



Diversity of taxon-specific traits of seasonally distinct unicellular eukaryotic assemblages in a eutrophic coastal area with marked plankton blooms

Savvas Genitsaris^{1,*}, Natassa Stefanidou², Ulrich Sommer³, Maria Moustaka-Gouni²

¹Section of Ecology and Taxonomy, School of Biology, National and Kapodistrian University of Athens, Zografou Campus, 15784 Athens, Greece

²Department of Botany, School of Biology, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

³Geomar Helmholtz Centre for Ocean Research Kiel, 24105 Kiel, Germany

ABSTRACT: In this work, we focused on the functional characterization of unicellular eukaryotic assemblages that had previously been taxonomically characterized by 18S rRNA gene amplicon sequencing in a eutrophic coastal site with marked plankton blooms. Biological traits of different functional groups were assigned to the retrieved operational taxonomic units (OTUs). The traits included size, trophic strategy, the presence of spines, mucilage production, colony formation, motility, spore formation, and potential harmfulness. Functional diversity indices were calculated and compared to analogous taxonomic diversity indices, indicating a strong positive coupling of richness and dominance and a negative coupling of evenness, even at a low taxonomic resolution (at the family/genus/species level). Biological trait trade-offs and co-occurrences of specific traits were evident during the succession of plankton blooms. The trophic strategy dominating in the assemblages frequently alternated between autotrophy, mixotrophy, and a few recorded cases of parasitism. Given that there was no indication of nutrient limitation, we suggest that biotic pressures force marine eukaryotes to exploit narrow niches by adopting specific strategies/traits that favour their survival. These traits act by increasing resource acquisition potential and via predator avoidance. This leads to a unique succession of blooms in the system, characterized by adaptations of the bloom taxa that are a direct response to the preceding assemblage.

KEY WORDS: High throughput sequencing · Marine protists · 18S rRNA gene · Functional diversity · Biological traits

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1. INTRODUCTION

Marine plankton comprises a large diversity of organisms with different phylogenetic origins, life strategies, evolutionary adaptations, and complex functional roles (Caron et al. 2012, Worden et al. 2015). The development of high throughput sequencing (HTS) tools has provided the means to thoroughly examine the prokaryotic and protistan taxonomic diversity (sensu DNA amplicon sequence

diversity) of plankton communities in numerous marine systems. Several studies applying HTS methods have documented the overlooked and underestimated high diversity of unicellular eukaryotic assemblages in the open ocean (de Vargas et al. 2015), coastal systems (Christaki et al. 2014, Genitsaris et al. 2015), polar environments (Cleary & Durbin 2016, Onda et al. 2017), the deep sea (Bik et al. 2012, Xu et al. 2018), anoxic zones (Parris et al. 2014), and estuaries (Balzano et al. 2015). This taxonomic-centred

*Corresponding author: genitsar@biol.uoa.gr

analysis has been used globally but this vast information of taxonomic diversity has only rarely been translated into functional diversity and its response to abiotic changes and biotic interactions (Genitsaris et al. 2015, 2016, Ramond et al. 2019). On the one hand, it has been shown that functional redundancy, i.e. a small pool of biological traits, can be found unexpectedly in protistan biodiversity hotspots (Seeleuthner et al. 2018, Ramond et al. 2021), creating challenges when facing exogenic adversities. On the other hand, the dominance of a few taxa may well provide the necessary ecological tools to counter top-down or bottom-up pressures and maintain ecosystem stability (Tilman et al. 2006, Sasaki & Lauenroth 2011), sensu resistance to disturbances (Tilman 1999). Thus, the coupling of taxonomy and function is not always apparent in protistan communities. Other evidence shows a positive signal between phylogenetic relatedness and environmental preferences among eukaryotes (Logares et al. 2020), permitting the description of specialization, functional redundancy, and community responses to abiotic and biotic pressures in aquatic systems (Weithoff et al. 2015, Leruste et al. 2018). However, it can also be argued that taxonomy and function are imperfectly coupled (Inkpen et al. 2017) and ‘constitute roughly independent axes of variation shaped by markedly different processes’ in the global ocean (Louca et al. 2016). Taxonomic diversity data, which is an *a priori* endpoint of amplicon sequencing, can reflect functional diversity only if it is shown that specific taxa bear specific traits. Adding to this, several species with complex lifestyles alter traits during their variable life stages. Thus, understanding the coupling of taxonomic and functional diversity in protistan communities will contribute to determining whether phylogenetic relatedness in eukaryotes corresponds to ecologic affinity (Seeleuthner et al. 2018). Ultimately, is it important to describe who is present in a marine system as long as we know their function? And if so, how can we handle taxa with complex life strategies that exhibit contrasting traits during their life cycles to face variable exogenic pressures?

A modern approach in marine microbial ecology to elucidating the drivers of marine protistan assembly requires a focus on describing the biological traits of individual taxa or groups that reflect the functional diversity of these assemblages (Jarzyna & Jetz 2016, Logares et al. 2020). A biological trait is a specific characteristic of a species that can be used to signify its life strategy, and consequently its ecological niche (Irwin & Finkel preprint doi:10.1101/148312). Several types of traits have been suggested to be meaningful

for describing marine protistan assemblages, such as cell size (Sommer et al. 2017), trophic preferences (i.e. strategies for nutrient acquisition; Troost et al. 2005), mucilage production and resting spore formation (Tréguer et al. 2018), colony formation (Irwin & Finkel preprint doi:10.1101/148312), motility (Litchman & Klausmeier 2008), and toxin production and other adaptations harmful to other species or to ecosystem health (Ger et al. 2016). However, this approach can present challenges in the assignment of trait values to specific taxa identified solely by genetic markers (Irwin & Finkel preprint doi:10.1101/148312) due to the variability in the acclimation of the same species in different abiotic and biotic surroundings at spatial and temporal scales (Litchman et al. 2007). Thus, a specific assemblage can be suitably described by several trait types, but in any case, their number is ultimately limited (Stubbs & Wilson 2004). This implies that the trait type is more important than the number of traits present (Zhu et al. 2017).

So far, the linking of taxonomic and functional diversity via the annotation of biological traits has been attempted mainly for specific species identified with classical microscopy tools in controlled environments and under the prism of climate change (Peter & Sommer 2012, Wilken et al. 2018), and to a lesser extent in natural systems (Santana et al. 2018). These attempts have associated increasing temperatures with minimal influence of top-down control on plankton size selection (Peter & Sommer 2012), and have demonstrated that mixotrophy and bacterivory are the preferred trophic strategies in extreme climate change scenarios (Wilken et al. 2018). Recent studies have used HTS data to connect deep marine protistan diversity with trophic strategies (Genitsaris et al. 2015, 2016, Stefanidou et al. 2018, Wang et al. 2020) and additional biological traits, such as those related to cell morphology and structure, physiology, and the life cycles of species, using published information and empirical expertise (Louca et al. 2016, Ramond et al. 2019, 2021). The implementation of this approach has the advantage of examining the functions corresponding to the overlooked, rare, and cryptic taxonomic diversity, and exploring the ecological drivers shaping marine unicellular assemblages in relation to environmental change and biotic pressures. Thus, the classic ecological concepts that are used to describe marine microbial functioning will be updated to include a variety of niches and strategies implemented by the neglected—but persistent and abundant—members, visible only with next generation tools (Christaki et al. 2014).

In this work, we focus on the functional characterization of unicellular eukaryotic assemblages which had previously been taxonomically characterized by microscopy and HTS in Thessaloniki Bay in the eastern Mediterranean Sea (Genitsaris et al. 2019, 2020). During the sampling period, a non-typical seasonal succession of frequent and conspicuous blooms of plankton unicellular eukaryotes, red tides, and surface mucilage aggregations were documented with both microscopy and HTS, and were associated with increased anthropogenic eutrophication pressures and ocean circulation effects (Krestenitis et al. 2012, Androulidakis et al. 2021).

In particular, the following questions were investigated:

- Is the taxonomic diversity of the marine unicellular assemblages coupled with the functional diversity?
- Do certain biological traits coincide? In other words, does the occurrence of a specific trait imply the occurrence of another trait of the same or another functional group?
- Are there trait trade-offs between the seasonally optimal traits during the succession of the unicellular eukaryotic assemblages in relation to the conspicuous blooms of specific taxa?

2. MATERIALS AND METHODS

2.1. Sample collection and environmental parameter measurements

The sampling strategy and the methods followed for measuring the environmental variables, i.e. temperature, salinity, nitrate/nitrite ($\text{NO}_3^-/\text{NO}_2^-$), ammonium (NH_4^+), silicate (SiO_4^-), phosphate (PO_4^-), have been described in detail elsewhere (Genitsaris et al. 2019, 2020). Briefly, surface samples were collected weekly from March 2017 to February 2018 at an urban coastal site (White Tower, WT) in Thessaloniki Bay in the Thermaikos Gulf, while monthly surface samples were simultaneously taken from 3 other adjacent sites at a distance of approximately 3 km between the closest ones, and 10 km between the 2 most distant (Aretsou Beach, AR; Music Hall Coast, MH; and Harbour, HB) (Fig. S1 at www.int-res.com/articles/suppl/a088p167_suppl1.pdf in Supplement 1). Overall, 47 samples were taken from WT, and 12 from each of the other 3 sites, i.e. from AR, MH, and HB, making a total of 83 samples. During all samplings, *in situ* measurements of water temperature and salinity were made with the use of the YSI Pro

1030 instrument (YSI), and $\text{NO}_3^-/\text{NO}_2^-$, NH_4^+ , SiO_4^- , and PO_4^- were measured colorimetrically with a Thermo Scientific™ Flash 2000 Elemental Analyzer (Thermo Fisher Scientific) at 882 nm, following the protocol of Hansen & Koroleff (1999).

2.2. Sequencing and read processing

Water subsamples from all samplings were pre-filtered through a 200 μm sterile mesh, subsequently filtered through 0.2 μm nucleopore filters, and then kept at -20°C until molecular analysis. The sequencing and read processing are reported elsewhere (Genitsaris et al. 2020). Briefly, DNA was extracted from the 83 samples using a NucleoSpin® Soil genomic DNA isolation kit (Macherey-Nagel), according to the manufacturer's instructions. The concentration and quality of recovered DNA was confirmed using the Thermo Scientific™ NanoDrop™ spectrophotometer (Thermo Fisher Scientific). The extracted DNA was subjected to PCR using specific primers targeting the V4 hyper variable region of the 18S rRNA gene (E572F: CYG CGG TAA TTC CAG CTC; E1009R: AYG GTA TCT RAT CRT CTT YG) (Comeau et al. 2011), the PCR products were purified, and amplicon samples were sequenced on Illumina MiSeq using 300+300 bp paired-end chemistry at the Integrated Microbiome Resource (IMR) at Dalhousie University, Halifax, Canada.

The produced reads were subjected to downstream processing using the *mothur* v.1.34.0 software (Schloss et al. 2009), following the proposed standard operating procedure (Schloss et al. 2011). The reads were subjected to quality control, the ambiguous and/or short reads were removed, and the remaining reads were dereplicated and aligned independently against the SILVA 132 database, containing 163 916 eukaryotic small subunit (SSU) rRNA gene sequences (Quast et al. 2013). The reads suspected of being chimeras were removed using the UCHIME software (Edgar 2010). The remaining reads were clustered into operational taxonomic units (OTUs) at 97% similarity level (Behnke et al. 2011), using the average neighbour method in *mothur*. The OTUs with a single read in the entire dataset were removed from the analysis (e.g. see Kunin et al. 2010, Richards et al. 2015, Genitsaris et al. 2016). The resulting dataset was rarefied to 14 258 reads with the *sub.sample* command in *mothur*, while 7 samples with lower total numbers of reads were kept as they were in the dataset, as the best compromise in order

to retrieve the majority of the biodiversity detected by sequencing, but also to include all samples in the analysis, even those with lower numbers of reads. Taxonomic classification was assigned using BLASTN searches against the Protist Ribosomal Reference database (PR2 database) with curated protistan taxonomy (Guillou et al. 2013), with >90% of the OTUs having >97% similarity with known sequences in PR2. The reads belonging to OTUs related to Metazoa and Streptophyta were removed from the dataset. The raw reads were submitted to GenBank-SRA under the accession number PRJNA552665.

2.3. Biological trait annotation strategy

Unicellular eukaryotes may belong to the same phylogenetic/taxonomic group but exhibit different functions. Based on this idea, the strategy applied here was to individually examine all 2812 OTUs of the dataset and, according to the taxonomic affiliations given by the PR2 database BLASTN search, proceed to literature searches concerning the biological descriptions for each taxon/OTU. Each OTU was then annotated to specific biological trait sets for different functional groups (following Genitsaris et al. 2016, Ramond et al. 2019) that were associated with resource acquisition, predator avoidance, and survival mechanisms (per Irwin & Finkel preprint doi:10.1101/148312). These included: (1) size as a master trait influencing metabolic rates, resource acquisition, and susceptibility to grazing (Finkel et al. 2010); (2) trophic preferences and optimum temperature for maximum growth rates, both traits related to resource acquisition; (3) the presence of spines, mucilage production, colony formation, and motility in relation to defence mechanisms; (4) spore/cyst formation as survival and dispersal strategy; and (5) potential harmfulness as a proxy to the ability to cause disruptive events (see Table 1).

About half of the retrieved OTUs, mainly affiliated to dinoflagellates (e.g. *Noctiluca scintillans*, *Gonyaulax* sp., *Gyrodinium* spp., *Gymnodinium* spp., *Prorocentrum* spp.) diatoms (e.g. *Chaetoceros* spp., *Skeletonema costatum*, *Pseudonitzschia* spp.), ciliates, haptophytes, cryptophytes, and chlorophytes, were observed by microscopy in the area during the same samplings (Genitsaris et al. 2020), and are usual inhabitants of the area (Nikolaides & Moustaka-Gouni 1990, Mihalatou & Moustaka-Gouni 2002, Giannakourou et al. 2005). Thus, their life strategies are well documented, and their functional

annotations were achieved with a high level of confidence. The rest of the OTUs, affiliated to taxonomic groups impossible to detect with microscopy, were considered at the lowest taxonomic level available, and their functional annotations were performed according to relevant literature. For example, OTUs belonging to the group of Marine ALveolates (MALV) were considered parasites (Skovgaard 2014), whereas the OTUs of Marine STRamenopiles (MAST) were grouped to nano-grazers (Massana et al. 2006), and OTUs associated with certain species of dinoflagellates, cryptophytes, and radiolarians were considered mixotrophs. These OTUs are reported to have both photosynthetic capacity and the ability to utilize heterotrophic strategies (see Supplement 2 at www.int-res.com/articles/suppl/a088p167_suppl2.xlsx for detailed taxon-specific trait annotation). For traits of size and those associated with predator avoidance and survival, the Ocean Biodiversity Information System (OBIS; <https://obis.org/>) and the Global Biodiversity Information Facility (GBIF; www.gbif.org/) were additionally consulted. The OTUs belonging to taxa with parasitic lifestyles were annotated as potentially harmful and the IOC-UNESCO Taxonomic Reference List of Harmful Microalgae (Moe-strup et al. 2009 onwards) was used to determine potential harmfulness for the remaining OTUs. Finally, the Protist Interaction Database (PIDA) on the planktonic protist interactome was also consulted (Bjorbækmo et al. 2020). Eventually, all traits were annotated to >67% of the total number of OTUs, except for optimum temperature (only 23.8% of the OTUs were assigned to this trait), which was not included in further analyses (see Table 1). Taxa that were affiliated to an optimum temperature preference included OTUs that belonged to all major high-level taxonomic groups (highlighted in Supplement 2).

2.4. Data analysis

The average linkage clustering method with Euclidean distances was applied to cluster the biological traits according to OTU trait annotation and respective OTU number of reads. The α -diversity estimators of taxonomic diversity (TD), Simpson's dominance ($1 - D$) and equitability (J) indices, were calculated with the PAST 3.16 software (Hammer et al. 2001). These TD estimators are indicators of the dominance of 1 or a few species and the level of homogeneity of the community examined, respectively. The functional diversity (FD) estimators, func-

tional richness (FRic), Rao's Quadratic Entropy (Rao's Q), and functional evenness (FEve), were calculated using the dbFD function of the 'FD' R package (Laliberté et al. 2014). The FD estimators were selected as analogous to the TD indices, representing traits rather than species present in the community. In this context, FRic reflects 'the amount of functional space occupied by a species assemblage' and can be interpreted as an indicator for a used/unused niche space. During our analysis, FRic was measured as the number of unique trait combinations and not as the convex hull volume, since only categorical traits were included. FEve corresponds to the level of homogeneity with which species abundances are distributed within the functional space (Mouchet et al. 2010). In other words, it measures whether mean species traits are distributed regularly within the occupied trait space (Schleuter et al. 2010). Rao's Q is a measure of the mean functional distance between 2 random individuals (Botta-Dukát 2005), analogous to Simpson's dominance index, which calculates the probability that 2 random individuals within a community belong to the same species. To explore a potential co-occurrence of biological traits throughout the study, Euclidean distances based on the number of reads per trait were implemented. In addition, to identifying optimal strategies in each assemblage, independently of trait dominance as shown by the number of reads of each trait or the characteristic trait of the dominant OTUs, the community weighted mean (CWM) trait values, reflecting the locally or temporally optimal trait in the community, were calculated with the 'FD' R package. Finally, network analysis was performed to detect significant relationships, both positive indicating co-dependence, and negative indicating exclusion, between the biological traits and the environmental parameters that were measured during the study. The relationships were characterized through maximal information-based nonparametric exploration (MINE) statistics by computing the maximal information coefficient (MIC), based on OTU number of reads and environmental values in pairwise comparisons (Reshef et al. 2011). The matrix of MIC values corresponding to a p -value < 0.05 , based on pre-computed p -values of various MIC scores at similar sample size to our dataset, was applied (MIC > 0.31 in this case). The network of trait–environmental parameter associations was visualized with Cytoscape 3.5.1 (Smoot et al. 2011). Positive and negative relationships were identified according to the approach of Hernández-Ruiz et al. (2018), who assigned negative relationships to the 'non-coexistence' non-linear typology of

pairs with values of the linearity metric MIC – $\rho^2 > 0.5$; and the '2 lines' and the 'line and parabola' non-linear typologies were assigned to positive relationships. Those correlations with MIC – $\rho^2 < 0.5$ were assigned as positive or negative based on their R -value.

3. RESULTS

The water temperature, salinity, and dissolved inorganic nutrient values in all samplings are shown in Table S1. Detailed information of the environmental parameter variability throughout the study can be found in Genitsaris et al. (2019, 2020). Briefly, water temperature and salinity fluctuated from 9.6 to 29.7°C and from 32.8 to 38.8, respectively. All nutrients recorded high concentrations, with extreme values for a few sampling dates, and were never limiting throughout the sampling period. High $\text{NH}_4\text{:NO}_3$ (average value 2.62) and N:P (25.1) ratios were calculated. During the study, previous macroscopic observation and microscopic analyses revealed frequent and successive phytoplankton blooms (described in detail in Genitsaris et al. 2019). Briefly, known mucilage-producing diatoms (mainly *Chaetoceros* spp., *Cylindrotheca closterium*, *Leptocyldrus minimus*, *L. danicus*, and *Skeletonema costatum*) alternated with red tides formed by the dinoflagellate *Noctiluca scintillans*, while extensive mucilage aggregates appeared in late June 2017 that consisted of several species, including the above-mentioned diatoms, the slime-producing dinoflagellate *Gonyaulax* cf. *fragilis*, and a foam-forming *Phaeocystis* sp. An increase in abundance of the known harmful dinoflagellate *Dinophysis* cf. *acuminata* was observed in October and November 2017, and a red tide caused by the photosynthetic ciliate *Mesodinium rubrum* in December 2017 was conspicuous throughout the sampling sites. Amplicon sequencing and taxonomic annotations detected 19 high-level (class) taxonomic groups at all sites, with Dinoflagellata being the most diverse group in terms of OTU richness, followed by Cercozoa, Ciliophora, and Bacillariophyta (see Genitsaris et al. 2020), largely in agreement with microscopy.

3.1. Coupling of taxonomic and functional diversity

In total, the 2812 OTUs retrieved after denoising and read processing were assigned to functional groups according to their biological trait annotations

(Table 1). Trait annotation was performed based on our knowledge of the life strategies of the common inhabitants of the Thermaikos Gulf in addition to extensive literature consultations. Taxonomic diversity, as reflected by α -diversity indices (OTU richness, Simpson's dominance, and equitability), fluctuated throughout the study. The number of OTUs ranged between 44, coinciding with a pronounced *N. scintillans* red tide, and 520 in a non-bloom period. Low values of Simpson's dominance (0.03 during the *N. scintillans* red tide) and low values of equitability (0.03 during the red tide) were calculated in the March bloom, representing the high dominance of

the *N. scintillans*-related OTU. However, the overall higher values during the rest of the samplings, excluding the samples from the mucilage aggregates in June that ranged from 0.56 to 0.98 for Simpson's dominance index and 0.31 to 0.75 for equitability, showed a rather evenly distributed unicellular eukaryote community. Functional indices also largely varied during the samplings: FRic ranged from 31 to 152, Rao's *Q* ranged from 0.01 during the spring red tide, indicating dominance of 1 single trait, to 0.24, and FEve ranged from 0.25 to 0.49 (Table 2, Fig. S2), suggesting that only a few traits were highly represented.

Table 1. Traits used to describe marine unicellular eukaryotes, trait annotations, and coverage of trait annotations (% of total OTUs that were assigned to a trait) for the total OTUs

Functional groups	Trait annotations	Functional categories	Coverage (%)
Minimum size (μm)	0.2–2 2–20 20–100 100–200 >200	Size as a master trait	67.6
Maximum size (μm)	0.2–2 2–20 20–100 100–200 >200		67.6
Trophic preference	Autotrophs Micrograzers Mixotrophs Nanograzers Picograzers Phagotrophs Decomposers Parasites	Resource acquisition	86.8
Optimum temperature ($^{\circ}\text{C}$)	0–10 10–15 15–20 20–25 25–30 >30	Optimum temperature is a proxy of maximum growth rates	23.8
Presence of spines	Yes No	Behaviour and defence mechanisms/predator avoidance	68.1
Mucilage production	Yes No		70.6
Colony formation	Yes No		79.8
Motility	Active Passive		86.8
Spore/cyst formation	Yes No	Life history, dispersal, and survival mechanisms	64.2
Potential harmfulness	Yes No	Ability to cause disruptive events	74.9

Positive coupling of taxonomic and functional indices was evident between OTU richness and FRic, and Simpson's dominance index and Rao's *Q*, while negative coupling between equitability and FEve was observed (i.e. a decrease in FEve with an increase in equitability; Fig. 1). Practically, when taxonomic richness and Simpson's dominance index increase, so do the respective functional indices. In contrast, taxonomic homogeneity does not correspond to functional evenness in our 1 yr dataset. This coupling of the taxonomic and functional diversity indices was tested on OTUs affiliated at lower taxonomic levels, i.e. at the level of family/genus/species. Similar trends of coupling between taxonomic and functional diversity indices were observed in both the entire dataset and the screened (reduced) dataset, the latter including only OTUs with high certainty taxonomic assignments at lower taxonomic levels (see Fig. S3), which corresponded to >70% of the total number of OTUs. This means that the functional assignment of traits of high-rank levels (e.g. phylum) does not simply mirror the taxonomy in our dataset.

3.2. Functional diversity of unicellular eukaryotic assemblages

The prevalence of specific biological traits in terms of number of reads showed seasonality and coincided with the traits characterizing the OTUs

Table 2. Number of OTUs, the α -diversity estimators (Simpson's dominance index, equitability) and the functional diversity estimators (functional richness, Rao's Q, functional evenness) of all samples at the White Tower, Aretsou Beach, Music Hall Coast and Harbour sampling sites

Sample date	Sample code	Number of OTUs	Simpson (1 – D)	Equitability (J)	Functional richness (FRic)	Rao's Q	Functional evenness (FEve)
White Tower							
15 March 2017	15MarWT	143	0.63	0.36	64	0.15	0.32
22 March 2017	22MarWT	44	0.03	0.03	31	0.01	0.49
29 March 2017	29MarWT	216	0.70	0.37	89	0.19	0.32
5 April 2017	05AprWT	182	0.77	0.45	89	0.20	0.34
12 April 2017	12AprWT	163	0.56	0.31	77	0.16	0.34
19 April 2017	19AprWT	223	0.89	0.61	89	0.19	0.30
26 April 2017	26AprWT	262	0.81	0.55	105	0.19	0.31
3 May 2017	03MayWT	281	0.84	0.57	105	0.19	0.30
9 May 2017	09MayWT	259	0.90	0.62	99	0.22	0.29
17 May 2017	17MayWT	205	0.60	0.37	83	0.14	0.30
24 May 2017	24MayWT	162	0.85	0.57	77	0.21	0.34
31 May 2017	31MayWT	158	0.83	0.56	78	0.19	0.37
7 June 2017	07JunWT	146	0.78	0.49	67	0.19	0.34
14 June 2017	14JunWT	208	0.84	0.54	85	0.21	0.27
21 June 2017	21JunWT	242	0.86	0.54	97	0.22	0.30
28 June 2017	28JunWT	94	0.36	0.22	48	0.10	0.36
4 July 2017	04JulWT	292	0.81	0.54	102	0.19	0.28
12 July 2017	12JulWT	256	0.84	0.53	99	0.24	0.29
19 July 2017	19JulWT	294	0.88	0.56	103	0.23	0.27
26 July 2017	26JulWT	292	0.95	0.67	103	0.23	0.26
2 August 2017	02AugWT	325	0.93	0.69	121	0.23	0.30
9 August 2017	09AugWT	202	0.90	0.59	84	0.24	0.32
23 August 2017	23AugWT	378	0.84	0.55	125	0.24	0.28
30 August 2017	30AugWT	292	0.94	0.69	110	0.24	0.30
6 September 2017	06SepWT	331	0.85	0.63	118	0.20	0.30
13 September 2017	13SepWT	243	0.93	0.66	92	0.23	0.29
20 September 2017	20SepWT	127	0.48	0.37	62	0.11	0.40
27 September 2017	27SepWT	376	0.87	0.64	127	0.20	0.28
4 October 2017	04OctWT	269	0.93	0.69	100	0.21	0.29
11 October 2017	11OctWT	331	0.95	0.73	126	0.22	0.30
18 October 2017	18OctWT	278	0.88	0.59	108	0.21	0.29
25 October 2017	25OctWT	276	0.84	0.54	114	0.19	0.31
1 November 2017	01NovWT	215	0.95	0.72	97	0.22	0.31
8 November 2017	08NovWT	252	0.74	0.47	103	0.16	0.30
15 November 2017	15NovWT	264	0.71	0.43	100	0.17	0.27
22 November 2017	22NovWT	313	0.82	0.59	111	0.18	0.27
29 November 2017	29NovWT	335	0.85	0.59	120	0.19	0.26
6 December 2017	06DecWT	267	0.88	0.63	105	0.20	0.31
13 December 2017	13DecWT	154	0.78	0.45	74	0.19	0.31
19 December 2017	19DecWT	202	0.80	0.48	88	0.19	0.32
27 December 2017	27DecWT	297	0.96	0.75	101	0.20	0.30
3 January 2018	03JanWT	257	0.93	0.67	100	0.23	0.29
10 January 2018	10JanWT	245	0.92	0.62	103	0.19	0.31
17 January 2018	17JanWT	520	0.98	0.74	152	0.20	0.22
24 January 2018	24JanWT	220	0.85	0.62	84	0.18	0.31
31 January 2018	31JanWT	319	0.91	0.64	113	0.22	0.27
6 February 2018	06FebWT	210	0.59	0.36	90	0.22	0.30
Aretsou Beach							
12 April 2017	12AprAR	137	0.73	0.49	63	0.17	0.30
9 May 2017	09MayAR	292	0.89	0.61	105	0.19	0.25
7 June 2017	07JunAR	166	0.77	0.48	77	0.19	0.31
28 June 2017	28JunAR	152	0.51	0.32	74	0.13	0.36
26 July 2017	26JulAR	220	0.92	0.62	97	0.24	0.31
23 August 2017	23AugAR	284	0.74	0.46	109	0.22	0.32
20 September 2017	20SepAR	325	0.96	0.72	112	0.22	0.27

Table continued on next page

Table 2 (continued)

Sample date	Sample code	Number of OTUs	Simpson (1 – D)	Equitability (J)	Functional richness (FRic)	Rao's Q	Functional evenness (FEve)
Aretsou Beach (continued)							
18 October 2017	18OctAR	401	0.96	0.72	130	0.21	0.26
15 November 2017	15NovAR	320	0.89	0.58	113	0.20	0.25
13 December 2017	13DecAR	107	0.60	0.34	61	0.16	0.36
10 January 2017	10JanAR	310	0.94	0.67	116	0.21	0.28
6 February 2017	06FebAR	211	0.82	0.49	84	0.24	0.32
Music Hall Coast							
12 April 2017	12AprMH	204	0.89	0.56	88	0.20	0.34
9 May 2017	09MayMH	279	0.88	0.63	100	0.20	0.27
7 June 2017	07JunMH	271	0.93	0.64	106	0.21	0.28
28 June 2017	28JunMH	226	0.86	0.53	90	0.20	0.30
26 July 2017	26JulMH	246	0.91	0.62	103	0.20	0.30
23 August 2017	23AugMH	256	0.76	0.49	97	0.24	0.32
20 September 2017	20SepMH	238	0.83	0.58	99	0.21	0.33
18 October 2017	18OctMH	368	0.94	0.69	115	0.20	0.25
15 November 2017	15NovMH	420	0.84	0.55	134	0.19	0.26
13 December 2017	13DecMH	251	0.77	0.43	94	0.19	0.28
10 January 2017	10JanMH	175	0.84	0.54	82	0.19	0.34
6 February 2017	06FebMH	215	0.86	0.53	94	0.24	0.29
Harbour							
12 April 2017	12AprHB	183	0.61	0.38	84	0.13	0.32
9 May 2017	09MayHB	291	0.90	0.59	104	0.21	0.26
7 June 2017	07JunHB	204	0.71	0.46	90	0.17	0.30
28 June 2017	28JunHB	162	0.64	0.38	72	0.17	0.33
26 July 2017	26JulHB	299	0.94	0.67	113	0.23	0.27
23 August 2017	23AugHB	286	0.90	0.45	111	0.24	0.33
20 September 2017	20SepHB	218	0.66	0.45	88	0.16	0.32
18 October 2017	18OctHB	150	0.49	0.33	68	0.12	0.37
15 November 2017	15NovHB	111	0.81	0.46	57	0.18	0.37
13 December 2017	13DecHB	268	0.73	0.40	106	0.19	0.30
10 January 2017	10JanHB	199	0.83	0.52	87	0.19	0.30
6 February 2017	06FebHB	152	0.80	0.50	72	0.22	0.33

responsible for the bloom events (i.e. OTUs related to the diatom genera *Chaetoceros*, *Cylindrotheca*, *Lep-
tocylin-
drus*, and *Skeletonema*, to the dinoflagellates *N. scintillans* and *Gonyaulax* spp., and to the haptophyte *Phaeocystis* sp.). During the *N. scintillans* red tides, large phagotrophs dominated as expected, and the non-harmfulness, no colony formation, active movement traits were also represented with high numbers of reads (Fig. 2). In the warmer months of May to August, medium-sized mucilage-producing colony-forming harmful autotrophs were dominant along with a large-scale mucilaginous aggregate phenomenon. By the end of this warm period, in August, the small-sized mixotrophs and autotrophs were the most abundant. With the succession of colder months in September to November, changes in traits towards active movement and between heterotrophic and autotrophic strategies were observed, along with the bloom of the known toxin-producing dinoflagellate *D. cf. acuminata*. Finally, in December

to February, traits of small size and parasitic strategy were initially abundant, along with a *M. rubrum* bloom, and were followed by the dominance of medium-sized colony-forming and spine-bearing species, with micro- and nanograzing strategies being prominent (Fig. 2).

Euclidean distances showed that certain traits were co-occurring throughout the sampling period; in particular, small size was grouped with parasitic trophic strategy, medium size was associated with autotrophic strategy and harmful traits, and large size with phagotrophic strategy. Potential harmfulness was clustered with spore formation and mucilage production, which points to ecosystem disruptive events, and not only toxin production ability. Additionally, trait trade-offs were recognized: active movement was linked with no colony formation as expected, and mixotrophy was associated with the absence of predator avoidance traits and survival characteristics such as spore formation (Fig. 2).

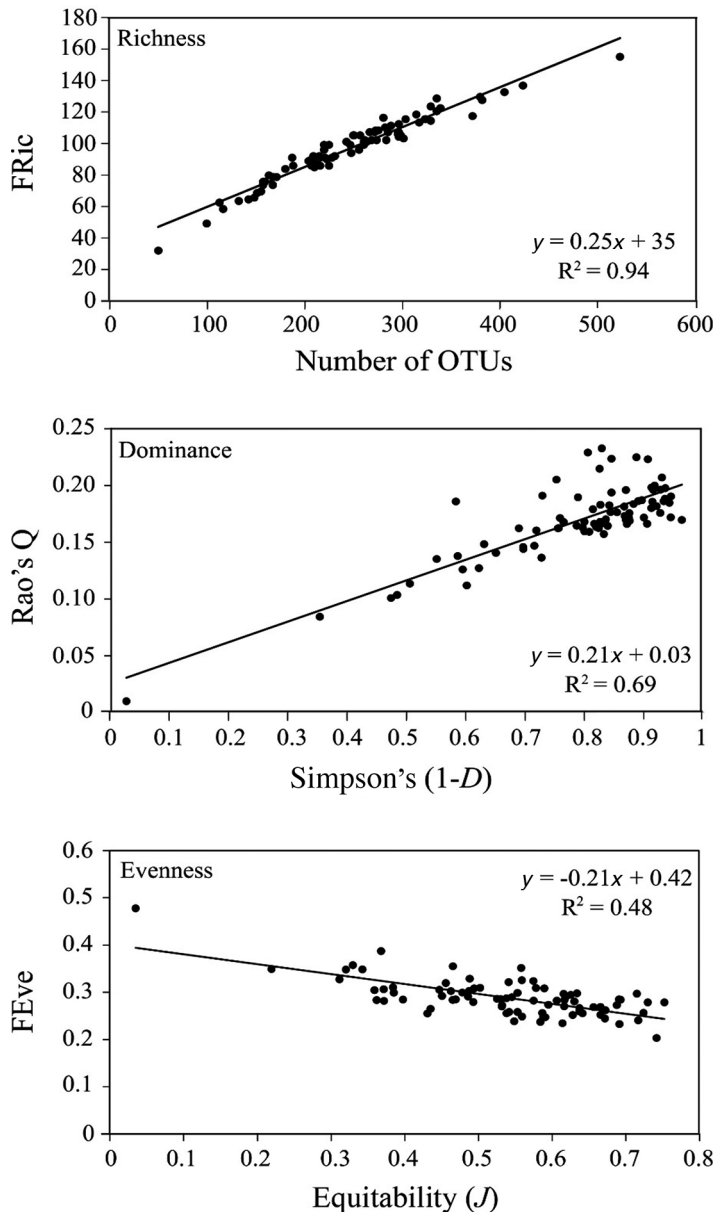


Fig. 1. Coupling of (a) number of OTUs and the functional richness index (FRic); (b) Simpson's dominance index and Rao's Q index; and (c) equitability (J) and the functional evenness index (FEve)

3.3. Optimal traits through seasonal succession

CWM values throughout the study suggested that, for certain functional categories (Table 1), the unicellular eukaryote assemblages were characterized by 1 optimal trait throughout the study, while for others, the optimal trait altered temporally. For example, no colony formation and absence of spines were preferred in almost all assemblages. On the other hand, the optimal traits for trophic preference, size distri-

bution, potential harmfulness, motility, mucilage, and spore formation varied across temporal succession of red tides, plankton blooms, and aggregates, forming locally optimal strategies (Fig. 3). Frequently, the optimal traits in the assemblages were identical to the characteristic traits of the most dominant OTUs. The optimal trophic preference of the assemblages in most samplings was mixotrophy, which alternated with autotrophy, especially during warm months and diatom blooms. Non-harmful taxa were abundant during most of the year, except for the June samples characterized by the mucilage aggregates, and the samples of the *M. rubrum* red tide dominated by parasites. However, on some occasions the optimal traits did not coincide with the traits of the most dominant OTUs. For example, the nanograzing strategy was the optimal trophic strategy in December to January during the *M. rubrum* red tide, a known mixotroph. Larger eukaryotes ($>100\ \mu\text{m}$) dominated especially after July; before July, the CWM values showed no clear size trait selection. Finally, actively moving taxa were favoured as optimal throughout the study, independently of the movement characteristics of the dominant OTUs, except in sporadic individual samplings (Fig. 3).

3.4. Relationship between traits and environmental parameters

Generalized linear model (GLM) analysis revealed significant positive correlations of NH_4 concentrations with Simpson's dominance and Rao's Q. On the contrary, significant negative correlations of SiO_4 and PO_4 with Simpson's dominance, equitability, and Rao's Q, and of SiO_4 with FEve were recorded (Table 3), reflecting the coupling of taxonomic and functional indices described in Section 3.1. Nutrient $\text{NH}_4:\text{NO}_3$ and N:P ratios showed no significant correlations with the diversity indices.

The network analysis, according to the MIC of pairwise comparisons between biological traits and environmental values, indicated 2 subnetworks of 33 edges between 27 nodes and 1 edge between 2 nodes, respectively (Fig. 4). Temperature was negatively associated with mixotrophs and parasites, while it was positively correlated with picograzers, autotrophs, potential harmfulness, presence of spines, and mucilage production, in association with the large mucilaginous aggregate event of June 2017. Small size ($<20\ \mu\text{m}$) exhibited negative correlations with the nutrients. Further, most nutrients, especially N, were negatively correlated with para-

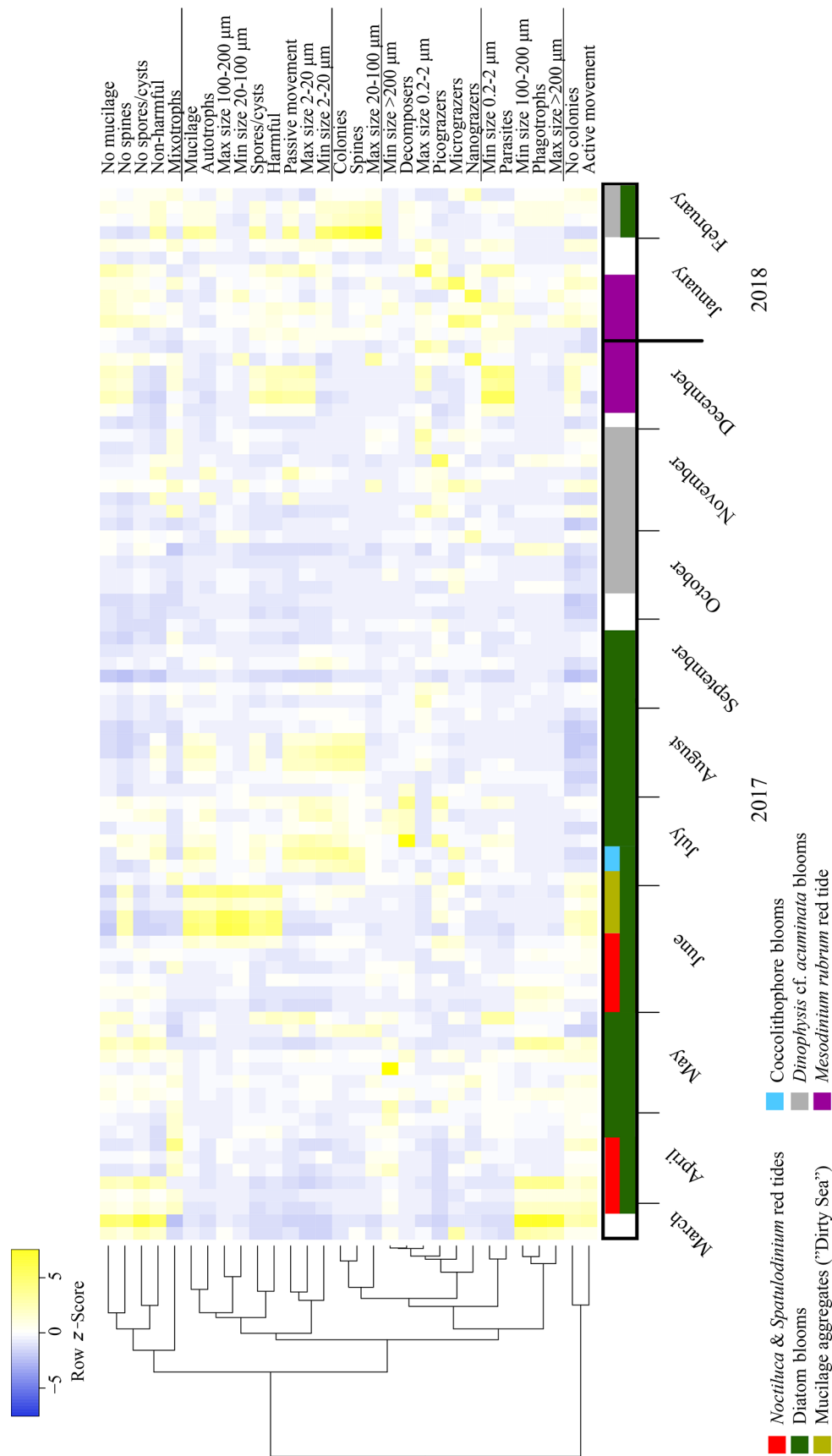


Fig. 2. Succession of the abundance as reflected by the number of reads assigned to each biological trait. The average linkage clustering method with Euclidean distances was used to cluster biological traits with similar trends. The coloured bar below the plot represents periods of plankton blooms as identified by microscopy and presented in Genitsaris et al. (2019). The z-score describes the relationship between the number of reads per trait and the mean of the total number of reads in each sample. When the z-score is 0, it indicates that the score of the data point is identical to the mean score, and a z-score value of 1.0 indicates a value that is 1 SD from the mean. Negative scores show that the z-score is below the mean

Table 3. Correlations of environmental variables and diversity indexes based on generalized linear model analysis. Significant correlations are given in **bold**. ***p < 0.001, **p < 0.01, *p < 0.05

Response variable	Independent variable	Coefficient	p
OTU richness	Temperature	0.01	0.51
	Salinity	0.01	0.80
	SiO ₄	-0.02	0.10
	PO ₄	-0.43	0.15
	NO ₂	-0.01	0.99
	NO ₃	-0.01	0.83
	NH ₄	0.04	0.24
	NH ₄ :NO ₃ ratio	-0.02	0.59
	N:P ratio	-0.01	0.18
Simpson's index	Temperature	0.01	0.74
	Salinity	-0.03	0.14
	SiO₄	-0.03	***
	PO₄	-0.48	***
	NO ₂	0.08	0.64
	NO ₃	0.02	0.06
	NH₄	0.03	*
	NH ₄ :NO ₃ ratio	0.02	0.23
	N:P ratio	-0.01	0.14
Equitability <i>J</i>	Temperature	0.01	0.49
	Salinity	-0.03	0.35
	SiO₄	-0.04	***
	PO₄	-0.51	*
	NO ₂	0.08	0.73
	NO ₃	0.02	0.18
	NH ₄	0.04	0.11
	NH ₄ :NO ₃ ratio	0.01	0.54
	N:P ratio	-0.01	0.42
Functional richness	Temperature	0.01	0.40
	Salinity	0.01	0.86
	SiO ₄	-0.02	0.08
	PO ₄	-0.25	0.18
	NO ₂	0.12	0.59
	NO ₃	0.01	0.76
	NH ₄	0.02	0.42
	NH ₄ :NO ₃ ratio	-0.01	0.75
	N:P ratio	-0.01	0.08
Rao's <i>Q</i> index	Temperature	0.01	0.31
	Salinity	-0.02	0.45
	SiO₄	-0.02	**
	PO₄	-0.50	**
	NO ₂	-0.14	0.45
	NO ₃	0.01	0.48
	NH₄	0.04	*
	NH ₄ :NO ₃ ratio	-0.01	0.99
	N:P ratio	-0.01	0.44
Functional evenness	Temperature	0.01	0.96
	Salinity	0.01	0.95
	SiO₄	0.01	*
	PO ₄	0.16	0.09
	NO ₂	-0.05	0.67
	NO ₃	-0.01	0.75
	NH ₄	-0.01	0.46
	NH ₄ :NO ₃ ratio	-0.01	0.76
	N:P ratio	0.01	0.37

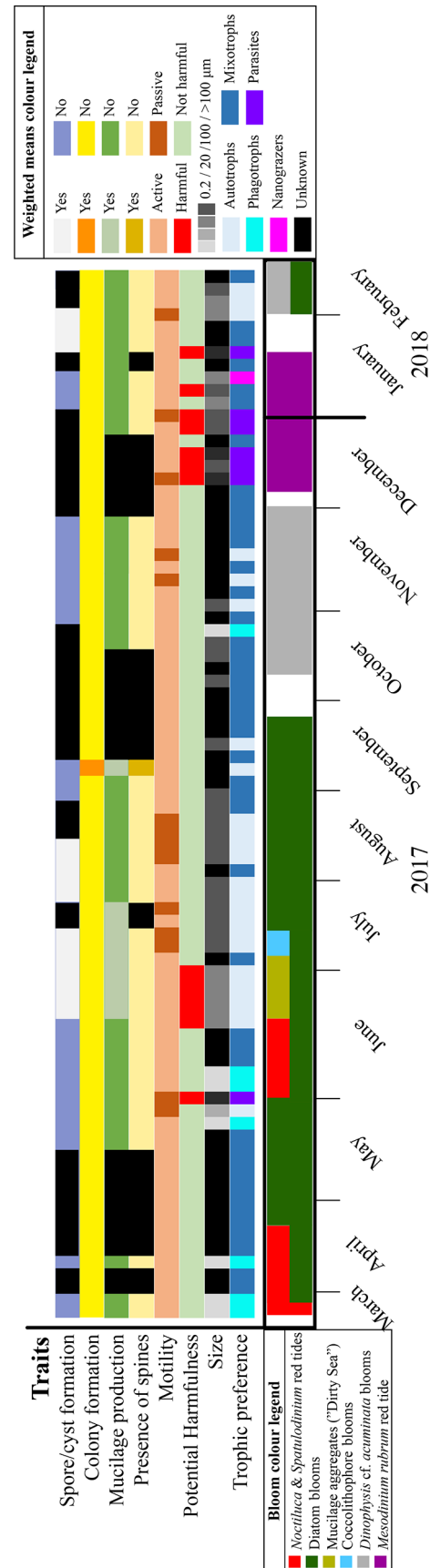


Fig. 3. Temporally optimal biological traits weighted by abundance levels (number of reads) in the community, along the succession of plankton blooms during the study

sites and decomposers, and negative correlations of PO_4 with autotrophs and colony formation were also observed (Fig. 4).

4. DISCUSSION

Previous microscopic (Genitsaris et al. 2019) and taxonomic amplicon analyses (Genitsaris et al. 2020) of the same samples revealed marked seasonal succession of unicellular eukaryotic assemblages but no spatial separation, reflecting the environmental homogeneity between the different sampling points. Overall, the environmental variables measured, especially temperature and salinity, were hypothesized to drive the seasonal variability, whereas nutrient availability was never limiting throughout the study. Taking this well-studied coastal area as a model system based on the repetitive and frequent disruptive blooms observed seasonally, we attempted to determine whether (1) the taxonomic annotation stage of the increasingly frequent amplicon-based studies is just an intermediate step towards ecological understanding of marine ecosystem function, and (2) a trait-based approach could be more informative and a favoured endpoint. Therefore, functional diversity indices analogous to commonly used taxonomic in-

dices in similar investigations were calculated and compared. We hypothesized that positive coupling would indicate that it is not important to identify what species are present but what they are doing. On the contrary, negative or no coupling would suggest that it is still important to describe taxonomic composition to answer ecological questions.

4.1. Coupling of taxonomic and functional diversity

The implementation of biological trait annotation to the taxonomic composition of marine unicellular eukaryotic assemblages is an approach to overcoming the limitations of ecological interpretations based solely on taxonomic diversity, as phylogenetic relatedness in eukaryotes does not necessarily reflect functional diversity (Seeleuthner et al. 2018). Here, we attempted to link taxonomic diversity indices with their corresponding functional diversity indices. Species richness and FRic are closely associated to the number of taxa identified in a community, while Simpson's dominance and Rao's Q indices are highly dependent on species abundance. This attempt revealed a strong positive coupling of species richness with FRic, and of Simpson's dominance with

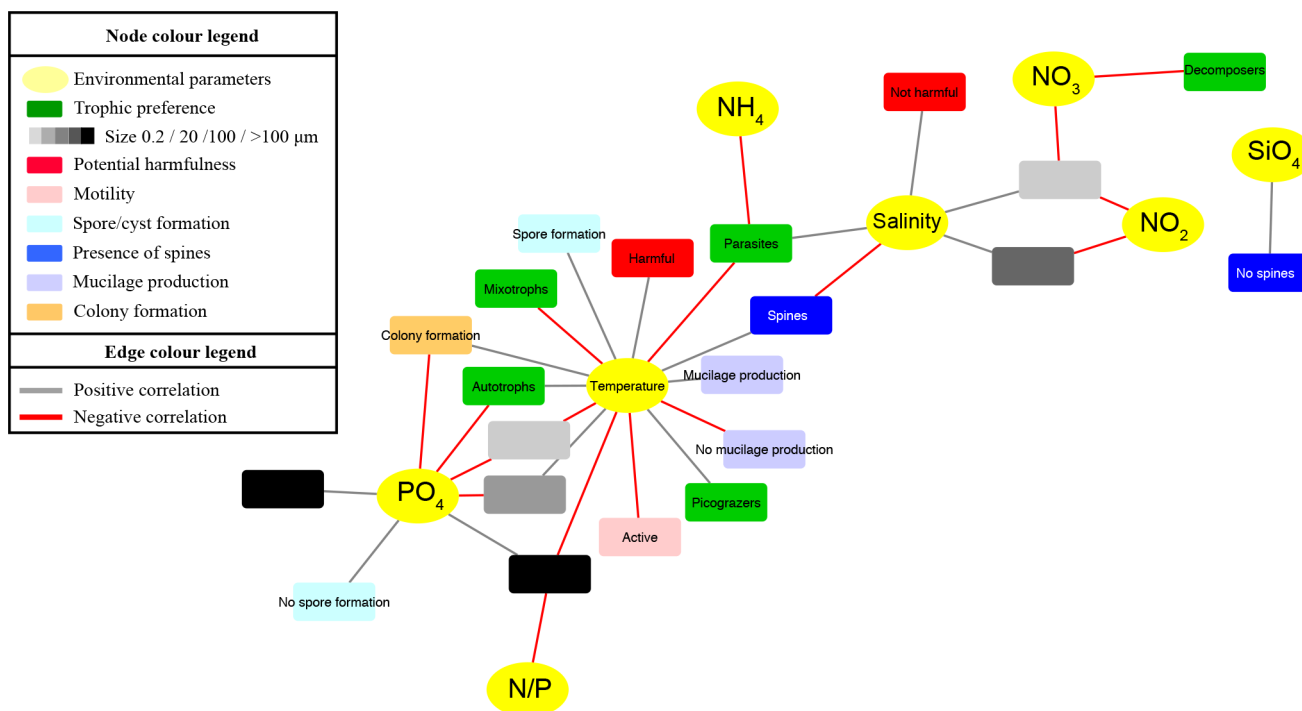


Fig. 4. Network diagram of the connections (edges) of environmental parameters with biological traits (nodes) with significant correlations (p -value < 0.05) based on the maximal information coefficient (MIC) scores during the study. Different colours represent different functional groups to which each biological trait belongs

Rao's Q , while a strong negative coupling of equitability with FEve was observed (Fig. 1). While Ramond et al. (2019) reported a conserved coupling of taxonomic and functional diversity across traits and size classes in unicellular eukaryotes in a similar analysis, functional redundancy by means of shared metabolic pathways is common in phylogenetically variable prokaryotes (Louca et al. 2016, Haggerty & Dinsdale 2017). This signal also seemed to extend into a marine protistan diversity hotspot where the confounding effects of dispersal and strong selective pressures favoured a limited number of traits (Ramond et al. 2021). In our 1 yr study, the positive coupling of the number of OTUs per sample with FRic suggests that an increase in taxonomically/phylogenetically different units increases the pool of traits, a common ecological concept. To highlight this, modern diversity and ecosystem functioning debates support the idea that it is actually 'the specific features of the species in an ecosystem that determine its function—not their number per se' (Grime 1997, p. 1260). Taxonomic dominance also followed a similar coupling trend with functional dominance, as reflected by Rao's Q index. Rao's Q is analogous to Simpson's dominance ($1 - D$) when the species exhibit identical traits (Pillar et al. 2013), i.e. when high taxonomic dominance by 1 or a few species is observed, then high dominance of 1 or a few traits is also expected, which is supported by our dataset. However, a negative coupling of taxonomic evenness—based on the equitability index—with FEve was observed, suggesting that homogeneity in relative abundance and read distribution among OTUs corresponds to underutilization of the niche space they occupy (per Mason et al. 2005). In effect, given that resource availability was not limiting during the study, the low functional evenness observed together with the homogenous abundance distribution of OTUs indicates that these resources are not fully exploited. It is plausible that, during our 1 yr study, different taxa favoured similar cost-effective strategies and this low number of apparent traits skewed the functional evenness towards the deployment of few but efficient life strategies, i.e. low FEve values. A more demanding environment could potentially coerce the employment of a larger range of traits per taxon which, although possibly costly, could provide better chances of survival. So, to return to the question posed in Section 1, our results showing negative coupling of taxonomic vs. functional evenness indicate that the full picture of the aquatic protistan community structure and function may ultimately be obscured. For example, if only the func-

tional diversity was available, the high evenness of the distribution of the unicellular eukaryotic assemblages in the area throughout the study would not be apparent, and valuable knowledge of the system would be lost due to potentially significant misconceptions of the state of the art. Thus, based on our results, knowledge of the taxonomic composition of assemblages is not merely a descriptive whimsy but an ecologically meaningful and necessary step towards understanding community function in aquatic systems.

4.2. Co-occurrence of biological traits and trait trade-offs during seasonal succession

'The paradox of the plankton' as initially described by Hutchinson (1961) is based on the fact that plankton diversity is too high to be explained by the limited range of resources: ultimately the strongest competitor should dominate and in a given plankton assemblage, only a few well-adjusted species should be detected. However, plankton assemblages are diverse because of gradients of nutrients, light, and turbulence, differential predation (Worm et al. 1999), constantly changing environmental conditions (Descamps-Julien & Gonzalez 2005), selective top-down control (Wiggert et al. 2005), and hydrographic variability (Károlyi et al. 2000). In order to successfully exploit a continuously variable environment and benefit from biotic interactions, a structure in the traits of plankton species is presupposed (Irwin & Finkel preprint doi:10.1101/148312), and trade-offs—sensu preference of one strategy at the expense of another—or co-occurrence patterns of specific traits are assumed. During the present study, different traits were favoured through plankton seasonal succession (Fig. 2), either by succession of the optimal traits for each bloom, or by non-random co-occurrence of traits (e.g. potential harmfulness co-occurred with autotrophy during the mucilage aggregations, and with parasitism during the *Mesodinium rubrum* red tide) (Fig. 3). This points to the concept that species exclusion and coexistence mechanisms (succession and co-occurrence of optimal traits, respectively) shape the community dynamics according to the optimal traits necessary for facing the exogenic pressures in highly variable environments (Kneitel & Chase 2004).

Euclidean distances indicated correlations of size with trophic strategies and potential harmfulness, with varying sizes favouring different traits (Fig. 2). The trophic preferences of the assemblages varied,

but frequently alternated among autotrophy, mixotrophy, and a few recorded cases of parasitism in specific samplings. In particular, small species were correlated with parasitism, medium-sized with autotrophy and potential harmfulness, and large species with phagotrophy (Fig. 2). Ramond et al. (2019) recognized patterns of trophic strategy distribution across size fractions, attributing these to the smaller energy requirements of picoplankton which helps them thrive in harsher environments (Worden et al. 2004). Indeed, cell size is a master trait, influencing physiological performance, such as growth rates, and the environmental requirements of plankters, i.e. resource acquisition, sinking, and susceptibility to grazing (Brown et al. 2004, Sommer et al. 2017). Following a similar approach to that used here in coastal protistan communities, Ramond et al. (2019) proposed that the persistence of parasites in the small fraction can be attributed to the release of several small spores from hosts (Guillou et al. 2008). Parasitism of marine protists has been an overlooked trait, but recent research suggests that this strategy is more widespread than previously thought (Christaki et al. 2017, Hassett 2020), and can constitute a large missing link in marine trophic relationships (Christaki et al. 2014). In addition, the predominance of mucilage-producing diatoms, in succession to the blooms of the large dinoflagellate *Noctiluca scintillans* with its phagotrophic lifestyle, was probably the cause of the observed medium/large size and trophic strategy linkages. In Thessaloniki Bay, plankton succession did not follow the typical succession of species as observed in coastal areas and expected simply by season alteration. Here, the typical spring diatom bloom observed in numerous marine systems extends throughout the year as nutrients are not limiting (Genitsaris et al. 2019), and light availability follows the Mediterranean 'non-biological winter' model postulate (Moustaka-Gouni et al. 2014). Thus, medium- and/or large-sized diatoms, benefiting from the nutrient-rich environment, are always an abundant food for large dinoflagellate predators, such as *N. scintillans*. We suggest that the persistent blooms of *N. scintillans* stemmed from the ubiquitous presence of available food regenerating excess NH_4 concentrations (Xiao et al. 2018), and in turn, this grazing pressure benefited the dominance of diatom species with anti-grazing adaptations, such as mucilage production, i.e. adaptations with potential harmful effects on ecosystem health. The functional group of spore formation was associated with potential harmfulness, while colony formation was clustered with the presence of spines, and both these traits are

linked to predator avoidance (Fig. 2). Even though higher predators were not examined in the present study, diverse and abundant zooplankters have been documented in the area (Siokou-Frangou et al. 2005, Zervoudaki et al. 2014), favouring the adaptation of these traits as survival mechanisms.

Furthermore, mixotrophy was associated with the absence of predator-avoidance traits and survival characteristics such as spore formation, and trait trade-offs between active movement and no colony formation were identified (Fig. 2). Both these trade-offs indicated that the preferred adaptation, i.e. active movement and mixotrophy, either required too much energy for the development of other ecologically beneficial traits, or is by itself enough to offer advantages in niche occupation antagonism. Active movement is usually favoured in stratified coastal waters (Jones et al. 1984); however, since our sampling sites were at the urban front of Thessaloniki Bay, with maximum depth no more than 4 m, stratification was not observed. On the other hand, actively moving dinoflagellate-related OTUs, linked to high N/P ratios (Genitsaris et al. 2019), were abundant throughout the present study (Genitsaris et al. 2020) and evidently, the active motility trait was frequently favoured. Even though trait trade-offs are extensively discussed in the literature (Edwards et al. 2011, Fiksen et al. 2013, Breton et al. 2017), a unified pattern of trait optimality in coastal unicellular assemblages is not yet evident: different pressures shape niche availability and niche exploitation is influenced by the available species and trait pools in each ecosystem.

Concerning the present study, the succession of plankton blooms and red tides in Thessaloniki Bay offered an original field of study where phytoplankton succession deviates from expected trends because of heavy eutrophication. Our method can be applied to other coastal sites with heavy anthropogenic influences, although it does have some limitations. For example, the approach offers little information on the physiological responses of marine unicellular eukaryotes, as these have usually been documented for cultivated species and cannot be easily annotated to molecular taxonomic units at the genus or species level (Ramond et al. 2019). Inherent limitations of HTS-generated microbial diversity, related to marker choice (Casey et al. 2021), PCR biases (Bik et al. 2012), and the differential effectiveness of DNA extraction protocols for the various microeukaryotic taxa (Muñoz-Colmenero et al. 2021), lead to semi-quantitative information that can skew both taxonomic and functional interpretations. Fur-

thermore, several taxa exhibit a wide range of intra-taxon trait variability depending on climate (Barton et al. 2015, Irwin et al. 2015), seasonal variability (Weithoff & Gaedke 2017), and life stage (Irwin & Finkel preprint doi:10.1101/148312). Highlighting the occurrence of contrasting traits within the same species during different lifecycle stages, the high presence of MALVs in the study area complicated trait annotation, since these taxa are characteristic endoparasites (Coats 1999) and thus have no active movement; they also spend part of their life as zoospores (Jephcott et al. 2016) actively swimming in search of their hosts. The solution in such cases should consider the dominant trait that is necessary for the survival and reproduction of the taxon and has greater influence on the functions of the community and the ecosystem. These considerations might hinder a more comprehensive picture of the complex ecological responses of the unicellular assemblages, but there is room for this approach to be integrated into future metagenomic and metatranscriptomic studies in aquatic systems that reveal trait–gene pairings and more accurate functional patterns.

4.3. Effects of environmental parameters on functional diversity and biological traits

The environmental variables measured, i.e. temperature, salinity, particulate organic phosphorus (POP), $\text{NO}_3^-/\text{NO}_2^-$, NH_4^+ , SiO_4^- , and PO_4^- were selected as potential key drivers governing the ecological niche of plankton (Boyd et al. 2010). Light availability may also have direct or indirect effects at the plankton community level (Ruiz-González et al. 2013, Yamamichi et al. 2018), but since our sampling sites were shallow (maximum depth <4 m), light was never a limiting factor. While nutrient concentrations were high and not limiting throughout the study, Simpson's dominance and Rao's Q indices showed significant negative correlations with SiO_4 , PO_4 , and POP, and positive correlations with NH_4 . Furthermore, significant negative correlations were calculated for SiO_4 and PO_4 with equitability, and a positive correlation between SiO_4 and FEve (Table 3). N-enrichment has been shown to promote phytoplankton biomass and increase activity (Rahav et al. 2018), which in turn may favour an increase in diversity estimators. However, when high and maintained nutrient availability is observed, it can indicate tracers of other complex processes (e.g. excretion by grazers and release from sediments, denitrification and nitrification, transport processes, and runoff

from land), which could lead to inter-taxon competition for nutrient uptake, and ultimately to lower diversity (Vallina et al. 2014). Furthermore, temperature variability did not appear to significantly impact on the diversity estimators (Table 3), in contrast to the metabolic theory that implies a strong relationship between temperature and diversity (Brown et al. 2004) following Bergmann's rule extension for marine phytoplankton (Sommer et al. 2017), which proposes that warming will increasingly lead to the dominance of small-sized cells. Generally, small or thermophilic organisms have faster ecological dynamics, resulting in a higher standing stock of species (Brown et al. 2004). This was not apparent in our dataset, possibly because the Mediterranean climate that characterizes the area causes a marked succession of the 4 seasons: diversity gradients due to temperature do not have enough time to form as temperature fluctuations happen fast (water temperatures between 9.6 and 29.3°C were recorded during the present study; Genitsaris et al. 2019). Furthermore, responses of plankton to bottom-up controls and/or other environmental variables can be shaped by the interactive effect of multiple factors (Boyd et al. 2010). In addition, the realization of the community niches, which are reflected by its taxonomic and functional diversity, is modelled by a complex combination of abiotic and biotic interactions (Brun et al. 2015), as well as species sinking rates (Acevedo-Trejos et al. 2015), which were not accounted for here.

Concerning relationships between environmental parameters and specific biological traits, temperature was negatively correlated with small size (<20 μm) and positively correlated with sizes of 20 to 100 μm (Fig. 4). The relationship between phytoplankton size and temperature based on Bergmann's rule has been described in detail by Sommer et al. (2017). Temperature was also positively linked to mucilage production, spore formation, and potential harmfulness. This can be attributed to the succession of mucilage-producing diatom blooms, and the mucilage aggregates that were ubiquitous during the warm months. N and P were also negatively correlated with small sizes and autotroph/decomposer strategies (Fig. 4). Competitive abilities for nutrient uptake are reversed with size, meaning that large individuals assimilate nutrients at decreased rates compared to smaller cells (Edwards et al. 2011). However, PO_4 was positively correlated with large size, suggesting a multivariate trait trade-off controlling size structuring. In other words, constraints between 2 traits might not be observed as a negative correlation if a considerable variation in a third trait

significantly influences this paired correlation (van Noordwijk & de Jong 1986). Overall, given that nutrients were never limiting throughout the present study, they do not act as a selective force on species and traits, but could be tracers of other processes which might be relevant for the succession of biological trait trade-offs or co-occurrences. Thus, in the community's trade-off axes, we should not neglect the biotic pressures expressed by the temporally optimal trophic strategies identified.

The seasonal succession of plankton is a complex process of community assembly that is shaped by variable external forces and internal interactions (Sommer et al. 1986). The major drivers can be summarized into physics (light and stratification), grazing by Metazoa, nutrient availability, and trophic cascades (Sommer et al. 2012). In this eutrophic coastal area, with non-limiting inflow of nutrients, non-stratified waters, and ample light, i.e. low environmental selective pressures, we observed a coupling of taxonomic and functional richness and dominance. On the contrary, a negative coupling of taxonomy and function with the evenness scores was evident, suggesting a skewed tendency towards few but effective life strategies. In these cases, taxonomic descriptions can be ecologically meaningful and a necessary step towards understanding community function. Similar trends of coupling between taxonomic and functional diversity indices were observed when both high-ranked taxonomic groups (e.g. phylum) and low-level resolution (family/genus/species) were considered, indicating that the functional assignment of traits does not simply mirror the taxonomy in our dataset. The low-pressure environment in the study area is compatible with the observed non-typical temporal succession of the unicellular eukaryotic plankters occurring as a result of biotic interactions. Thus, a plausible scenario is that biotic pressures force marine eukaryotes to exploit narrowed niches by adopting specific strategies/traits that favour their survival. This increases taxonomic and functional diversity and opens pathways for the autogenic succession of blooms, characterized by survival adaptations of the blooming taxa that are a direct response to the preceding assemblage/bloom.

Data availability. The 18S rRNA gene sequencing data are available in the NCBI-SRA database under the accession number PRJNA552665.

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