     | 0.78           |
| avr-18  | 3     | 0.76           | 3     | 0.52           |
| mai-18  | 2     | 0.95           | 3     | 0.58           |
| juin-18 | 1     | 0.81           | 1     | 0.54           |
| juil-18 | 3     | 0.73           | 1     | 0.54           |
| août-18 | 1     | 0.54           | 1     | 0.49           |
| sept-18 | 3     | 0.73           | 1     | 0.5            |
| nov-18  | 1     | 0.65           | 1     | 0.65           |
| déc-18  | 2     | 0.9            | 1     | 0.55           |
| févr-19 | 3     | 0.54           | 3     | 0.77           |
| mars-19 | 2     | 0.98           | 3     | 0.7            |
| avr-19  | 2     | 0.96           | 2     | 0.93           |
| mai-19  | 2     | 0.93           | 2     | 0.91           |
| juin-19 | 3     | 0.69           | 1     | 0.56           |
| juil-19 | 1     | 0.78           | 1     | 0.6            |
| août-19 | 3     | 0.63           | 3     | 0.51           |
| sept-19 | 1     | 0.78           | 1     | 0.5            |
| nov-19  | 1     | 0.75           | 3     | 0.65           |
| déc-19  | 1     | 0.75           | 2     | 0.87           |
| janv-20 | 3     | 0.63           | 3     | 0.79           |
| févr-20 | 3     | 0.85           | 2     | 0.87           |
| mai-20  | 3     | 0.81           | 2     | 0.87           |
| juin-20 | 3     | 0.82           | 3     | 0.66           |
| juil-20 | 2     | 0.91           | 1     | 0.7            |
| août-20 | 3     | 0.62           | 1     | 0.52           |

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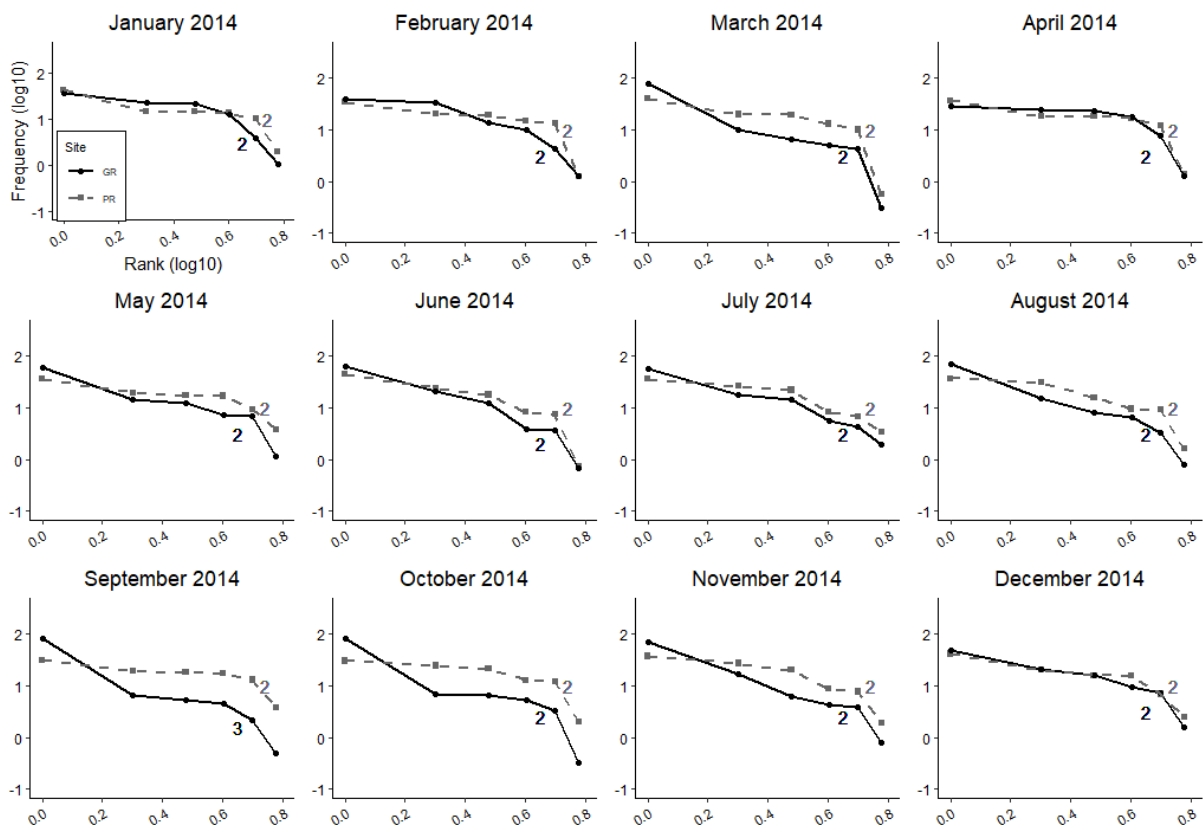
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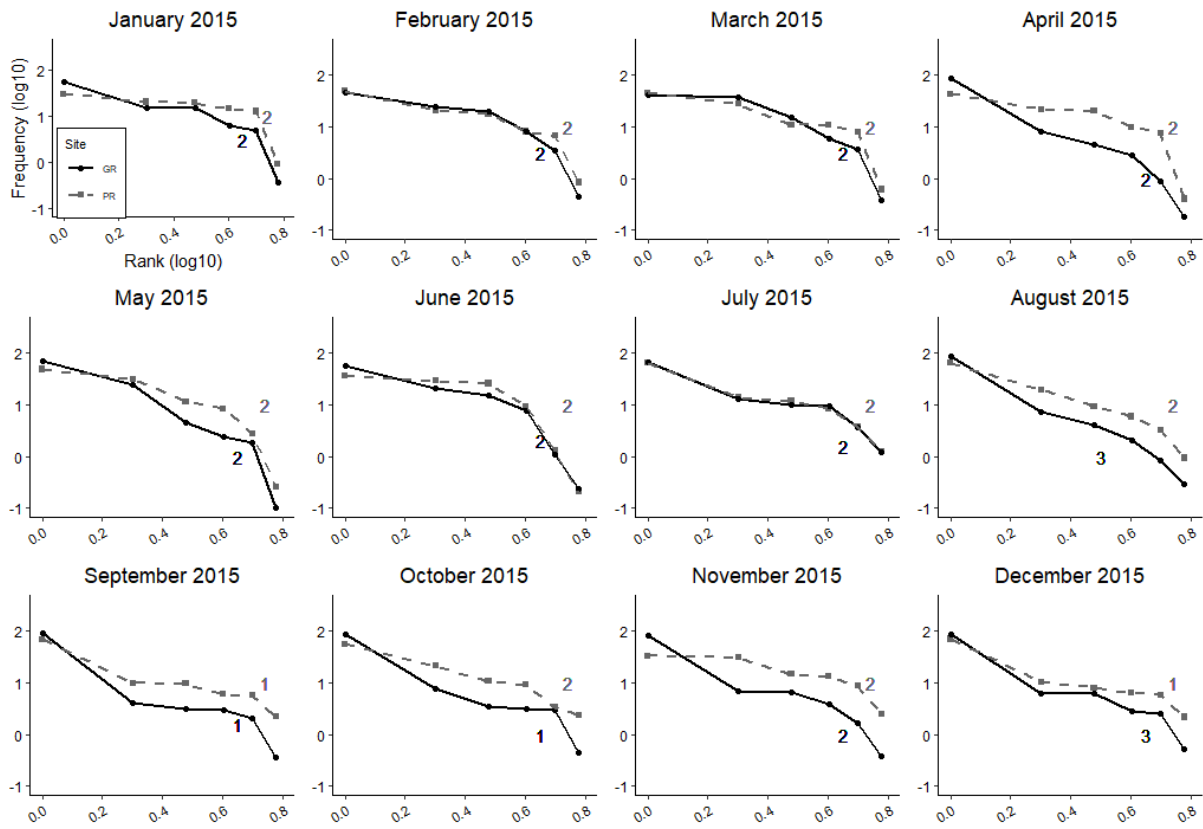
Figure 1: Rank frequency diagrams of pico- and nanoplankton assemblage in the Little Bay (dashed grey lines) and the Large Bay (solid black lines) in 2013. Both axes are on a logarithmic scale.

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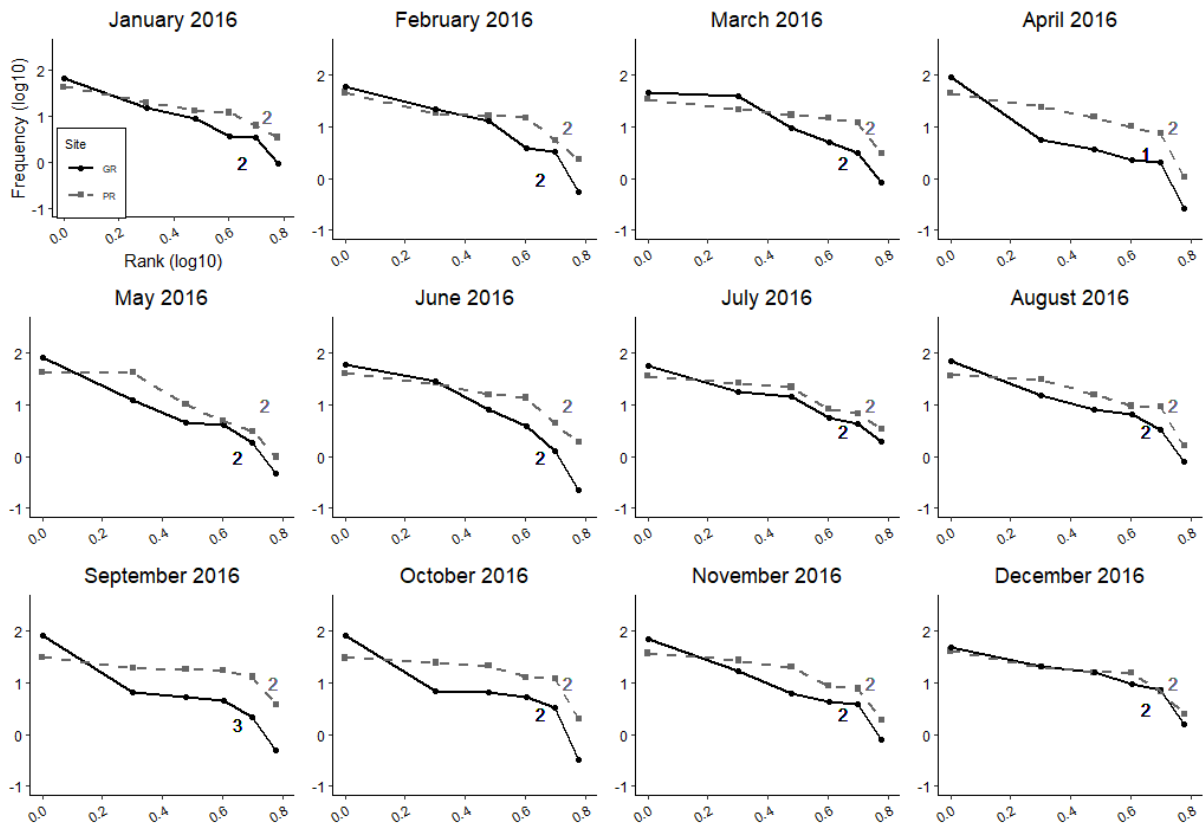
709 Figure 2: Rank frequency diagrams of pico- and nanoplankton assemblage in the Little Bay (dashed  
710 grey lines) and the Large Bay (solid black lines) in 2014. Both axes are on a logarithmic scale.



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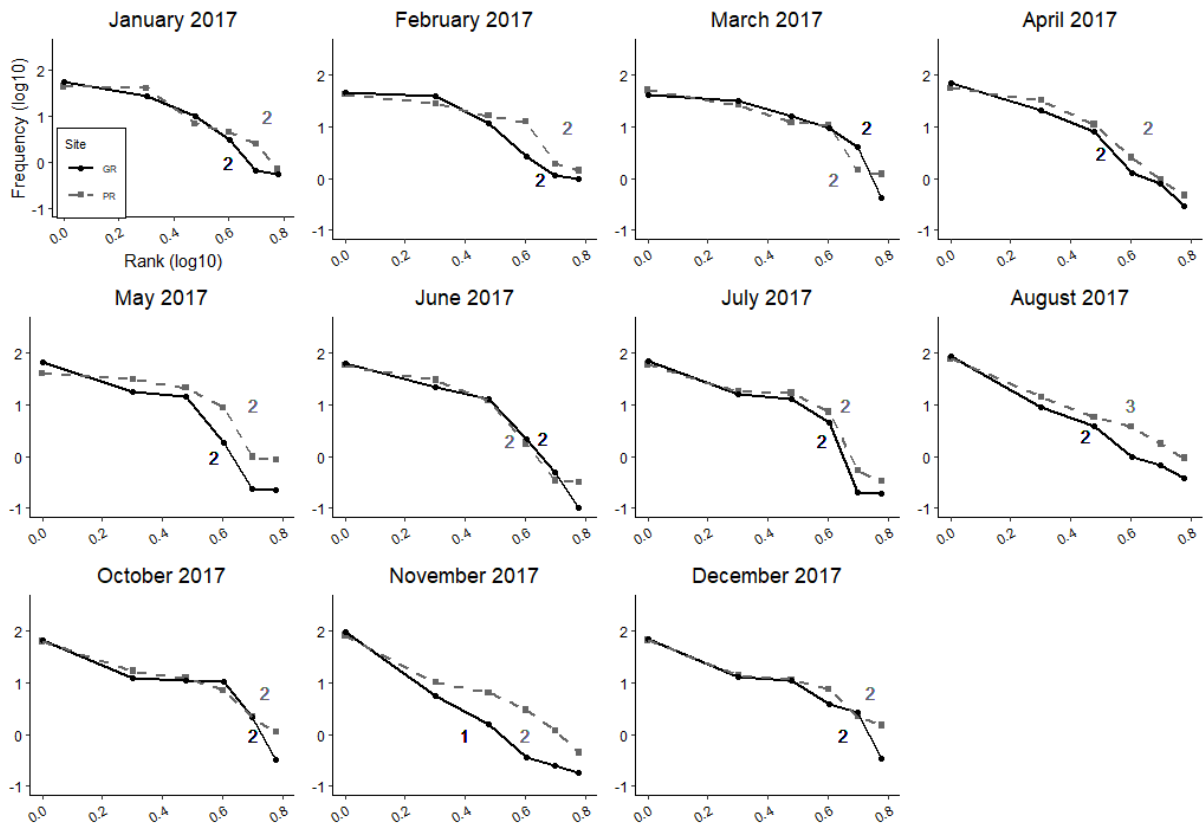
712 Figure 3: Rank frequency diagrams of pico- and nanoplankton assemblage in the Little Bay (dashed

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



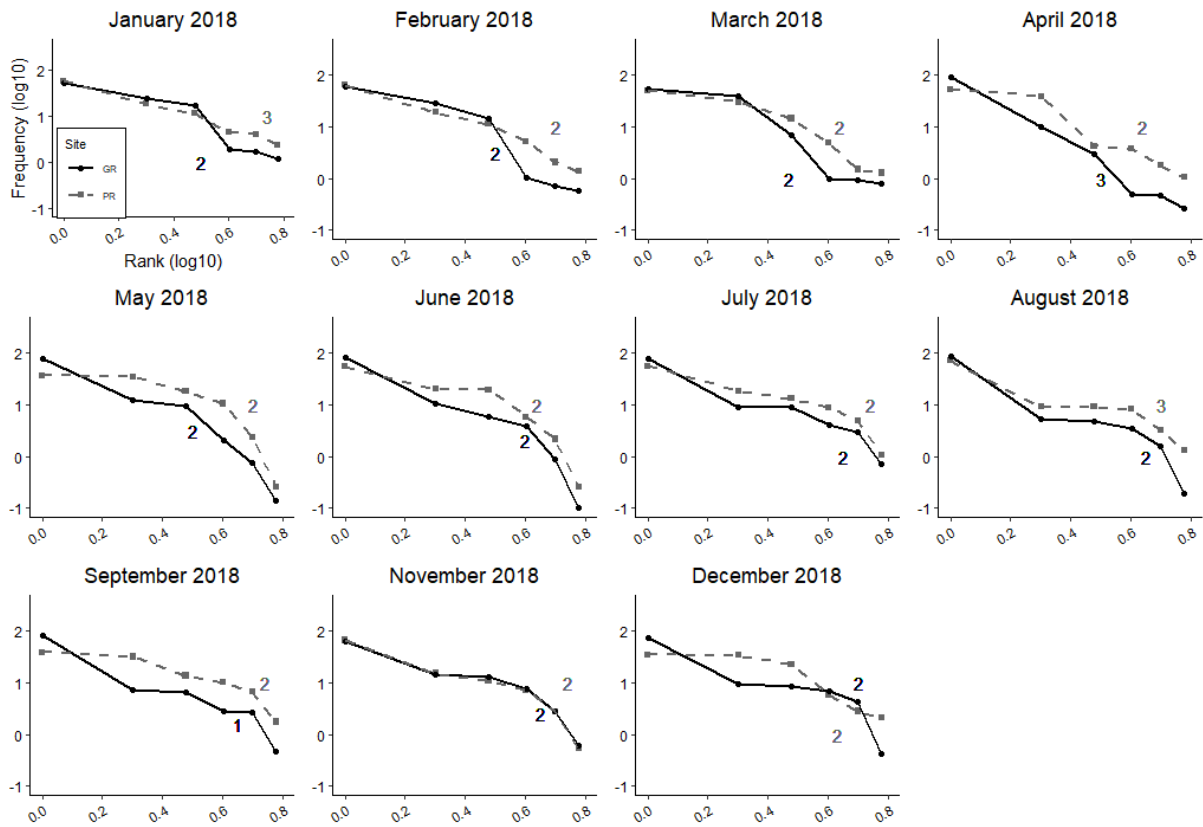
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715 Figure 4: Rank frequency diagrams of pico- and nanoplankton assemblage in the Little Bay (dashed  
 716 grey lines) and the Large Bay (solid black lines) in 2016. Both axes are on a logarithmic scale.



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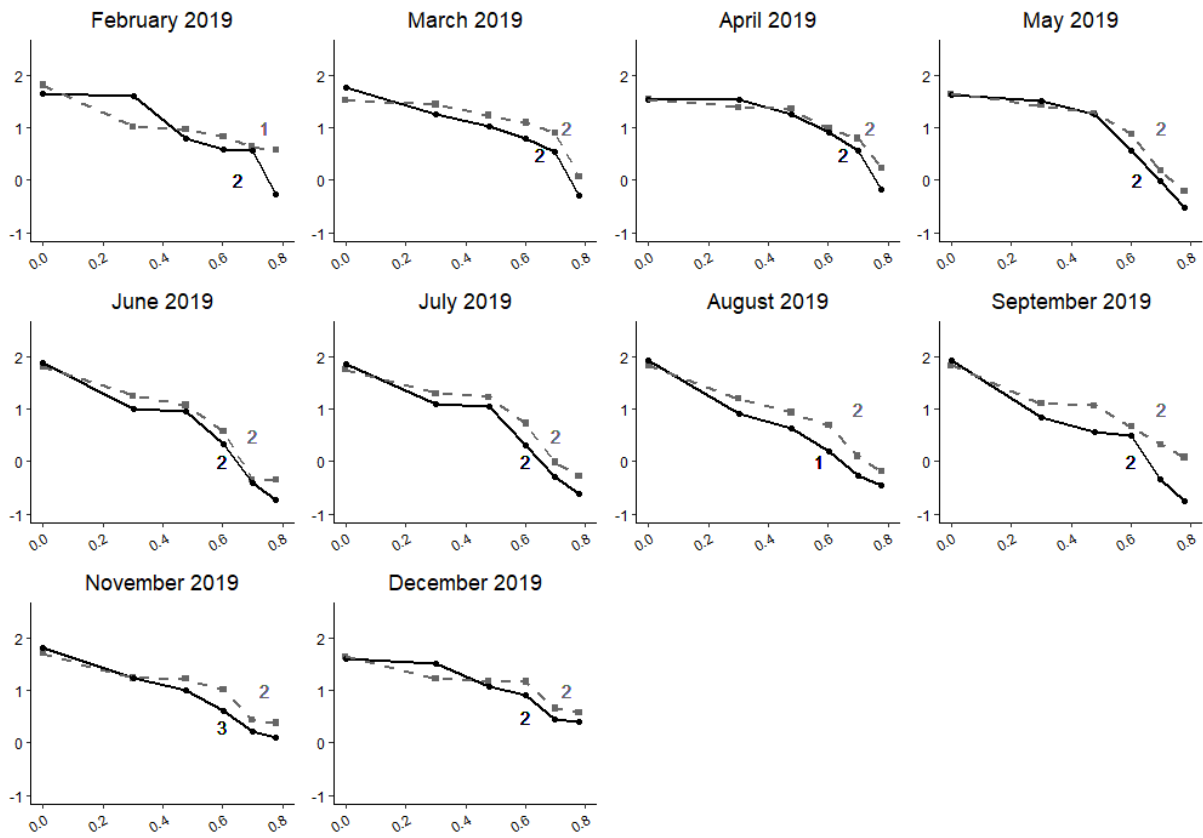
718 Figure 5: Rank frequency diagrams of pico- and nanoplankton assemblage in the Little Bay (dashed  
 719 grey lines) and the Large Bay (solid black lines) in 2017. Both axes are on a logarithmic scale.



720

721 Figure 6: Rank frequency diagrams of pico- and nanoplankton assemblage in the Little Bay (dashed  
 722 grey lines) and the Large Bay (solid black lines) in 2018. Both axes are on a logarithmic scale.

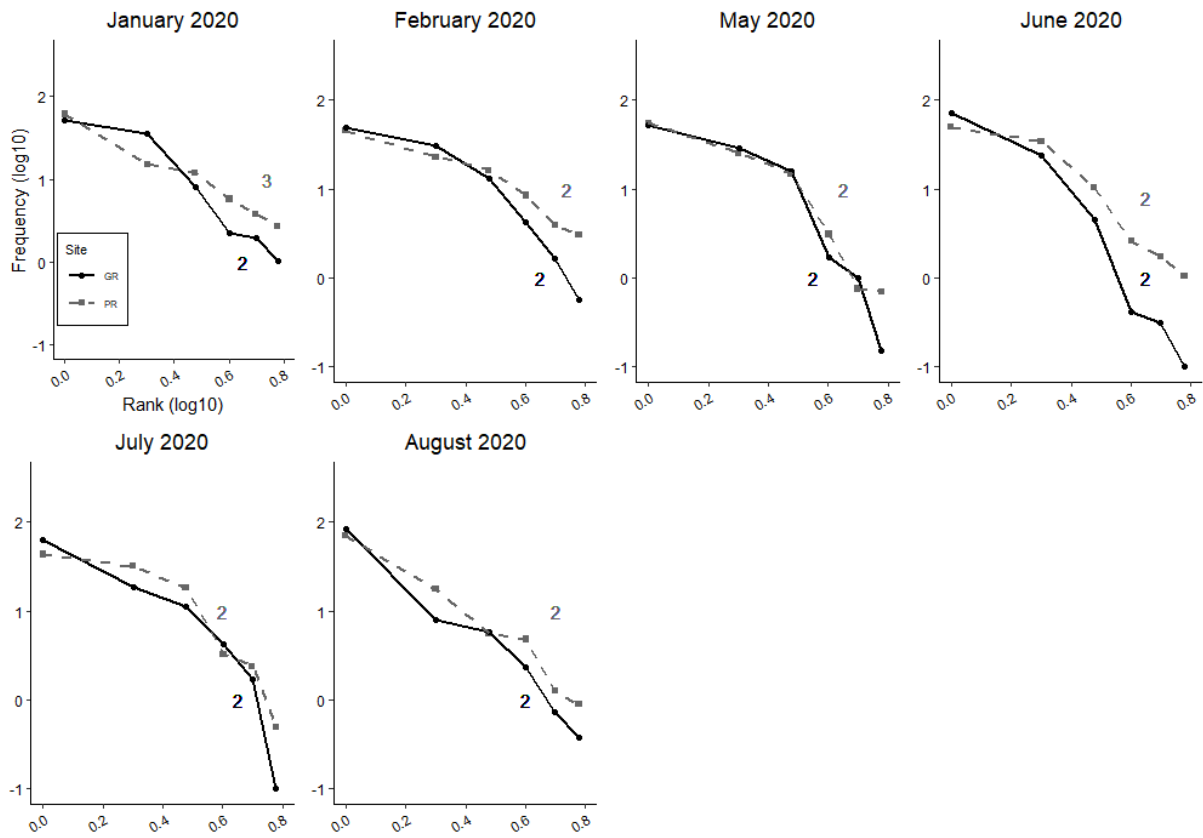




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724 Figure 7: Rank frequency diagrams of pico- and nanoplankton assemblage in the Little Bay (dashed

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



726

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

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