1 **Seasonal succession of small planktonic eukaryotes inhabiting**
2 **surface waters of a coastal upwelling system**

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21 **Running title:** Seasonality of small eukaryotes in surface waters

22 **Keywords:** Small eukaryotes, coastal upwelling, diversity, illumina, seasonality,
23 succession, networks

24 **Originality-Significance Statement:**

25 This work explores temporal changes in the taxonomic composition of the
26 smallest fraction of marine planktonic eukaryotes (0.2-20 μm cell-size) in a coastal
27 upwelling system. We found that seasonal changes in small-eukaryote community
28 composition were significantly related to changes in both abiotic factors and community
29 function in surface waters. We provide a first picture of small-eukaryote community
30 successional patterns in surface waters of this highly productive marine system, which
31 are likely to be driven by both abiotic and biotic factors. Our results also suggest the
32 potential structuring and stabilizing role of biotic interactions in the response of
33 microbial communities to environmental disturbance.

34 **Summary**

35 Small eukaryotes (0.2-20 μm cell-size) represent a significant fraction of the
36 microbial plankton community in shelf waters of NW-Spain. The community
37 composition of small eukaryotes living at the surface and at the base of the photic zone
38 was analysed by means of 18S rDNA high-throughput sequencing on a circa-monthly
39 basis over a 23-months period. *Ostreococcus* was the most abundant taxon in surface
40 waters, showing marked peaks in read abundance in spring and late summer, while
41 Syndiniales dominated at the base of the photic zone. A well-defined seasonal pattern of
42 community composition, linked to the succession of the dominant taxa, was found in
43 surface waters. Seasonality was less apparent at the base of the euphotic zone. Temporal
44 changes in abiotic factors significantly correlated with changes in community
45 composition in surface ($r=0.71$) and at the base of the photic zone ($r=0.38$). Changes in
46 community composition significantly correlated with changes in community function-
47 related variables (including biomass, primary production and respiration) only in

48 surface water ($r=0.36$). Co-occurrence network analyses revealed 45 significant
49 interspecies associations among the 50 most abundant taxa with highly connected OTUs
50 belonging to cryptophyceans. The network topology, with small-world characteristics,
51 suggests a stabilizing role of biotic interactions to environmental disturbance.

52 **Introduction**

53 Protists cover a wide spectrum of cell sizes, morphologies and functions and
54 present an extraordinarily high diversity (Schaechter, 2012). Furthermore, they play a
55 wide variety of ecological roles as primary producers, predators, symbionts, parasites or
56 decomposers (Sherr *et al.*, 2007). Marine protists perform a significant fraction of
57 carbon fixation in the global ocean and have a major impact in biogeochemical cycles
58 (Li, 1994; Field, 1998; Jardillier *et al.*, 2010; Massana, 2011). Linking microbial
59 community composition with carbon cycling represents a major challenge for better
60 understanding marine ecosystems and building predictive ecosystem models (Allison
61 and Martiny, 2008; Strickland *et al.*, 2009; Landa *et al.*, 2016). The development of
62 molecular tools based on 18S rDNA amplicon sequencing have considerably
63 contributed to our knowledge of protist diversity in the last two decades (López-García
64 *et al.*, 2001; Moon-van der Staay *et al.*, 2001; Massana *et al.*, 2004; Richards *et al.*,
65 2005; Not *et al.*, 2007; Guillou *et al.*, 2008; Lepere *et al.*, 2008; Massana and Pedrós-
66 Alió, 2008; Massana, 2011). Compared to the number of long-term (>1 year) studies
67 exploring seasonal patterns of marine prokaryotic community composition (e.g.
68 Fuhrman *et al.*, 2006, 2008, 2015; Gilbert *et al.*, 2012; Chow *et al.*, 2013; Cram *et al.*,
69 2015), the description of taxonomic composition seasonal variability is still incipient for
70 small planktonic eukaryotes in the marine realm (McDonald *et al.*, 2007; Kim *et al.*,
71 2014; Genitsaris *et al.*, 2015).

72 To date, most efforts to identify the drivers of microbial-plankton community
73 composition have primarily focused on environmental selection, exploring the role of
74 abiotic factors such as temperature, salinity, solar radiation, day length or nutrients (e.g
75 Countway *et al.*, 2010; Kirkham *et al.*, 2013; Dasilva *et al.*, 2014; Marquardt *et al.*,
76 2016; Piredda *et al.*, 2017). It is however well-known that both biotic and abiotic factors
77 are relevant drivers of community composition (Martiny *et al.*, 2006; Morin, 2011). The
78 importance of biotic interactions between microbial taxa has been recently proposed
79 using co-occurrence network analyses (e.g. Chow *et al.*, 2013; Lima-Mendez *et al.*,
80 2015; Genitsaris *et al.*, 2016; Milici *et al.*, 2016)

81 The Northwest Iberian coast, located at the northern end of the Eastern
82 Boundary Upwelling Ecosystem (EBUE) of the Canary current, is a temperate region
83 where alternation between water column stratification and mixing driven by the
84 seasonal variations in solar irradiance is modified by wind-driven upwelling-
85 downwelling events (Casas *et al.*, 1997). The upwelling of subsurface cold and

96 To test this hypothesis, we explored the annual cycle of the small planktonic
97 eukaryotes (0.2-20 μm) through a monthly sampling over ca. two years in shelf waters
98 of the NW Iberian EBUE in a fixed station at two sampling depths, surface and base of
99 the euphotic zone. We analysed the temporal variability of protist taxonomic
100 composition as well as the potential role of abiotic factors and biotic interactions in
101 shaping their assemblages. We also inspected the linkages between abiotic factors,
102 community composition and functions driven by the smallest protists (including
103 biomass, production and respiration rates). We anticipate strong temporal changes in the
104 taxonomic composition of small eukaryotes as a response to seasonally varying abiotic
105 factors (Hernando-Morales *et al.*, 2018). Furthermore, we expect more marked seasonal
106 changes in surface waters compared with the base of the euphotic zone given that the
107 seasonal variation of many abiotic factors is attenuated with depth (Hernando-Morales
108 *et al.*, 2018).

109 **Results**

110 *Abiotic conditions and microbial function-related variables*

111 Samples were collected at two depths in a shelf station off the Ría de Vigo at
112 approximately monthly intervals from January 2014 to November 2015. Environmental
113 conditions during the two sampling years were different. Year 2014 was characterized
114 by more precipitations during autumn and winter (Fig. 1a) and warmer surface waters
115 during late summer and autumn (Fig. 1b) compared to year 2015. Enhanced riverine
116 discharge produced a marked halocline from January to April 2014 (Fig. 2c) and surface
117 inorganic and organic nutrient concentrations in winter were higher in 2014 than in
118 2015 (Table S1, Fig. 1d). The strong thermal stratification during September-October
119 2014 triggered relatively low nutrient concentrations in the upper 40 m of the water
120 column (Fig. 1d) that resulted in lower levels of chlorophyll-*a* fluorescence in autumn

2014 compared to 2015 (Fig. 1e). Upwelling-favourable conditions, as inferred from positive upwelling index values (Fig. 1a), dominated from March to August in both years, while downwelling conditions were more pronounced in January-February 2014 than in January-February 2015. Surface dissolved organic nitrogen (DON) concentrations were the highest in March 2014 and both surface dissolved organic carbon (DOC) concentration and the fluorescence intensity of peak M, characteristic of humic-like substances, during winter were higher in 2014 than in 2015 (Table S1). Average DOC concentration and peaks M and T (characteristic of protein-like substances) intensities were significantly lower (t-test, $p < 0.01$) at 30 m than in surface waters (Table S1).

Surface primary production and chlorophyll-*a* (Chl-*a*) were high in spring and late summer and low during winter and the summer period, except for primary production rates in spring 2015 which were low (Fig. 2). Especially remarkable was the primary production peak in September 2015, which was extremely high compared with the other months. Primary production and Chl-*a* concentration at 30 m followed similar temporal trends than in surface waters. Microbial community respiration was highest in April and late summer 2015 (Fig. 2). The biomass of pico- and nanophytoplankton, as estimated from size-fractionated Chl-*a* concentration, was much less variable than that of microphytoplankton ($>20 \mu\text{m}$ cell size) (Fig. 2). The joint contribution of the pico- and nanoplankton size fractions (small plankton) to total Chl-*a* concentration, total primary production and total community respiration averaged 67%, 66% and 73%, respectively (Fig. 2). The contribution of small plankton ($0.2\text{-}20 \mu\text{m}$) to Chl-*a* and primary production was significantly higher during 2014 than during 2015 (t-test, $p < 0.037$).

Small-eukaryote community diversity and composition

146 A total of 42 DNA samples were successfully amplified for partial 18S rRNA
147 gene sequencing (Table S2). The average number of distinct OTUs per sample (i.e.
148 richness) was 371 ± 98 (Table S2). Richness was significantly higher at 30 m depth than
149 in surface water (t-test, $p < 0.03$) (Fig. S1, Table S2). Temporal variability in richness
150 suggests a seasonal pattern in surface waters, with higher richness during the winter
151 periods, while such seasonal pattern was less evident at 30 m depth (Fig. S1, Table S2).

152 Almost 95% of the eukaryotic OTUs were affiliated to 18 high-level taxonomic
153 groups (Fig. 3). In surface waters, Mamiellophyceae clearly dominated the small-
154 eukaryote community representing 32% of the reads, followed by Dinophyceae (17%)
155 (Fig. 3). Other abundant taxa in surface waters were MALV-II (9%), Diatoms (8%), and
156 Cryptophyceae (7%). At 30 m depth the small-eukaryote community was dominated by
157 the marine alveolate lineages MALV-II and MALV-I, representing 33% and 12% of the
158 reads respectively, followed by Mamiellophyceae (20%) and Dinophyceae (9%) (Fig.
159 3). In surface waters, Mamiellophyceae contributed $>30\%$ of the total reads in spring
160 and late summer months, while Dinophyceae contributed $>20\%$ of the total reads in
161 winter, early summer, and autumn months. Small-sized cryptophyceans were more
162 abundant in 2014 than 2015 (Fig. 3). Fungal reads belonging to Ascomycota were only
163 relevant in surface waters from November 2014 to January 2015. Radiolarians
164 belonging to Acanthaera were relatively abundant at 30 m depth in August and
165 September 2015 (Fig. 3).

166 A distance based redundancy analysis (dbRDA) based on Bray-Curtis
167 similarities between samples showed that the small-eukaryote community composition
168 was strongly influenced by the sampling season and by the optical depth (Fig. S2).
169 There were significant differences in community composition between surface and 30 m
170 depth (PERMANOVA test, $p = 0.001$). As expected, the similarity among surface and 30

171 m depth communities increased when the upper water column was well mixed, mostly
 172 during winter and autumn months. The best DistLM solution included irradiance,
 173 upwelling index, temperature, picoplankton chlorophyll-a, and nanoplankton primary
 174 production as significant predictors of the small eukaryotic assemblages (Fig S2). These
 175 variables explained only 28% of the fitted model variation.

176 *Exploring seasonality of abiotic factors, community composition and function*

177 Mean pairwise Bray-Curtis dissimilarity of samples was higher at 30 m depth
 178 (0.81) than in surface samples (0.72) (Fig. 4). By contrast, mean pairwise abiotic factor
 179 Euclidean distance of samples was higher in surface (5.2) than at 30 m depth (5.0).
 180 Overall, the lowest mean pairwise Bray-Curtis dissimilarity (0.61) occurred between
 181 surface communities 1 month apart. The lowest community dissimilarity values were
 182 returned annually (1 and 12 months lag) and highest community dissimilarity values
 183 were observed between samples that were 7 and 19 months apart. The sinusoidal
 184 function with a 12 months period of mean pairwise dissimilarity with increasing time
 185 lag suggests seasonality for small eukaryotes community composition in surface waters
 186 ($r^2=0.55$) (Fig. 4a). The abiotic and community function-related variables also followed
 187 a seasonal pattern in surface waters ($r^2=0.57$ and $r^2=0.15$, respectively) (Fig. 4 b, e).

188 Temporal changes in community composition were correlated with temporal
 189 changes in abiotic factors both in surface waters ($r^2=0.50$, $p<0.001$) and at 30 m depth
 190 ($r^2=0.15$, $p<0.05$). Furthermore, changes in community function were correlated with
 191 changes in abiotic factors in surface waters ($r^2=0.184$, $p<0.05$) but did not correlate in
 192 30 m waters ($p>0.05$). A significant link between small eukaryotes community
 193 composition and function was found in surface waters when calculating the partial
 194 correlation between composition and function, controlling for the effects of the abiotic
 195 factors ($r^2=0.13$, $p<0.05$).

196 *Co-occurrence patterns of abundant and frequent eukaryote populations*

197 In order to explore the potential role of biotic factors on small-eukaryote
 198 community assembly we examined the co-occurrence patterns among the 50 most
 199 abundant and frequent OTUs at both sampling depths. Most of the relationships were
 200 non-linear (Table 1). From the 50 eligible nodes, 34 and 22 were included in surface
 201 and 30 m depth networks, respectively (Fig. 5). Both the characteristic path lengths
 202 (CPL) between nodes and the clustering coefficients (CL) were significantly higher than
 203 in random networks of the same size (Z-test, $p < 0.001$). The considerably higher
 204 clustering coefficient of real vs. random networks ($CL/CL_r > 4$) suggests “small-world”
 205 network properties. Both networks had a power law distribution of nodal degrees, which
 206 indicates the existence of more highly connected nodes than expected from an even
 207 distribution of interactions. The clustering coefficients, the characteristic path lengths,
 208 the average number of neighbours and the number of significant associations (edges)
 209 among OTUs were higher in surface than at 30 m depth (Table 1). The proportion of
 210 negative associations was higher in surface than at 30 m depth. Such non-coexistence
 211 connections, reflecting either niche partitioning or negative ecological interactions,
 212 typically involved heterotrophic-heterotrophic or MALV-autotrophic pairs of OTUs,
 213 with some exceptions like the negative interactions between *Chaetoceros sp.* or
 214 *Ostreococcus tauri_1* and two cryptophyceans (Fig. 5).

215 In surface waters, diatoms, chlorophytes and cryptophyceans showed strong
 216 positive linear correlations among them (Fig. 5a). Within the Mamiellales, a strong
 217 positive association exists between *Bathycoccus prasinos_1* and *Micromonas pusilla_2*.
 218 By contrast, *Ostreococcus tauri* OTUs were not associated to other Mamiellales, except
 219 for a weak positive connection between *Ostreococcus tauri_1* and *Micromonas*
 220 *pusilla_1*. A cryptophycean OTU (Cryptomonadales_2) presented the highest number

of connections with other OTUs (degree=8), followed by the cryptophycean *Geminigera cryophila_3* (degree=6). Other chlorophytes, such as *Micromonas pusilla_2* or *Ostreococcus tauri_1*, and the cryptophycean *Geminigera cryophila_1* also showed a relatively connectivity in surface waters (degree=5) (Fig. 5a).

At 30 m, the network appeared fragmented in 7 components, including 6 small components composed of only 2 nodes (Fig. 5b). MALV clades, especially MALV-II, were more represented at 30 m depth than in surface. Strong positive associations dominate among cryptophyceans and Mamiellales. As in surface waters, *Ostreococcus tauri_1* was not connected with other Mamiellales. The most connected node was a *Micromonas* OTU, although the nodal degrees were lower than in surface waters (nodal degrees=6).

As some of the observed significant associations could just reflect either shared or distinctive environmental requirements, we explored niche separation between the co-occurring OTUs by analysing their relationships with the abiotic factors (Fig. 6). Overall, the Mamiellophyceae taxa showed remarkably different correlations with abiotic variables, particularly within the *Micromonas* genus (Fig. 6). The two *Ostreococcus tauri* ribotypes showed strong positive correlations with peak T, which is a proxy for labile dissolved organic matter. The *Micromonas* ribotypes showed weak correlation with the explored variables except for *Micromonas pusilla_2*, which show similar correlation as the two *Bathycoccus prasinos_1* (high correlations with nitrate and silicate, negative correlations with irradiance and peak T) (Fig. 6). Cryptophyceans, in general, showed negative correlations with salinity, upwelling index and irradiance, and positive correlations with precipitation, silicate and dissolved organic matter (DOC and peak M), but the patterns of significant correlations differed among OTUs (Fig. 6). Overall, several OTUs belonging to MALV-II clades showed negative correlations with

246 dissolved organic matter, while MALV-III clades showed positive correlations with
 247 temperature, precipitation and DOC concentration (Fig. 6). The two ribotypes of the
 248 diatom *Minidiscus trioculatus* showed contrasting correlations with abiotic factors
 249 showing a strong negative correlation with irradiance and a strong positive correlation
 250 with DOC, respectively (Fig. 6). Dinoflagellate were also a heterogeneous group with
 251 multiple and different correlations depending on the species. As an example,
 252 dinoflagellates_1 to 3 showed strong positive correlations with silicate concentration,
 253 precipitation and peak M, and negative with salinity and the upwelling index, while
 254 dinoflagellates_5 and 6 showed strong negative correlation with precipitation (Fig. 6).

255 *Species succession of small eukaryotes in surface waters*

256 The clear seasonal pattern observed for the small-eukaryote community in
 257 surface waters is coherent with the temporal variability of the relative read abundance of
 258 the 15 most abundant OTUs in surface waters compared to 30 m depth (Fig. S3). The 15
 259 most abundant OTUs contributed, on average 42% of the total reads in surface waters.
 260 A significant correlation between the relative read abundance of these OTUs for each
 261 month in 2014 and the corresponding month of 2015 was found ($r=0.71$, $p<0.001$,
 262 $n=135$). Therefore, to better illustrate the succession of the dominant taxa in surface
 263 waters, we averaged the abundance data for each month from the two sampling years
 264 (Fig. 7). *Ostreococcus tauri* dominated throughout the studied period, showing higher
 265 read abundance during spring and summer, with a decrease occurring in the spring to
 266 summer transition (Fig. 7). By contrast, *Bathycoccus prasinus* showed higher read
 267 abundance in winter months (Fig. 7). Despite Cryptophyceae were more abundant in
 268 2014 than in 2015 (Fig S3), they were systematically more abundant in winter and
 269 autumn months (Fig. 7), except the Cryptomonadales_2 OTU, that peaked in June (Fig.
 270 7). The most abundant dinoflagellates and diatoms also showed strong temporal

variability in their read abundances in surface waters sporadically blooming along the sampling period (Fig. 7). Some OTUs preferentially peaked in summer months, such as the diatom *Chaetoceros* sp or dinoflagellate_5, while the diatom *Minidiscus trioculatus* and dinoflagellates_1 and 3 had a winter-autumn distribution (Fig. 7).

Discussion

The present two-year analysis of small-eukaryote community composition in a highly productive and dynamic ecosystem reveals strong temporal variation irrespective of the relative temporal stability in terms of biomass (Espinoza-González *et al.*, 2012), and describes the seasonal succession of the dominant species within the small-eukaryote community in surface waters. Our data suggest that, while community seasonal patterns are significantly related to seasonal changes in abiotic factors, interspecies interactions may also play an important role in species succession in surface waters of this coastal area.

Small-eukaryote community composition

Previous studies based on pigment analyses, cytometry cell sorting and molecular approaches led to the consensus that coastal phototrophic picoeukaryotes are dominated by Mamiellophyceae (Not *et al.*, 2004; Zhu *et al.*, 2005; Worden, 2006; Masquelier *et al.*, 2011). Taxa belonging to the Mamiellales also dominated in our study (on average 32% and 19% of total reads in surface and at 30 m depth, respectively) (Fig. 3). *Ostreococcus* clearly dominated over *Micromonas* and *Bathycoccus*, particularly during the spring and summer (Fig. 7) as previously observed in surface waters of other coastal upwelling areas (Collado-Fabbri *et al.*, 2011; Rii *et al.*, 2016; Simmons *et al.*, 2016). A compilation of fluorescence in situ hybridization (FISH) data by Massana (2011) revealed that heterotrophic picoeukaryotes, are typically dominated by MALV

295 and MAST groups. As in preceding studies, the most abundant heterotrophic
296 picoeukaryotes affiliated with MALV clades (Massana, 2011; Dasilva *et al.*, 2014; Wu
297 *et al.*, 2014; de Vargas *et al.*, 2015; Massana *et al.*, 2015). MALV I and II taxa were
298 more abundant at 30 m depth than in surface waters (Fig. 3), a pattern also observed by
299 Wu *et al.* (2014) in the Pearl estuary, South China Sea. Dinophyceae (13%) and
300 Diatoms (5%) were not abundant within the small-eukaryote community in agreement
301 with other V4 18S rDNA sequencing surveys in temperate coastal waters (Massana *et*
302 *al.*, 2015).

303 *Abiotic and biotic factors drive seasonality of small eukaryotes*

304 Previous research in the sampling area suggests that the background microbial
305 plankton community is composed of small sized cells (<20 μm) to which large cells are
306 added, triggering large increases in total biomass, in response to upwelling episodes
307 (Espinoza-González *et al.*, 2012). We also found that major changes in Chl-*a*
308 concentration were primarily driven by microplankton increments during the productive
309 period (from April to September) (Table S1, Fig. 2). Comparatively, pico- and nano-
310 sized primary production showed higher temporal variability (Fig. 2), indicating
311 changes in small phytoplankton function regardless of the stability in autotrophic
312 biomass (as estimated from Chl-*a*), which could be associated to changes in the
313 community composition. As expected, the diversity, richness, evenness and taxonomic
314 composition of small eukaryotes (<20 μm) showed a marked temporal variability (Figs.
315 3, S3, and Table S2) as previously observed for prokaryotes (Fuhrman *et al.*, 2015) and
316 large plankton (>10-20 μm) communities inhabiting the euphotic zone of this
317 productive area (Bode and Varela, 1998; Bode *et al.*, 2015). A marked temporal
318 variability in small (<10 μm) eukaryotes diversity and taxonomic composition was also

319 reported in other coastal areas over single annual cycles (Marquardt *et al.*, 2016;
320 Kataoka *et al.*, 2017).

321 The detection of seasonality requires >2 years observations to confirm the
322 existence of repeatable patterns (Fuhrman *et al.*, 2015). While there are many published
323 long-term studies on seasonal patterns of prokaryotic plankton communities (e.g.
324 Fuhrman *et al.*, 2006; Carlson *et al.*, 2009; Vergin *et al.*, 2013; Chow *et al.*, 2013;
325 Alonso-Sáez *et al.*, 2015; Cram *et al.*, 2015) similar studies focusing only on small (<20
326 μm) eukaryotes are still lacking in marine systems. Despite we only covered a period of
327 23 months we were able to detect clear seasonal patterns in surface waters of the NW
328 Iberian EBUE. The seasonality observed in Figure 4 was more robust for the first 12
329 months due to the progressively lower number of pairwise samples comparisons >12
330 months apart. A long-term study in coastal temperate surface waters by Kim *et al.*
331 (2014) also found repeatable seasonal patterns for microbial eukaryotic communities
332 (<80 μm size fraction). Similarly, Genitsaris *et al.* (2015) also found repeatable seasonal
333 patterns for <150 μm protist communities.

334 Although seasonal community composition changes were significantly
335 correlated to changes in abiotic conditions, the relatively low correlation ($r^2=0.5$) points
336 to other non-measured environmental parameters, biological interactions or multiple
337 dynamics. The latter can happen when taxa are correlated with environmental variables
338 that present disparate dynamics.

339 Several recent studies, based on co-occurrence network analyses, strongly
340 support the role of interactions as important drivers of protistan community variability
341 (Genitsaris *et al.*, 2015; Lima-Mendez *et al.*, 2015). Co-occurrence/non-coexistence
342 patterns among populations may reflect either niche overlap/partitioning or

343 positive/negative ecological interactions such as commensalism, mutualism or
344 competition. Discerning the driving mechanisms of coexistence or exclusion from
345 correlation analysis is highly difficult, as two closely interacting species will show very
346 similar or opposing correlations with environmental factors if they occur together or
347 exclude each other, respectively. Yet, we found that the OTU pairs involved in most of
348 the significant associations showed unrelated correlations with the abiotic variables
349 considered in this study (Figs 5, 6), suggesting truly ecological interactions.
350 Notwithstanding, further studies are necessary to corroborate the biological interactions
351 between species pairs detected by network analyses.

352 As previously observed for prokaryotic communities (Chow *et al.*, 2013;
353 Fuhrman *et al.*, 2015; Hernando-Morales *et al.*, 2018), seasonal patterns were stronger
354 in surface waters than at the base of the euphotic zone (Fig. 4), which is consistent with
355 the higher seasonal variation in the abiotic factors (Fig. 4b, e). A higher number of
356 interspecies associations in surface waters compared to the base of the euphotic zone is
357 also coherent with a higher variability in the abiotic environment (Table 1, Fig. 5).
358 Complex protistan species co-occurrence networks were also associated to highly
359 variable environmental pressures by Genitsaris *et al.* (2016) in a coastal ecosystem.
360 Both the surface and 30 m depth network had high clustering coefficients and short
361 characteristic path lengths compared to random networks (Table 1) representing small-
362 world (SW) network characteristics (Humphries and Gurney, 2008). The SW topology
363 is associated to a higher resistance of networks to environmental change (Albert *et al.*,
364 2000; Montoya *et al.*, 2006) and to random species loss (Peura *et al.*, 2015). Even if
365 both surface and 30 m depth networks showed a SW topology, the lower connectance at
366 the base of the photic zone compared to surface (Table 1) could imply a lower
367 community stability (Thébault and Fontaine, 2010; Mougi and Kondoh, 2012). Thus,

biological interactions, might explain the lower mean dissimilarity of small-eukaryote communities in surface compared to 30 m depth regardless of similar mean environmental distances at both sampling depths (Fig 4). The strong month-to-month community structure variability observed at 30 m depth (Fig. 4 d) could also be related to non-explored abiotic factors or to non-included biological interactions. The highly fragmented and poorly connected interspecies association network at the base of the euphotic zone (Fig. 6b) strongly suggest that biological interactions with organisms not included in our study (e.g. viruses, prokaryotes, large protist or metazoans) likely play a relevant role as structuring forces of the small eukaryotes community. The fact that communities at this depth were dominated by MALV taxa, described as parasites of dinoflagellates, ciliates, copepods, crabs, and even fish eggs (Guillou *et al.*, 2008; Chambouvet *et al.*, 2008; Worden *et al.*, 2015; Strassert *et al.*, 2017), support this hypothesis.

381 *Succession of dominant pico- and nanoeukaryotes in surface waters*

Our dataset allowed a first tentative description of annual succession of the dominant species within the smallest fraction of eukaryotes in surface waters of the NW Iberian EBUE (Fig. 7), in a similar way as previously described for large (mostly >10 μm) phytoplankton in the nearby Rías Baixas based on inverted microscope observations (Margalef *et al.*, 1956; Figueiras and Niell 1987; Figueiras *et al.*, 2002; Nogueira and Figueiras 2005). On top of the seasonal variation in abiotic conditions, the growth of a particular assemblage can modify the available resources by consuming nutrients and/or attenuating irradiance, thus promoting biological interactions, which may force species selection. The highly connected interspecies association network observed in surface waters (Fig. 5a) strongly suggest that biological interactions play an important role in small eukaryotes succession. The high number of connections (i.e.

high degree) of *Cryptomonales_2* point to a pivotal role of this taxon in this upwelling system, despite contributing moderately to total read abundance (Fig. 5, 7). This taxon, *Geminigera cryophila_3* and dinoflagellate_5, all potentially mixotrophs, show negative interactions with the abundant *Ostreococcus tauri_1* taxon, suggesting that predation-prey interactions may be important regulators of *Ostreococcus* abundance.

During winter, low light availability associated to low solar radiation and intensive water column mixing favoured a small eukaryotes community largely dominated by dinoflagellates and cryptophyceans (Fig. 7). Low light conditions are especially suited for heterotrophic or mixotrophic protists. Many dinoflagellates are heterotrophic and both, dinoflagellates and cryptophyceans, may be mixotrophs (e.g. Kraberg and Stern 2017). In addition, the pigment composition of cryptophyceans allow them to better exploit low light intensities (Stern *et al.*, 2017). Significant negative correlations with light found for dinoflagellate_3, cryptomonadales_1 and *Geminigera cryophila_1* are coherent with their high abundance in January and February (Figs. 6, 7). Although less abundant, the winter distribution of *Bathycoccus prasinus* and the diatom *Minidiscus trioculatus* (Fig. 7) is consistent with previous studies (Marie *et al.*, 2006; Buck *et al.*, 2008; Monier *et al.*, 2016; Simmons *et al.*, 2016; Joli *et al.*, 2017). Many positive associations exist among the winter components including cryptophyceans, mamiellales and diatoms (Fig. 5a), likely reflecting biological interactions rather than simple niche overlapping, as the correlation patterns with environmental variables are not identical for any of the potentially interacting pairs (Fig. 5a, 6). *Ostreococcus tauri* becomes increasingly abundant during the onset of the spring bloom, when nutrients are abundant and light becomes increasingly available, reaching maximum abundances in May. *Micromonas* taxa also proliferate in spring, while *Bathycoccus prasinus*, dinoflagellates and cryptophyceans and diatoms abundance

progressively declines. A similar seasonal pattern was observed for *Ostreococcus* in the
 Chilean EBUE (Collado-Fabbri *et al.*, 2011). The high abundance of *Ostreococcus tauri*
 in upwelling systems suggests adaptation to high new nutrients availability, although we
 failed to detect a correlation with nitrate concentration, the positive correlation with the
 upwelling index (Fig. 6) support their ability to exploit new nutrients. Associated to
 nutrient exhaustion in late spring (Fig. 1d, Table S1), the abundance of *Ostreococcus*
 starts to decline coinciding with the proliferation of Cryptomonadales_2 a
 dinoflagellate_5. Our dataset suggests that the decline in *Ostreococcus* abundance
 occurring in late spring likely results from the combination of top-down processes and
 nutrient exhaustion. The extremely low nutrient concentration in May and June (Table
 S1) may favour the growth of heterotrophic or mixotrophic species. During summer,
 when the thermocline consolidates, nutrient supply to the euphotic zone is associated to
 upwelling events, supporting the increase of *Ostreococcus* abundance, reaching a
 second abundance peak in August. During summer, cryptophycean and *Micromonas*
 taxa sharply decline (Fig. 7). These results are in agreement with the study by
 Masquelier and colleagues (2011) in the English Channel, where they found a higher
 abundance of *Micromonas* in well mixed waters. The summer-autumn transition is
 characterized by the progressive erosion of the thermocline by mixing, leading to an
 autumn community dominated by cryptophyceans, dinoflagellates and diatoms
 (*Chaetoceros* sp. and *Minidiscus trioculatus*_1) and the progressively higher abundance
 of *Micromonas* taxa followed by *Bathycoccus prasinos* within the Mamiellales.

Concluding remarks

Disentangling the extraordinary complexity of microbial communities requires a
 holistic perspective including a full and detailed description of all potentially
 influencing abiotic and biotic factors. We here combined temporal analyses of small-

eukaryote community taxonomic composition with a thorough description of contextual variables and the exploration of interspecies associations to depict a tentative picture of the seasonal succession patterns of the small eukaryotes in surface waters of the NW Iberian EBUE likely driven by both abiotic and biotic factors. Our results also suggest the potential stabilizing role of interactions in the response of microbial communities to environmental disturbance. Further research, incorporating higher and lower trophic levels and higher spatial resolution and temporal coverage is required in order to confirm the observed patterns and improve our understanding of the dynamics of the ubiquitous small-sized component of microbial communities.

Experimental procedures

Samples were collected on board R/V José Navaz at a shelf station off the Ria de Vigo (position 42.14° N, 8.96° W; water depth 97 m) at approximately monthly intervals from January 2014 to November 2015. We did not sample in July and August 2014 because of technical problems with the vessel and in December 2015 due to rough weather conditions. Full-depth vertical profiles of temperature, salinity and chlorophyll-fluorescence were obtained with a SBE-25 CTD equipped with a Seapoint in situ fluorometer at each sampling date. Water was sampled with 5 L Niskin bottles that were attached to the hydrographic wire. Samples for inorganic nutrients analyses were collected at 1, 5, 10, 20, 30, 50 and 75 m depth. Dissolved organic carbon (DOC) and nitrogen (DON) concentrations and dissolved organic matter (DOM) fluorescence, size-fractionated chlorophyll-*a* concentration, size-fractionated primary production and size-fractionated microbial community respiration were determined at two depths, surface (1 m) and approximately the base of photic zone (30 m) (Teira *et al.*, 2015). Samples for pico- and nanoeukaryote community composition analyses were also collected at 1 and 30 m.

Upwelling intensity was estimated by calculating the offshore Ekman transport from coastal winds. Daily values of this upwelling index (UI) were computed by the Instituto Español de Oceanografía (<http://www.indicedeafloramiento.ieo.es>) in a $2^{\circ} \times 2^{\circ}$ geostrophic cell centred at 42°N , 10°W , using data from atmospheric pressure at sea level, derived from the WXMAP model (González-Nuevo *et al.*, 2014). Precipitation and solar irradiation data were obtained from the Regional Weather Forecast Agency-Meteogalicia (<http://www.meteogalicia.gal>) in the nearest station (42.2°N , 8.9°W).

Inorganic nutrients and chlorophyll-a. Aliquots for inorganic nutrients determination (ammonium, nitrite, nitrate, phosphate and silicate) were collected in 50 mL polyethylene bottles and frozen at -20°C until analysis by standard colorimetric methods with an Alliance Future segmented flow analyser (Grasshoff *et al.*, 1999). Size-fractionated Chl-*a* concentration was determined from acetone extracts of plankton retained by 20, 3 and $0.2\ \mu\text{m}$ polycarbonate filters and measured by the fluorometric method (Parson *et al.*, 1984).

Dissolved organic matter. Water for the analysis of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) was filtered through $0.2\ \mu\text{m}$ filters (Pall, Supor membrane Disc Filter) in an all-glass filtration system under positive pressure of N_2 , collected into pre-combusted (450°C , 12 h) 10 mL glass ampoules, and acidified with H_3PO_4 to $\text{pH} < 2$ before heat sealing. Samples were measured in a Shimadzu TOC-V analyzer (Pt-catalyst) following the method of Álvarez-Salgado and Miller (1998) method. Dissolved organic nitrogen (DON) was obtained by subtracting ammonium + nitrite + nitrate from TDN. Dissolved organic matter (DOM) fluorescence measurements were performed in a Perkin Elmer LS 55 luminescence spectrometer. Fluorescence intensities were measured at two fixed excitation/emission wavelengths of 320 nm/410 nm (peak M), characteristic of marine humic-like substances, and of 280

493 nm/350 nm (peak T), characteristic of protein-like materials (Coble, 1996), following
494 Nieto-Cid *et al.* (2006).

495 **Primary production.** Size-fractionated primary production was measured by
496 inoculating four 75 mL acid-washed bottles (three with light and one dark) of surface
497 and 30 m seawater with (370 KBq) 10 μCi of $\text{NaH}^{14}\text{CO}_3$ and incubated under in situ
498 irradiance and temperature conditions for 3 h. The samples were sequentially filtered
499 through a 20, 3 and 0.2 μm 47 mm polycarbonate filters using low vacuum pressure (<
500 50 mmHg). The filters were fumed overnight with HCl to remove the ^{14}C that was not
501 assimilated into organic matter or was assimilated as particulate inorganic carbon (i.e.
502 carbonates). Four mL of scintillation cocktail were then added and radioactivity in each
503 sample was measured on a Wallac β -scintillation counter. The DPMs (disintegrations
504 per minute) of the dark bottle were subtracted from the light bottle DPMs in order to
505 calculate the primary production. Primary production rates were calculated using the
506 method described by Mara $\tilde{\text{n}}$ on *et al.* (2001).

507 **Microbial community respiration.** The INT ((iodo-phenyl)-3(nitrophenyl)-
508 5(phenyl) tetrazolium chloride) reduction rate was used as estimator of respiration. Size-
509 fractionated in vivo ((iodo-phenyl)-3(nitrophenyl)-5(phenyl) tetrazolium chloride) INT
510 reduction rates as described in Mart $\acute{\text{n}}$ ez-Garc $\acute{\text{a}}$ *et al.* (2009)). Four 150 mL dark bottles
511 were filled from each depth. One bottle was immediately fixed by adding formaldehyde
512 (2% w/v final concentration) and used as killed-control. Samples were inoculated with a
513 sterile solution of 7.9 mM INT to a final concentration of 0.2 mM and incubated at in
514 situ temperature and in dark conditions during 1.5 h. After incubation, samples were
515 filtered sequentially through 20, 3 and 0.2- μm pore size polycarbonate filters, which
516 were stored at -20°C in 1.5 mL cryovials until further processing as described in
517 Mart $\acute{\text{n}}$ ez-Garc $\acute{\text{a}}$ *et al.* (2009). In order to transform ETS activity into C respiration, a

518 CR/ETS ratio of 12.8 (Martínez-García *et al.*, 2009) and a respiratory quotient of 0.8
519 (Williams and Giorgio, 2005) were used.

520 **Pico- and nanoeukaryote community composition.** Community composition
521 was assessed by sequencing the V4 region of the 18S rRNA gene. Water samples (2-3
522 L) were sequentially filtered through 20 and 3 µm pore size polycarbonate filters and
523 0.2 µm pore size sterivex filter and immediately frozen in liquid nitrogen and conserved
524 at -80 °C. Microbial community DNA retained in the 3 µm (referred to as
525 nanoeukaryotes) and 0.2 µm filters (referred to as picoeukaryotes) was extracted,
526 respectively, with the PowerSoil DNA isolation kit (MoBio Laboratories Inc., CA,
527 USA) and the PowerWater DNA isolation kit (MoBio Laboratories Inc., CA, USA),
528 following the manufacturer's protocol. The DNA concentration was quantified using a
529 Qubit® 2.0 fluorometer and the Qubit dsDNA HS Assay Kit (Thermo Fischer Scientific
530 Inc., Massachusetts, USA). DNA from 84 samples was amplified using the primers
531 TAREukFWD1 (5'-CCAGCASCYGCGGTAATTCC-3') and TAREukREV3 (5'-
532 ACTTTCGTTCTTGATYRA-3') (Logares *et al.*, 2014) and sequenced in an Illumina
533 MiSeq platform (paired-end reads; 2×250bp) at the Research and Testing Laboratory
534 (Lubbock, TX, USA). Illumina MiSeq reads were processed following an in-house
535 pipeline (Logares, 2017). Raw reads were corrected using BayesHammer (Nikolenko *et*
536 *al.*, 2013) following Schirmer *et al.* (2015) method. After correction, paired-end reads
537 were subsequently merged with PEAR (Zhang *et al.*, 2014) and the longer sequences (>
538 200bp) were quality-checked (maximum expected errors 0.5) and dereplicated using
539 USEARCH (Edgar, 2010). UPARSE V8.1.1756 (Edgar, 2013) was used to delineate
540 OTUs at 99% similarity. To obtain OTU abundances, reads were mapped back to OTUs
541 at 99% similarity using an exhaustive search (-maxaccepts 20 -maxrejects 50,000).
542 Chimera check and removal was performed both de novo and using the SILVA

reference database (Quast *et al.*, 2013). BLAST (Altschul *et al.*, 1990) was used to taxonomic assignment of 18S OTUs using representative sequences against three reference databases, PR2 (Guillou *et al.*, 2013) and two in-house marine protist databases (available at <https://github.com/ramalok>) based in a collection of Sanger sequences from molecular surveys (Pernice *et al.*, 2013) and on 454 reads from the BioMarKs project (Massana *et al.*, 2015). Metazoan, Charophyta and nucleomorphs OTUs were removed. Blast hits were filtered prior to taxonomy assignment considering a percentage of identity >90%, a coverage >70%, a minimum alignment length of 200bp and an e-value > 0.00001. Computing analyses were performed at the MARBITS bioinformatics platform of the Institut de Ciències del Mar (ICM; <http://marbits.icm.csic.es>). Sequences are publicly available at the European Nucleotide Archive (<http://www.ebi.ac.uk/ena>) under the accession numbers PRJEB23729.

Statistical analysis. Firstly, we subsampled the OTU table to the number of reads present in the sample with the lowest amount of reads, which was 2,385. Bray-Curtis pairwise dissimilarity matrices obtained from raw (i.e, including all the reads) and subsampled OTU tables were significantly correlated (Mantel test, $r = 0.927$, sig. 0.001).

Along the study period, a total of 4058 unique OTUs were identified, from which 1192 exclusively belong to picoeukaryotes and 1080 OTUs to nanoeukaryotes. As many OTUs were present in both size fractions, we combined datasets derived from the 0.2 and the 3 μm filters for community analyses. We normalized the reads from each filter size by the filter DNA yield, as recommended in Dupont *et al.* (2015).

The Bray-Curtis similarity of the eukaryote community was calculated from transformed read abundances (squared root transformed). The resulting similarity matrix was used to construct a distance based redundancy analysis (dbRDA) (Legendre

568 and Andersson, 1999). Permutation multiple analyses of the variance (PERMANOVA)
569 were performed in order to statistically explore whether small eukaryote community
570 composition significantly differ among sample groups (i.e., different depths or sampling
571 period), by comparing Bray-Curtis similarities among samples (all groups together and
572 pair-wise comparisons) using an 'add-on' package for PRIMER, PERMANOVA+
573 (Anderson, 2001; McArdle and Anderson, 2001; Anderson *et al.*, 2008). Furthermore,
574 parsimonious models were built to identify the best factors explaining variation in the
575 community composition (distance linear-based model (DistLM) using the best
576 procedure with Akaike's information criterion (AIC) (McArdle and Anderson, 2001).

577 In order to analyse the seasonality and the association between abiotic factors,
578 community composition and community function we made the dissimilarity matrices
579 based on the Bray-Curtis dissimilarity for community composition, and on the
580 Euclidean distances for the other matrices. The abiotic-factor similarity matrix included
581 the following variables: precipitation, upwelling index, solar radiation, temperature,
582 concentration of inorganic nutrients (nitrite, nitrate, ammonium, phosphate and silicate),
583 DOC and DON, peak T and peak M intensities. The community function matrix
584 included the chlorophyll-a concentration, primary production and respiration of the
585 pico- and nano-sized community fractions. To analyse the seasonality, the averaged
586 pairwise dissimilarity was calculated for groups of samples from each depth occurring
587 from 1 to 22 months apart (time lag) in order to explore seasonality (Fuhrman *et al.*,
588 2015). Seasonality can be assumed if dissimilarity values are lowest between
589 assemblages 1 and 12 months apart and highest 6 and 18 months apart following a
590 sinusoidal model. It is important to note that the number of samples is increasingly
591 smaller as the time lag increases. The Pearson correlation coefficient and partial
592 correlation coefficient were used to analyse the association between averaged

593 dissimilarities and distances and p-value was standardized as proposed by Good (1982)
594 in order to overcome the reduced number of data points.

595 We conducted network analyses to examine co-occurrence patterns and detect
596 keystone OTUs in eukaryote communities in surface and at 30 m depth. We chose the
597 Maximal Information-based Nonparametric Exploration, MINE, approach (Reshef *et*
598 *al.*, 2011) as it can detect nonlinear relationships between pairs of taxa. A static versus a
599 dynamic approach was used because the sampling time points were not equidistant and
600 interpolation would introduce biases (Faust *et al.*, 2015; Peura *et al.*, 2015). Following
601 general recommendations for network analyses (Berry and Widder, 2014), we filtered
602 infrequent and rare OTUs. We selected the 50 most abundant OTUs among those that
603 were present in more than 50% of the samples. The selected OTUs had a per sample
604 mean relative abundance >0.25%. We selected the same number of potential nodes
605 (OTUs) for both surface and 30 m depth networks in order to compare their topological
606 properties. MINE package computed the maximal information coefficient (MIC), the
607 linearity metric $MIC-\rho^2$ and the R coefficient of the linear model between each pair of
608 OTUs. The significance of MIC values was assessed from pre-computed uncorrected p-
609 values for different MIC scores for N=20 which were corrected using the Benjamini-
610 Hochberg procedure (Benjamini and Hochberg, 1995) at a false discovery rate of 5%.
611 All significant MIC scores were > 0.62. The MIC value indicates the strength of the
612 interaction between OTUs but do not provide information on the sign (positive or
613 negative). We identified strong non-linear relationships as those with a $MIC-\rho^2 > 0.5$.
614 The “non-coexistence” non-linear typology was considered negative, while the “two
615 lines” and the “line and parabola” non-linear typologies were assigned as positive (see
616 Resheff *et al* 2011 for details on non-linear typologies). Those connections with $MIC-\rho^2$
617 <0.5 were assigned as positive or negative based on the R value. Matrices with

618 significant MIC values were used to visualize the networks using Cytoscape 3.4.0
 619 (Shannon *et al.*, 2003). The networks were compared against 100 randomized versions
 620 generated using the Erdős-Rényi model. NetworkAnalyzer tool was used to calculate
 621 network topological parameters. OTUs with high degree (number of connections an
 622 OTU share with other OTUs) are referred to as network “hubs”, which can be viewed as
 623 “keystone” OTUs (Eiler *et al.*, 2012; Peura *et al.*, 2015; Comte *et al.*, 2016).

624 To explore the influence of abiotic factors we calculated the Pearson correlation
 625 coefficient between the OTUs included in the network analyses and all the available
 626 abiotic variables. We chose Pearson instead of MIC because > 95 % of the significant
 627 correlations were linear.

628 **Acknowledgments**

629 This research was supported by the Xunta the Galicia through DIMENSION
 630 project (grant EM2013/023) and by Spanish Ministry of Economy, Industry and
 631 Competitivity (MINECO) through ENVISION project (grant CTM2014-59031-P) in
 632 collaboration with the “Instituto Español de Oceanografía” (IEO) RADIALES project.
 633 Esther Barber-Lluch was funded by an F.P.I. MINECO fellowship. We thank all the
 634 people involved in the projects DIMENSION, in particular to E. Barber-LLuch and A.
 635 Prieto for their help with sampling and analytical work, especially to G. Casas, A.
 636 Miranda, M. P. Lorenzo and A. Fuentes-Lema. From IIM-CSIC, V. Vieitez performed
 637 the inorganic nutrient analyses and M.J. Pazó the carbon, nitrogen and fluorescence of
 638 dissolved organic matter measurements. We also thank the crew of the R/V J. M. Navaz
 639 for their help during the work at sea. R. Logares was supported by a Ramón y Cajal
 640 fellowship (RYC-2013-12554, MINECO, Spain). Bioinformatics analyses were
 641 performed at the MARBITS platform of the Institut de Ciències del Mar
 642 (ICM; <http://marbits.icm.csic.es>). We thank Caterina R. Giner, Ramon Massana and

643 Vanessa Balagué at the ICM-CSIC for their assistance in sequence analyses and data
644 interpretation.

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961

962 **Figure Captions**

963 Fig. 1: (a) Temporal variation of the upwelling index ($\text{m}^3 \text{s}^{-1} \text{km}^{-1}$ of coast), solar
964 radiation ($10 \text{ KJ m}^{-2} \text{day}^{-1}$) and precipitation (L m^{-2}) in the sampling area during the
965 study period, (b) temperature ($^{\circ}\text{C}$), (c) salinity, (d) nitrate concentration ($\mu\text{mol L}^{-1}$) and
966 (e) chlorophyll-*a* fluorescence at the sampling site during 2014 and 2015. Dots
967 represent data points used for interpolation.

968 Fig. 2: Size-fractioned chlorophyll-*a* (a and b), primary production (c and d) and
 969 community respiration (e and f) during the study period in surface waters (a, c and e)
 970 and at 30 meters depth (b, d and f).

971 Fig. 3: Temporal variation in the relative contribution of reads to the major taxonomic
 972 groups of (a) surface small-eukaryotes and (b) 30 meters small-eukaryotes during the
 973 study period. The last bar in each panel represented averaged composition over the
 974 sampled period.

975 Fig. 4: Panels a and d show the temporal patterns in pairwise Bray-Curtis community
 976 composition dissimilarity between samples collected N months apart (a) in surface
 977 waters and (d) at 30 meters depth. Panels b and e show the temporal patterns in pairwise
 978 abiotic-factor Euclidean distance between samples collected N month lag (b) in surface
 979 waters and (e) at 30 meters depth. Panels c and f show the temporal patterns in pairwise
 980 community function Euclidean distance between samples collected N months apart (c)
 981 in surface waters and (f) at 30 meters depth. Grey circles are single comparison
 982 between samples pairs and the black circles represent the average for each lag. Non-
 983 linear regression resulted in a good fit of a sinusoidal function (black curve lines) with a
 984 12-month period only for surface datasets.

985 Fig. 5: Maximal Information-based Nonparametric Exploration (MINE) Networks
 986 based on the abundance of the 50 most abundant and frequent eukaryotes OTUs (a) in
 987 surface waters and (b) 30 meters depth. The size of nodes is proportional to the node
 988 degree and the width of edges is proportional to the MIC value. Red and blue edges
 989 represent positive and negative associations, respectively.

990 Fig. 6: Pearson correlation of the MINE selected OTUs with abiotic variables: salinity
 991 (Sal), upwelling index (UI), solar radiation (Irr), precipitation (Pre), ammonium (NH₄),

992 nitrite (NO_2), silicate (SiO_2), nitrate (NO_3), phosphate (PO_4), temperature of water
 993 (Twat), dissolved organic nitrogen (DON), protein-like dissolved organic matter
 994 fluorescence (peak T), dissolved organic carbon (DOC), humic-like dissolved organic
 995 matter fluorescence (peak M). The dendrogram represents clustering of OTUs based on
 996 their correlations with abiotic factors (Euclidean distance). The colour code used for
 997 different groups of OTUs corresponds to that used in figure 5.

998 Fig. 7: Seasonal succession of the 15 most abundant taxa in surface waters. The
 999 abundance data from the two sampling years were averaged by month.

1000

1001 **Supplementary Figure Captions**

1002 Fig. S1: Temporal variation of richness and Shannon diversity index during the study
 1003 period of (a) surface small-eukaryotes and (b) 30 meters small-eukaryotes.

1004 Fig. S2: Distance based redundancy analysis (dbRDA) of small-eukaryote community
 1005 structure based on Bray-Curtis similarity. Filled and open symbols represent samples
 1006 from surface and 30 meters depth, respectively. Triangles and squares represent samples
 1007 from 2014 and 2015, respectively. Numbers correspond to the twelve months of the
 1008 year. Coloured areas in the plot highlight the divergence between surface (light green)
 1009 and 30 m depth (light brown) samples in spring-summer months compared to autumn-
 1010 winter samples (blue area). Only contextual variables that explained variability in
 1011 community structure selected in the DistLM model (best procedure with AIC selection
 1012 criterion) were fitted to the ordination. Abbreviations: picoplankton chlorophyll-*a*
 1013 (Chla.p); nanoplankton primary production (PP.n); upwelling index (UI); temperature of
 1014 water (Twat); and irradiance (Irr).

1015 Fig. S3: Relative abundance of reads of the most abundant OTUs (a) in surface waters
 1016 and (b) at 30 meters depth.

Fig. 1: (a) Temporal variation of the upwelling index ($\text{m}^3 \text{ s}^{-1} \text{ km}^{-1}$ of coast), solar radiation ($10 \text{ KJ m}^{-2} \text{ day}^{-1}$) and precipitation (L m^{-2}) in the sampling area during the study period, (b) temperature ($^{\circ}\text{C}$), (c) salinity, (d) nitrate concentration ($\mu\text{mol L}^{-1}$) and (e) chlorophyll-a fluorescence at the sampling site during 2014 and 2015. Dots represent data points used for interpolation.

190x275mm (300 x 300 DPI)

Fig. 2: Size-fractionated chlorophyll-a (a and b), primary production (c and d) and community respiration (e and f) during the study period in surface waters (a, c and e) and at 30 meters depth (b, d and f).

190x275mm (300 x 300 DPI)

Fig. 3: Temporal variation in the relative contribution of reads to the major taxonomic groups of (a) surface small-eukaryotes and (b) 30 meters small-eukaryotes during the study period. The last bar in each panel represented averaged composition over the sampled period.

276x206mm (300 x 300 DPI)

Fig. 4: Panels a and d show the temporal patterns in pairwise Bray-Curtis community composition dissimilarity between samples collected N months apart (a) in surface waters and (d) at 30 meters depth. Panels b and e show the temporal patterns in pairwise abiotic-factor Euclidean distance between samples collected N month lag (b) in surface waters and (e) at 30 meters depth. Panels c and f show the temporal patterns in pairwise community function Euclidean distance between samples collected N months apart (c) in surface waters and (f) at 30 meters depth. Grey circles are single comparison between samples pairs and the black circles represent the average for each lag. Non-linear regression resulted in a good fit of a sinusoidal function (black curve lines) with a 12-month period only for surface datasets.

275x190mm (300 x 300 DPI)

Fig. 5: Maximal Information-based Nonparametric Exploration (MINE) Networks based on the abundance of the 50 most abundant and frequent eukaryotes OTUs (a) in surface waters and (b) 30 meters depth. The size of nodes is proportional to the node degree and the width of edges is proportional to the MIC value. Red and blue edges represent positive and negative associations, respectively.

210x297mm (300 x 300 DPI)

Fig. 6: Pearson correlation of the MINE selected OTUs with abiotic variables: salinity (Sal), upwelling index (UI), solar radiation (Irr), precipitation (Pre), ammonium (NH4), nitrite (NO2), silicate (SiO2), nitrate (NO3), phosphate (PO4), temperature of water (Twat), dissolved organic nitrogen (DON), protein-like dissolved organic matter fluorescence (peak T), dissolved organic carbon (DOC), humic-like dissolved organic matter fluorescence (peak M). The dendrogram represents clustering of OTUs based on their correlations with abiotic factors (Euclidean distance). The colour code used for different groups of OTUs corresponds to that used in figure 5.

275x190mm (300 x 300 DPI)

Fig. 7: Seasonal succession of the 15 most abundant taxa in surface waters. The abundance data from the two sampling years were averaged by month.

214x131mm (300 x 300 DPI)

Table 1. Topology of the co-occurrence networks in surface and at 30 m depth. CL, clustering coefficient; CPL, characteristic path length; MIC, maximum information coefficient; CL:CLr and CPL:CPLr ratio between de CL or CPL of the real network and the mean CL or CPL of 100 random networks of the same size.

	Surface	30 m
Eligible nodes	50	50
Nodes	34	22
Edges	45	17
Non-Linear edges	34	5
Negative edges	16	3
CL	0.249	0.170
CPL	3.72	2.02
Average number of neighbors	2.65	1.55
Diameter	10	4
Minimum MIC	0.62	0.69
Distribution of nodal degrees	Power law	Power law
CL:CLr	4.02	4.64
CPL:CPLr	1.11	0.66
Connectance	0.018	0.007