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Interannual dynamics of putative parasites (Syndiniales Group II) in a coastal ecosystem

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Abstract

Temporal dynamics of Syndiniales Group II were investigated combining 18S rDNA amplicon sequencing and direct microscopy counts (fluorescence in situ hybridization-tyramide signal amplification [FISH-TSA]) during 5 years. The study was undertaken in meso-eutrophic coastal ecosystem, dominated by diatoms, the haptophyte *Phaeocystis globosa* and exhibiting relatively low dinoflagellate abundance (max. 18.6×10^3 cells L^{-1}). Consistent temporal patterns of Syndiniales Group II were observed over consecutive years highlighting the existence of local populations. According to sequencing data, Syndiniales Group II showed increasing abundance and richness in summer and autumn. Dinospores counted by microscopy, were present at low abundances and were punctuated by transient peaks. In summer dinospore highest abundance (559×10^3 L^{-1}) and prevalence (38.5%) coincided with the peak abundance of the dinoflagellate *Prorocentrum minimum* (13×10^3 L^{-1}) while in autumn Syndiniales Group II likely had more diversified hosts. Although, several peaks of dinospore and read abundances coincided, there was no consistent relation between them. Ecological assembly processes at a seasonal scale revealed that stochastic processes were the main drivers (80%) of the Group II community assembly, though deterministic processes were noticeable (20%) in June and July. This latter observation may reflect the specific Syndiniales—dinoflagellate interactions in summer.

INTRODUCTION

Parasitism greatly influences ecosystem functioning by altering food web structure carbon flow (Hudson et al., 2006; Worden et al., 2015). For example, by damaging their phytoplanktonic hosts, parasites decrease the primary production that sustains the trophic web (e.g., Kagami et al., 2007; Rasconi et al., 2011). Consequently, they produce labile dissolved organic matter that can be used by heterotrophic prokaryotes and zoospores that are consumed by zooplankton (Gleason et al., 2008). In particular, Syndiniales which are known to infect a wide range of organisms have attracted additional attention in recent decades due to their repeated occurrence in sequence datasets (e.g., Guillou et al., 2008; López-García et al., 2001). High-throughput sequencing of marine planktonic communities have shown that the putative parasites Syndiniales are likely to be the most abundant and diversified members of the planktonic parasite community (e.g., Christaki et al., 2017; Guillou et al., 2008). Current knowledge concerning spatio-temporal structuring and the host range of Syndiniales is scarce, due to the difficulties of identifying the parasite–host consortia.(e.g., Sassenhagen et al., 2020 and references therein). Within the order of

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Syndiniales, the dominance of Group I is well established in coastal and open-ocean waters (e.g., Christaki et al., 2017; Guillou et al., 2008; Sassenhagen et al., 2020; Sehein et al., 2022). Direct observations evidenced association of members of Group I with ciliates (review by Skovgaard, 2014) and single cell amplification has suggested that Group I could be associated to diatoms (Sassenhagen et al., 2020). Syndiniales Group II association with dinoflagellates has been directly evidenced through microscopic observations of hybridized cells (e.g., Chambouvet et al., 2008; Salomon et al., 2009; Siano et al., 2011) thanks to specific fluorescence in situ hybridization (FISH) probes (Chambouvet et al., 2008) and has been also indirectly inferred via statistical approaches using metabarcoding data (e.g., network analysis, Christaki et al., 2017; Anderson & Harvey, 2020). The life-cycle of these parasites is well understood since 1964 (Cachon, 1964). It is characterized by an alternation between a biflagellated free-living infective stage (the dinospore) and an intracellular stage (the trophont) which grows and can expand up to filling the whole host cell. The maturation of the trophont lasts a few days and can eventually kill the host and release a motile worm-shaped multinucleated and multiflagellated structure (the vermiforme). It can then release within a few hours hundreds of dinospores, each potentially capable of infecting a novel host (Cachon, 1964; Coats & Park, 2002).

Owing to the potential of Syndiniales Group II to terminate toxic dinoflagellate blooms most of the previous studies focusing on these algae's have been restricted to environments and periods in relation to dinoflagellate blooms (Alves-de-Souza et al., 2012; Chambouvet et al., 2008; Velo-Suárez et al., 2013). High prevalence of Syndiniales is usually associated with marked densities of host organisms, stratification, and may be influenced by the availability in nutrients (Alves-de-Souza et al., 2015; Sehein et al., 2022) though it has also been reported that dinospores (free-living, small flagellated Syndiniales forms, typically <10 μm) were able to infect dinoflagellates at relatively low dinoflagellate abundances (Salomon et al., 2009) and in oligotrophic waters (Siano et al., 2011).

The main objective of this study was to investigate the temporal dynamics of Syndiniales Group II in a productive constantly mixed coastal system—the eastern English Channel (EEC). The EEC is characterized by diatom communities punctuated by spring blooms of the haptophyte *Phaeocystis globosa* and exhibiting relatively low dinoflagellate abundances (e.g., Grattepanche, Breton, et al., 2011). A previous temporal survey in the same area revealed the occurrence of Syndiniales and provided insights into their temporal dynamics inferring their potential hosts through network analysis (Christaki et al., 2017). However, co-occurrence networks infer putative associations but do not necessarily reveal real interactions. Direct observations are needed to identify microbial interactions such as parasitism.

Parasitic taxa usually present short transient peaks, that is, their abundance is usually low or below detection limit and they occasionally increase to a noticeable abundance at the community level. They can feature annual and inter-annual distributions identified as 'Conditionally Rare Taxa' (CRT, Shade et al., 2014). The frequency of a CRT's abundance over time exhibits a bimodal distribution (Shade et al., 2014). Furthermore, because the overall community assembly processes is critical in tracking and predicting future changes in planktonic communities (e.g., Ramond et al., 2021; Skouroliakou et al., 2022; Xu et al., 2022, and references therein), an exploratory analysis was undertaken for Syndiniales Group II at a seasonal scale applying the null model analysis framework (Stegen et al., 2012, 2013). Community assembly describes how processes interact to determine species composition and local biodiversity of a community (e.g., Chase & Myers, 2011). The rationale here was that while ecological deterministic processes are conducive to modelling, stochastic ones are far less predictable. The main question in this study is whether stochastic and deterministic ecological processes varied across seasons for Syndiniales Group II. Our hypothesis was that stochastic ecological processes occur a larger scale in the microbial communities and may therefore prevail in Syndiniales Group II community assembly (e.g., Skouroliakou et al., 2022).

Overall, this study focused on the following specific questions: (i) On a technical aspect, is sequencing an accurate method to infer dinospore abundances? (ii) Do Syndiniales Group II, have seasonal patterns and do they relate to dinoflagellate dynamics? (iii) Do stochastic or deterministic ecological assembly processes prevail in the Syndiniales Group II community assembly?

To answer these questions, a multiple year temporal survey at two different frequencies was conducted to better capture the temporal changes of planktonic communities (bi-weekly during 2016–2020 and at a higher frequency during 2018–2020). 18S rDNA amplicon Illumina Mi-Seq sequencing and dinospore enumeration were combined with the FISH-TSA method, microscopical examination of microplankton, and flow cytometry for nano- and picoplankton, along with measurements of environmental parameters.

EXPERIMENTAL PROCEDURES

Study site and sample collection

Samples were collected at 2 m depth at five neighbouring stations between March 2016 and October 2020 (Figure S1) using 12 L Niksin bottles. From 2016 to 2020, the SOMLIT coastal S1 and the offshore S2

TABLE 1 Station and sample description. Max. depth corresponds at highest tide. To note that S1 and R1 being closer to the coast were easier to sample under difficult weather conditions (see also Figure S1). Environmental parameters and 18S rDNA amplicon sequencing were realized for all 2016–2020 samples. Microscopy counts of microplankton, flow cytometry and FISH-TSA for 2018–2020 samples at all stations (total 269 samples).

Sta.	Date	Long (°E)	Lat (°N)	Max. depth	Distance from the coast (km)	Sampling frequency	No of samples
S1	2016–2020	1.3117	50.4075	27	2	Bi-weekly	63
S2	2016–2020	1.2460	50.4075	56	9.8	Bi-weekly	39
R1	2018–2020	1.3360	50.4760	19	2.6	Once/twice a week	79
R2	2018–2020	1.3231	50.4760	23	4.3	Once a week	42
R4	2018–2020	1.2780	50.4760	52	10.9	Once/twice a week	46

stations (French Network of Coastal Observatories; https://www.somlit.fr/) were sampled bi-weekly (Table 1). Stations (R1, R2 and R4) belonging to the 'DYPHYRAD' transect situated about 15 km north of the SOMLIT stations (Figure S1, Table 1) were sampled weekly. Higher frequency samplings (2–3 times a week) were also carried out after the end of the spring bloom in June–July and in autumn in September–October at stations R1 and R4. The higher frequency was applied in an effort to catch rapidly changing dinoflagellate and Syndiniales Group II abundance dynamics. *P. globosa* spring bloom occurs every year in April–May (e.g., Breton et al., 2006, 2017, 2021; Breton et al., 2021; Christaki et al., 2014, 2017; Grattepanche, Vincent, et al., 2011). The post spring bloom and autumn periods were chosen because higher Syndiniales Group II and /or dinoflagellate abundances were observed during these periods in previous studies (e.g., Christaki et al., 2017; Grattepanche, Vincent, et al., 2011). Only three samples were collected in August due to the unavailability of the boat crew during their annual leave. For this reason, the August data are presented but not further discussed.

Environmental variables

Seawater temperature (°C) and salinity were measured in situ using a conductivity-temperature-depth profiling system (CTD Seabird SBE 25). The average subsurface daily photosynthetically active radiation (PAR) experienced by phytoplankton in the water column for a six-day period before sampling was obtained using a global solar radiation (GSR, Wh m^{-2}) recorded by the Copernicus Atmosphere Monitoring Service (CAMS) radiation service (http://www.soda-pro.com/web-services/radiation/cams-radiation-service). GSR was converted into PAR by assuming PAR to be 50% of GSR and by considering 1 W m^{-2} = 0.36 E m^{-2} d^{-1} (Morel & Smith, 1974). Inorganic nutrient concentrations (nitrate, NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}), and silicate ($Si(OH)_4$) were analysed according to Aminot and Kérouel (2004). Chlorophyll *a* (Chl-*a*) concentrations were measured by fluorometry (Lorenzen, 1966).Wind speed (m s^{-1}), wind direction (deg), and rainfall (kg m^{-2}) were obtained from the National Aeronautics and Space Administration (NASA) and Goddard Space Flight Center (http://gmao.gsfc.nasa.gov/reanalysis/MERRA-2, resolution 0.625°×0.5°, longitude × latitude). Wind stress (Pa) was calculated from wind speed as described in Smith (1988). Additional details on environmental data acquisition and sample analysis can be found at https://www.somlit.fr/.

DNA barcoding and bioinformatic analysis

Four to seven litres of seawater depending on the quantity of particulate matter in the water, that is, until clogging of the filter) were filtered onto 0.2 μm polyesthersulfone (PES) membranes (142 mm, Millipore, USA) after a pre-screening step through 150 μm nylon mesh (Millipore, USA) to remove metazoans. Filters were stored at −80°C for 18S rDNA amplicon Illumina MiSeq sequencing. DNA extraction was performed according to the DNAeasy PowerSoil kit protocol (QIAGEN, Germany). To describe protist diversity, the V4 hyper-variable region of the 18S rDNA gene (527 bp) was amplified using the primers EK-565F (5′-GCAGTTAAAAAGCTCGTAGT) and UNonMet (5′-TTTAAGTTTCAGCCTTGCG) biased against Metazoa (Bower et al., 2004). Pooled and purified amplicons were then paired-end sequenced on an Illumina MiSeq 2 × 300 platform by Genewiz (South Plainfield, NJ, USA).

Quality filtering of reads, identification of amplicon sequencing variants (ASVs), and taxonomic affiliation based on the PR2 database (Guillou et al., 2013) were done in the R-package DADA2 (Callahan et al., 2016). A total number of 41,179 ASVs were identified from 6,366,087 reads in 287 samples containing Metazoa, Streptophyta, Excavata, Alveolata, Amoebozoa, Apusozoa, Archaeoplastida, Hacrobia, Opisthokonta, Rhizaria and Stramenopiles. ASVs affiliated to Excavata, Metazoa, Streptophyta, unaffiliated ASVs and

singletons were removed, obtaining a phyloseq object containing 20,651 taxa by eight taxonomic ranks. After eliminating samples with less than 5000 reads, the number of reads per sample was rarefied to the lowest number of reads (5234) which produced 15,250 ASVs distributed in 269 samples. Then, only taxa affiliated to the order Syndiniales Group II were kept, resulting in a new phyloseq object composed of 663 taxa detected in 269 samples (for more details on DNA barcoding and bioinformatic analysis, see Supplementary Information S1). Raw sequencing data have been submitted to the Short Read Archive under BioProject number PRJNA851611.

Fluorescent in situ hybridisation (FISH-TSA)

Fluorescent in situ hybridization (FISH) coupled with tyramide signal amplification (TSA) was used to enumerate Syndiniales Group II dinospores and observe infected hosts. Dinospores and infected hosts were enumerated on the same hybridized filters. Samples (300 ml) were fixed with paraformaldehyde (1% final concentration) for 1 h at 4°C and then filtered onto 0.6 μm polycarbonate membranes. The filters were dehydrated by several successive ethanol baths (50%, 70% and 100%) and stored at −80°C until analysis. The oligonucleotide probe ALV01 (5′-GCC TGC CGT GAA CAC TCT-3′) was used to target Syndiniales Group II dinospores (Chambouvet et al., 2008). A total of 199 filters over the period 2018–2020 were processed with FISH-TSA following the protocol described in (Piwosz et al., 2021 and references therein). Following the TSA reaction, the filters were counter stained 15 min with calcofluor (100 ng ml^{-1}) to visualize armoured dinoflagellates. All counts were performed with an epifluorescence microscope at 100× (Zeiss Imager M2) with different fluorescence filters (for calcofluor, excitation: 345 nm; emission: 475 nm), propidium iodide (excitation: 536 nm; emission: 617 nm) and FITC (excitation: 495 nm; emission: 520 nm). For dinospore counts, 150 optical fields at ×100 corresponding to 873 ± 149 μl of initial sample (390–1333 μl) were observed for each filter (for more details see Supplementary. Information S1).

Microscopic and cytometric counts (morphological data)

For diatoms and *P. globosa* colonies and free cells counting, 110 ml water samples were collected and fixed with Lugol's-glutaraldehyde solution (1% v/v, which does not disrupt *P. globosa*'s colonies, Breton et al., 2006). For dinoflagellates, another 250 ml were fixed with acid Lugol's solution (1% v/v) (data for dinoflagellates are available for 2018–2020, Table 1). Microplankton was identified to the genus or species level when possible using an inverted microscope (Nikon Eclipse TE2000-S) at 400× magnification after sedimentation in a 10, 50 or 100 ml Hydrobios chamber, as described previously in (Breton et al., 2021). The abundance of pico- and nano-phytoplankton (PicoNano, 0.2–20 μm) were enumerated by flow cytometry with a CytoFlex cytometer (Beckman Coulter). For all samples, 4.5 ml were fixed with paraformaldehyde (PFA) at a final concentration of 1% and stored at −80°C until analysis (Marie et al., 1999). Phytoplankton cells were detected according to the autofluorescence of their pigments (Chl-a). Heterotrophic nanoflagellates (HNFs) were enumerated after staining with SYBR-Green I following (Christaki et al., 2011).

Abundance distribution and community assembly of Syndiniales Group II

Shade et al. (2014) suggested a simple method for detecting CRT based on the coefficient of bimodality (b) defined by Ellison (1987),

$$b = \left(1 + \text{skewness}^2\right) / (\text{kurtosis} + 3) \quad (1)$$

According to Shade et al. (2014) when $b > 0.9$, taxa are CRT. The "*b*" coefficient was calculated for abundant ASVs representing a relative abundance ≥0.01 in the Syndiniales Group II (this corresponded ≥500 reads, and 72% of the total Syndiniales Group II reads). For comparison, the bimodality coefficient was also calculated for the 55 ASV with ≥100 reads (84% of the total reads).

To explore the ecological assembly processes regulating Syndiniales Group II at a seasonal scale null models based on metabarcoding data were applied according to Stegen's framework (Stegen et al., 2012, 2013) reviewed by Zhou and Ning (2017). Briefly, the community assembly analysis is based on the comparison of observed community turnovers (shifts in composition across samples), phylogenetic turnovers (shifts in composition weighted by the phylogenetic similarity between taxa), and turnovers expected by chance (in null-models). This information is used to estimate whether the differences between pairs of communities are explained by dispersal, selection or ecological drift. According to this framework, deterministic processes are divided into homogeneous selection (i.e., consistent abiotic or biotic conditions filter which parasites can persist) and heterogeneous selection (i.e., high compositional turnover caused by shifts in environmental factors or hosts). Stochastic processes are divided into homogeneous dispersal (i.e., low compositional turnover caused by high dispersal rates),

TABLE 2 Prevalence (%) of infected hosts identified in individual samples where it was possible to count at least 50 morphologically recognizable potential hosts were observed (for station position, see Table 1 and Figure S1). The clades present at each date are detailed in Figure S5.

Station, date	R1, 5 June 2018	R1, 6 June 2018	R2, 7 June 2018	R1, 8 June 2018	S1, 12 June 2018	R1, 14 November 2018	R1, 5 July 2019	R1, 9 October 2019
Prevalence (%)	11.5	10.5	4.7	38.5	17.6	9.1	12.8	14.3
Infected protist	*Prorocentrum minimum*	*P. minimum*	*P. minimum*	*P. minimum*	*P. minimum* *Amphidinium* sp.	various	*Scripsiella* sp. *P. minimum*	various

dispersal limitation (i.e., high compositional turnover caused by a low rate of dispersal), and ecological drift that can result from fluctuations in population sizes due to chance events (Table 2). The implicit hypothesis is that phylogenetic conservatism exists, which means that ecological similarity between taxa is related to their phylogenetic similarity (i.e., phylogenetic signal) (Losos, 2008). The Mantel correlogram was applied to detect phylogenetic signal (e.g., Liu et al., 2017; Doherty et al., 2020, for details see Supplementary Information S1). The phylogenetic temporal turnover (betaNRI) between pairwise communities among sampling dates was quantified to investigate the action of deterministic and stochastic ecological processes with microeco R-package v.0.6.0 (Liu et al., 2021), using the trans_nullmodel function. The phylogenetic distance between pairwise communities (beta mean pairwise distance: βMPD) was computed with null models based on 999 randomizations with the random shuffling of the phylogenetic tree labels, as in Stegen et al. (2013). All definitions of the different assembly processes corresponding to the values of βNRI are given in Table 2. Non-weighted metrics were used as metabarcoding data are semi-quantitative and the rarefied dataset was considered to prevent any bias due to potential undersampling (Ramond et al., 2021). All analyses were carried out using R 4.1.0 (R Core Team, 2021a, 2021b). For more details, see Supplementary Information S1.

RESULTS

Environmental variables

The environmental variables showed seasonal patterns typical of temperate marine waters. Nutrient inputs originated mainly from local rivers and reached relatively high values during autumn and winter with the highest values in February (Figure S2A). The N/P ratio was highly variable, ranging from 0.4 to 316, strongly deviating most of the time from the Redfield ratio (N/P = 16, Redfield, 1958). Overall, the environmental variables were of the same range and showed the same seasonal variations at all stations. A comparison of the mean ranks (Kruskal Wallis and Nemenyi post hoc test) of environmental variables between the different stations revealed significant differences in salinity, phosphate, silicate, and Chl-*a* between the stations (Figure S2B). Principal component analysis (PCA) performed on the environmental dataset showed a seasonal pattern opposing winter and summer conditions. Overall, summer and autumn samples formed tighter groups on the PCA biplot than spring and winter samples which were more dispersed (Figure S3).

Planktonic eukaryotic diversity and abundance

Microscopic counts of diatoms and *P. globosa*, and flow-cytometric counts of pico-nanophytoplankton showed that diatoms were always abundant with highest abundances from winter to early summer (range 4×10^3–5×10^6 cells L^{-1}, Figure 1A). *P. globosa* bloomed in April and May, reaching a peak of 3.6×10^7 cells L^{-1} (Figure 1B). Pico-nanophytoplankton showed clear seasonal variations, with the highest abundance in early summer after the phytoplankton spring bloom and then in autumn (Figure 1C). HNFs highest values were recorded each year in May–June after the spring phytoplankton bloom (range 0.2×10^6–1.8×10^7 cells L^{-1}, Figure 1D). Dinoflagellates -although they presented the highest number of reads—were three orders of magnitude less abundant than diatoms (ranging from 0.76 to 18.6×10^3 cells L^{-1}). Dinoflagellates reached their lowest abundances in winter and highest in summer after the end of *P. globosa* bloom (Figure 1E). *Gymnodinium spp*. was the most abundant dinoflagellate genus in almost all samples while two other dinoflagellates (*Prorocentrum minimum* and *Scrippsiella spp*.) showed moderate increases in summer (Figure S4). Ciliates presented relatively low abundances from <100 to 7.4×10^3 cells L^{-1} (Figure 1F).

Sequence data showed that the most abundant group in terms of number of reads was Dinophyceae (30%), followed by Baccilariophyta (25%) and Syndiniales (10%) (Figure 2A). Within Syndiniales, Group I was the most abundant (61%, 6 Clades, 285 ASVs, Figure 2B) while Group II was the most diversified (35%, 27 Clades, 663 ASVs). Within Syndiniales Group II, Clades 10–11, and 8 dominated (21% and 25% of the reads, respectively, Figure 2C). Syndiniales Group II showed highest abundance and richness in June, July and September and lowest in winter; the highest

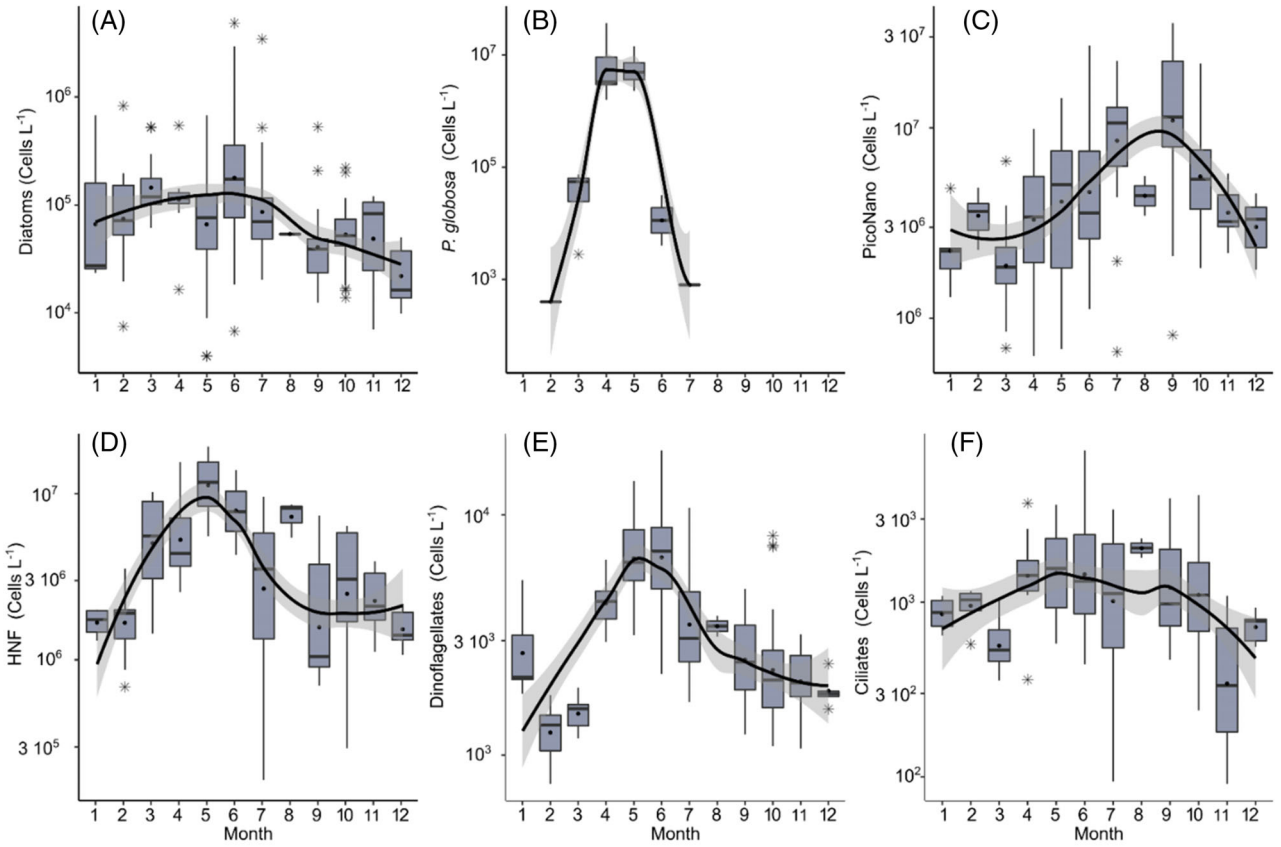


FIGURE 1 Seasonal variation of protist abundance (cells per litre): (A) Diatoms, (B) *Phaeocystis globosa*, (C) PicoNano: pico-nanophytoplankton, (D) HNF: heterotrophic nanoflagellates, (E) dinoflagellates, and (F) ciliates, identified in the eastern English Channel at the SOMLIT and DYPHYRAD stations from March 2016 to October 2020. Y scale is log10 transformed. Solid black lines in the boxplot represent the median, black dots the mean, and the black stars the outliers. The solid line and ribbon represent LOESS smoothing and the 95% confidence interval. Data for ciliates and dinoflagellates were available for 2018–2020.

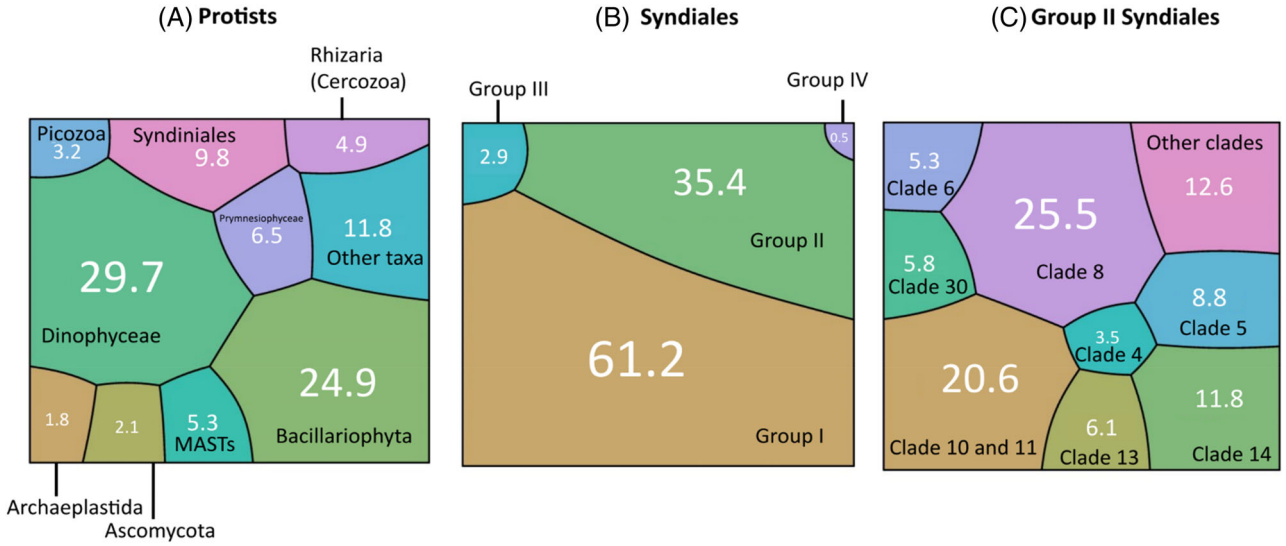


FIGURE 2 Voronoi diagrams representing the relative abundance, given in percentage, of different protist taxonomies from 2016 to 2020 in the eastern English Channel at the SOMLIT (S1, S2) and DYPHYRAD (R1, R2 and R4) stations. The area of each cell being proportional to the taxa relative abundance (the specific shape of each polygon carries no meaning). This type of visualization is similar to pie charts but represents better small contributors (A) Relative abundance of the nine identified supergroups, (B) Relative abundance of Dino-Group Syndiniales within the taxonomic Class Syndiniales, (C) Relative abundance of Clades within Group II Syndiniales. Visualisation performed using the online tool http://www.bioinformatics.com.cn/srplot.

number of reads occurring in June 2018 (Figure 3A,B). Fifteen samples showed high Syndiniales Group II abundance (>600 reads, Figure 3A). Clade 8 was the most abundant clade in terms of reads, followed by Clades 10–11, 14, 30, 5 and 13, respectively (Figure S5). The most important peaks were observed at the beginning of summer and autumn (Figure 3A,B). While the summer peaks were mostly dominated by Clade 8, a variety of clades and ASV dominated in the autumn peaks (Figure S5). The highest values of Syndiniales Group II read abundance and richness were recorded at station R1 which was the closest to the coast but also the most visited (Figure S6A,B, Table 1). The cumulative number over time of ASVs affiliated to Syndiniales Group II constantly increased. The cumulative plot based on new ASVs between two consecutive dates revealed that the slope of the cumulative curve became steeper with the influx of new ASVs in summer and autumn as well as in autumn 2020 due to a particular high number of new ASV (Figure S7).

Dinospores cells were counted by microscopy (on FISH-TSA hybridized filters) ranged from undetectable to 5.6 $\times$ 10^5 L^{-1} (Figure 3C) and generally followed a seasonal pattern similar to the one of dinoflagellate microscopy abundances (Figures 1E and 3C). The highest values of dinospore abundance were also recorded at station R1 (Figure S6C and Table 1). Although dinospore abundances followed similar seasonal patterns with Syndiniales Group II reads (Figures 3A–C and 2E), the number of reads cannot be used as a predictor of the number of dinospores and vice versa as there was no consistent numerical relationship between them (Figure S8).

Syndiniales Group II occurrence and potential hosts

The maximum likelihood tree of the 20 most abundant Syndiniales Group II ASVs (i.e., ASVs representing at least 1% of the reads affiliated to Syndiniales within the rarefied dataset), showed that ASVs belonging to Clade 8 (ASV13 and ASV124) were the most phylogenetically distant from the rest of the Clades present. Clades 10, 11 and 14 were those phylogenetically closer to each other (Figure 4). The majority of the most abundant ASVs were present every year at low or very low abundances and showed a few transient peaks. Indeed, 13 out of the 20 ASVs were found every year, while 7 were absent at least 1 year and up to 3 years (Figure 4). Among these most abundant ASVs, several ones can be characterized as 'highly persistent' such

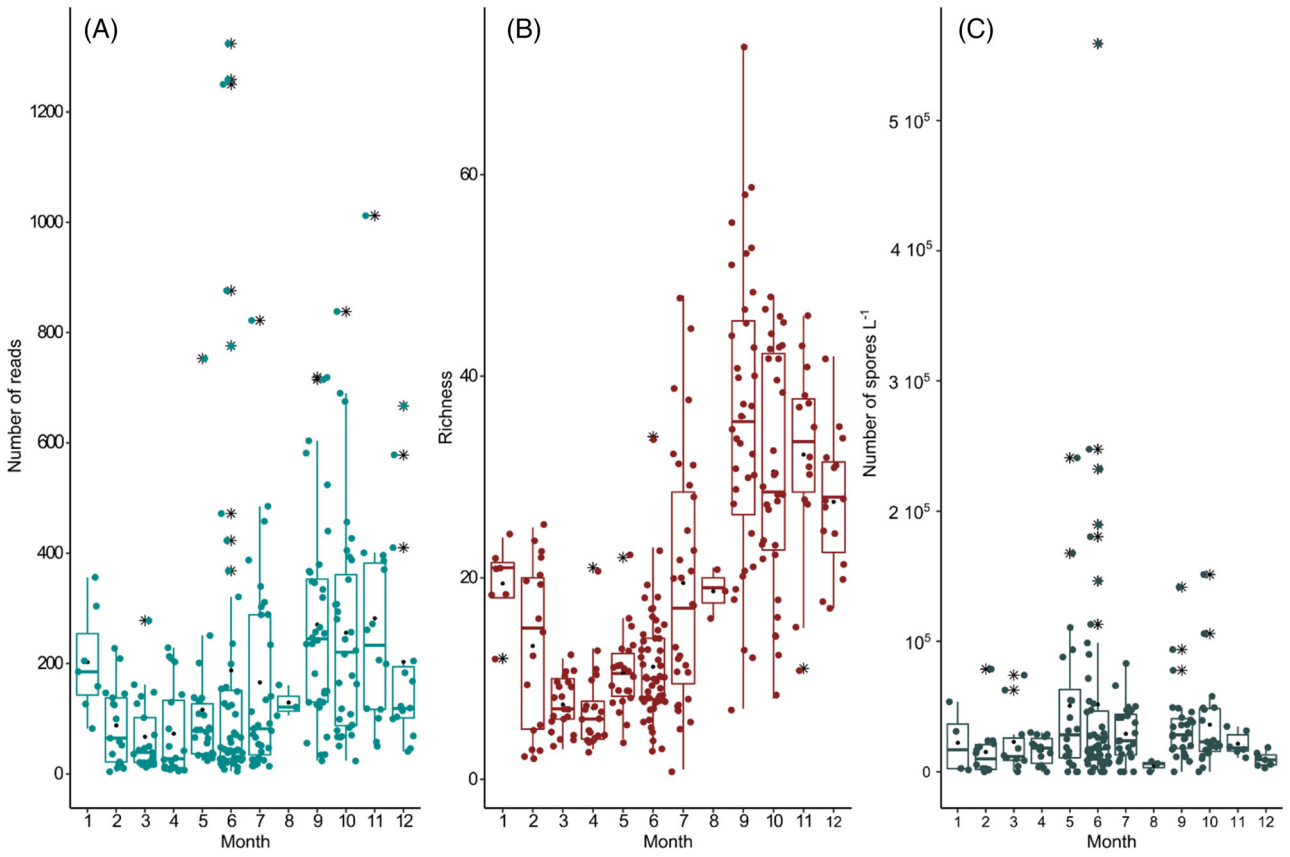
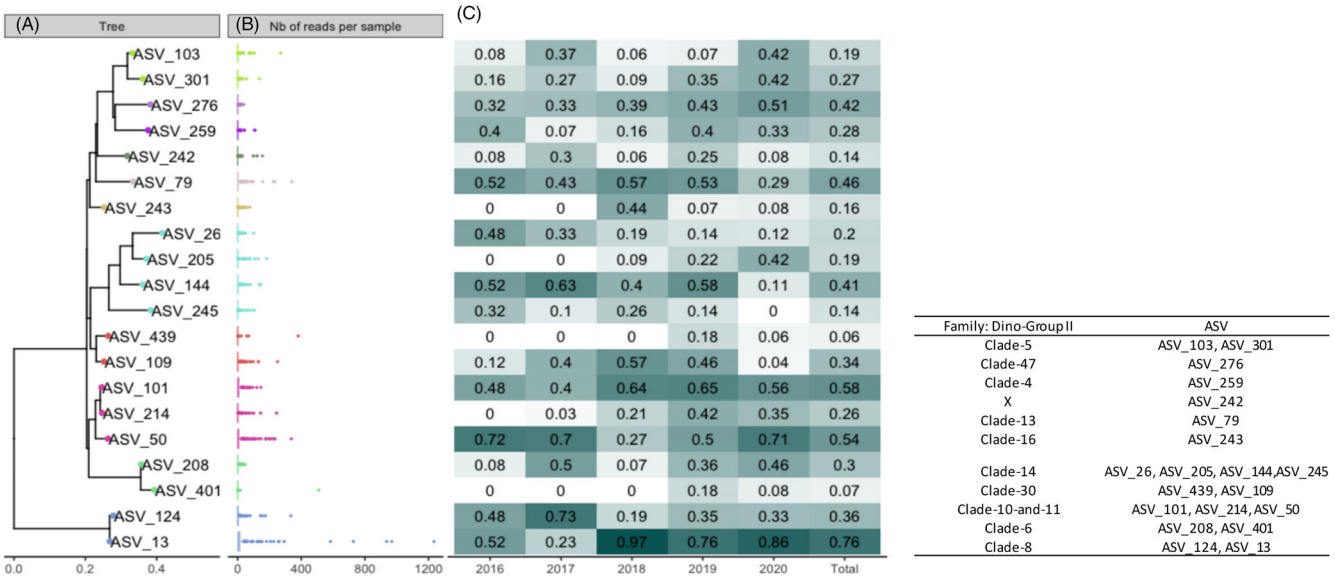


FIGURE 3 Seasonal variation of Group II Syndiniales in the eastern English Channel at the SOMLIT and DYPHYRAD stations. (A) Number of reads, (B) Richness, (C) Dinospores. Solid lines in the boxplot represent the median, black dots the mean, and the black stars the outliers.



Family: Dino-Group II	ASV
Clade-5	ASV_103, ASV_301
Clade-47	ASV_276
Clade-4	ASV_259
X	ASV_242
Clade-13	ASV_79
Clade-16	ASV_243
Clade-14	ASV_26, ASV_205, ASV_144,ASV_245
Clade-30	ASV_439, ASV_109
Clade-10-and-11	ASV_101, ASV_214, ASV_50
Clade-6	ASV_208, ASV_401
Clade-8	ASV_124, ASV_13

FIGURE 4 Maximum likelihood tree of the 20 most abundant Group II Syndiniales amplicon sequencing variant [ASVs] (ASVs representing at least 1% of the Syndiniales affiliated reads on the rarefied dataset); (B) Boxplots of the number of reads per sample. Note that for all boxplots the median value is close to zero and high abundances are visible as outliers (C) ASV's relative abundance in samples where at least one read of each ASV was present for each year (2016–2020). The intensity of the colour corresponds to the relative abundance. To note that the number of samples differs between the years as follows 2016: 26 samples, 2017: 30 samples, 2018: 76 samples, 2019: 72 samples and 2020: 65 samples.

as ASV13 (Clade 8), ASV50 (Clade 4) and ASV101 (Clade 4), since they were present every year and in more than 50% of all samples (Figure 4). At the opposite, ASV401 (Clade 6), ASV439 (Clade 47) were found only in 2019 and 2020 (5% of the samples) where they showed a unique peak.

Prevalence of infection was assessed when hosts were abundant enough to make accurate microscopy observations. Hosts were clearly identified only during two infection events (Table 2). The first observed infection occurred during the *P. minimum* increase (up to 1.3×10^3 L^{-1}) coinciding with the dominance of Clade 8 (ASV 13) and the second was related to *P. micans* and *Scrippsiella spp*. and coinciding again to the dominance of Clade 8 in July 2019 (Table 2). Infected cells were rare and/or not possible to identify based on their morphology in the other samples although dinospores were detected. Host morphologies were diverse and represented athecate and thecate dinoflagellate cells and a few cells resembling to ciliates. Trophont and veriform like Syndiniales were rarely observed in the samples (Figure S9). It is worth noting, that microscopy observations of *P. globosa* and diatom cells, which reached very high abundances during the study, showed no sign of infection by Syndiniales Group II.

Abundance distributions and Syndiniales Group II community assembly

For the 20 most abundant ASVs (≥0.01 relative abundance), the coefficient of bimodality varied from 0.62 to 0.99 with only three ASVs identified as CRT ($b > 0.9$ ASV401 and ASV439). Applying the same calculation to the ASVs having ≥100 reads, the result was similar as the factor "*b*" varied between 0.62 and 0.99 and only five additional ASVs were detected as conditionally rare ($b > 0.9$, see also Figure S10).

The potential of stochasticity versus determinism regulating the Syndiniales Group II community was explored, and then, the associated ecological processes were quantified.

The Mantel correlograms indicated phylogenetic signals at short phylogenetic distances (10%–30%, Figure S11). The phylogenetic temporal turnover (βNRI) values ranged from −2 to 4.6 (Figure 5A). The monthly variability of βNRI showed that the median value was between −2 and 2, defining stochastic assembly processes in Syndiniales Group II. However, βNRI values were somewhat greater than 2 defining '*determinism*' in June and July (Figure 5A Table 2). The null model analysis suggested that 'ecological drift' was the major ecological process regulating the community assembly of Syndiniales Group II (80%) with heterogenous selection accounting for 20% (Figure 5B, Table 3).

DISCUSSION

In the present study, microscopy and flow cytometry were used to quantify pico- and nano- microplankton as well as dinospores, while metabarcoding data provided an extended evaluation of their diversity. The present

study is original in its observation frequency—less than a week—and the duration of the survey—over a multiple of years. In addition, contrary to most previous studies, it was conducted in a meso-eutrophic coastal area with an expected low-host abundance of dinoflagellates and Syndiniales Group II.

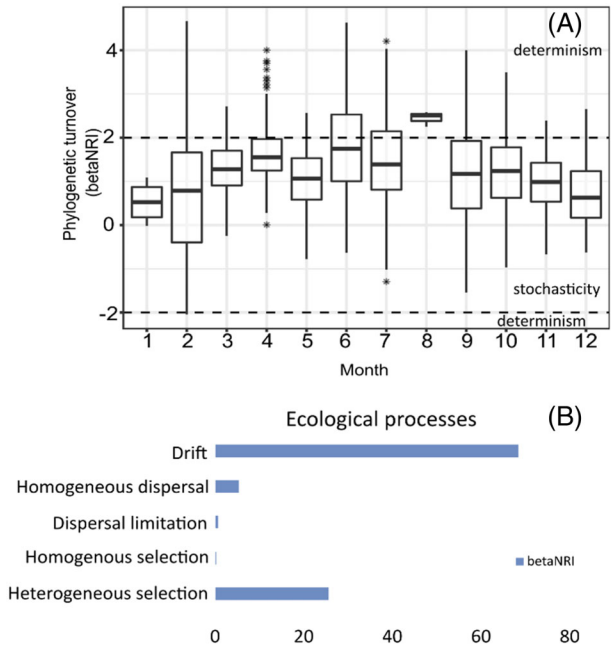


FIGURE 5 (A) Phylogenetic turnover index (βNRI) Group II Syndiniales amplicon sequencing variants (ASVs) in the eastern English Channel at the SOMLIT and DYPHYRAD stations from March 2016 to October 2020. Solid black lines represent the median and black dots the mean. βNRI>2, βNRI<−2 indicating deterministic processes and −2 < βNRI < 2 indicate in stochastic processes (see also Table 2). (B) Relative importance of the ecological processes driving Group II Syndiniales communities in the eastern English Channel at the DYPHYRAD and SOMLIT stations from March 2016 to October 2020. Note that only three samples were available for August—data not interpretable.

Environmental context

During this study, environmental variables showed clear seasonal patterns typical of the EEC (e.g., Grattepanche, Breton, et al., 2011; Breton et al., 2017, Figure S2A). This coastal area is highly dynamic, characterized by a mega-tidal hydrological regime and strong tidal currents parallel to the coast, with coastal water drifts towards the shoreline. Thus, the less salty and more turbid near-shore waters remain separated from the open sea by this tidal front (Brylinski et al., 1991; Lagadeuc et al., 1997; Sentchev et al., 2006). The stations nearer to the coast (R1, S1)—being more influenced by coastal run-off—showed several extreme values of environmental variables (Genitsaris et al., 2016, Figure S2B) and also the highest chlorophyll-a concentrations (Figure S2B) and dinospore abundance (R1, Figure 3C). Since dinoflagellates proliferate in relatively warm, stratified and nutrient enriched waters (Smayda, 2002), the highly dynamic hydrological conditions and the absence of stratification can explain the relatively low dinoflagellate abundances in this area. In the EEC, while dinoflagellates showed their highest abundances during the summer months (Grattepanche, Vincent, et al., 2011), these abundances remain relatively low compared to diatoms and nano-picoplankton (Figure 2) and to other close or distant coastal areas (Table 4).

Sequence versus abundance data, is sequencing an accurate method to infer dinospore abundances?

The comparison of the abundances of taxa obtained by sequencing (relative abundance of reads) and microscopy (cell abundance) is not straightforward. In silico analysis considering zero mismatches revealed that the ALV01 probe sequence matched with 80% of the

TABLE 3 Definitions of the different assembly processes, and respective model conditions referenced from Webb 2002, Stegen et al. (2012), (2013) and Zhou and Ning (2017).

	Deterministic processes		Stochastic processes		
	Homogeneous selection	**Heterogeneous selection**	**Dispersal limitation**	**Homogeneous dispersal**	**Drift**
Definition	Consistent biotic (i.e., hosts) or abiotic factors cause low compositional turnover	Shifts in biotic (i.e., hosts) or abiotic factors cause high compositional turnover	Movement of individual is restricted	High rate of movement of an individual from one location to another	Population size fluctuates due to chance events
Phylogenetic turnover index	βNRI < −2	βNRI > 2	–2 < βNRI < 2		
Taxonomic turnover index	—	—	RC_{bray} >0.95	RC_{bray} < −0.95	−0.95 < RC_{bray} <0.95

TABLE 4 Comparative table between the present and several previous studies Widdicombe et al. (2010) and Grattepanche, Breton, et al. (2011) studies are reported for comparison with the dinoflagellate abundances of the present study.

Dinoflagellates 10^3 L^{-1}	Dinospores 10^3 L^{-1}	Prevalence (% of infected protists)	Site	Observations	References
Max ~400	Max ~800	Mean 21 Max 46	Penzé estuary (northern Brittany, France)	June, during dinoflagellate bloom	Chambouvet et al. (2008)
Mean 70 Min <1 Max >500 *Prorocentrum* up to 3360	ND	ND	Western English Channel (coastal)	Year round, Weekly, 15 years	Widdicombe et al. (2010)
Mean 5.3 Min 0.3 Max 32.4	ND	ND	Eastern English Channel (station S1, Figure S1)	Year round, Bi-weekly 52 samples	Grattepanche, Vincent, et al. (2011)
ND	Min 4.2 Max 1500	Min ~1 Max 25	Mediterannean	Summer, East–West transect	Siano et al., 2011
Min <10 Max >100 *Prorocentrum* up to 103	Max 1680	Min undetectable Max ~12	Reloncaví Fjord, southern Chile (coastal)	Summer, bi-weekly during *Prorocentrum* bloom	Alves-de-Souza et al., 2012
Range ~1–1000	Min <0.1 Max 1000	Min undetectable Max ~70	Salt pond (Eastham, MA USA)	March–May, 1–3 days, 13 samples	Velo-Suárez et al. (2013)
ND	Min 0 Max 335	Min 0 Max 2.56	Salt pond (Falmouth, MA, USA)	March–October, twice a week	Sehein et al., 2022
Mean 4.5 Min 0.76 Max 18.6 *Prorocentrum* up to 15	Mean 35.5 Min 0 Max 559	Min undetectable Max 38.5	Eastern English Channel (coastal, Figure S1)	Year round, One/ twice a week, 205samples	This study

ASVs (corresponding to 83.5% of the total reads) affiliated to Syndiniales Group II found in this study. These results suggest that a relative increase in Syndiniales Group II reads was generally the consequence of an increase in dinospore abundance measured by FISH-TSA counts. This was the case in summer, in general, and autumn 2018 and 2019, in particular. Conversely, there was no relation between the dinospore abundance counted by microscopy and the read abundances (Figure S8). This result implies that the dinospore abundance cannot be used as a predictor of relative read abundance and vice-versa. This discrepancy can be explained by: (i) Metabarcoding captures all cells while FISH-TSA targets only active cells. Massana et al., 2015 reported that Group I and Syndiniales Group II were found about 4-times more in RNA relative to DNA data; (ii) there is a lag time of about 2–3 days between the infection and the spore release (e.g., Alves-de-Souza et al., 2015; Coats & Park, 2002).

Syndiniales Group II communities and temporal patterns

In this study, Group II was, as expected, the most diversified Syndiniales group (Guillou et al., 2008), accounting for 27 out of the 44 identified clades. Syndiniales Group II reads were always recorded and, in a few cases, very abundant. The sampling effort during this study was intensified during the last 2 years of the survey (2018–2020), with an increase in the frequency of 25–30 samples/year to 70–75 samples/year, producing a total of 269 samples. The cumulative plots of ASV affiliated to Syndiniales Group II did not reach a plateau and new ASV between two consecutive dates (defined as 'new arrivals' herein) were detected until the end of the study (Figure S8A,B). These intriguing observations may be explained by the introduction of new taxa via transportation of water masses travelling northwards in the English Channel. However, our data also showed consistent temporal patterns providing an indication of the existence of 'local populations'. The repeating patterns of the most abundant clades and ASVs have already been observed in two previous studies (Chambouvet et al., 2008; Christaki et al., 2017). Indeed, dominant taxa of Syndiniales Group II varied from 1 year to the other, though most of them were detected every year (Figure 4). The bimodality coefficient (b) showed that only three among the 20 most abundant ASVs (≥0.01 relative abundance) were identified as 'Conditionally Rare Taxa' ($b > 0.9$) that is, their abundance was usually low or below detection limit and they occasionally increased to an

abundance 'appreciable' at the community level (sensu Shade et al., 2014, see also M + M). The majority of ASVs exhibited a "*b*" coefficient lower than 0.9, suggesting that they had seasonal and/or irregular dynamics (Shade et al., 2014).

In a previous two and a half years survey in the same area, Syndiniales Group II was dominated by Clades 30, 8 and 10–11 (16, 13 and 7% of total Group II reads, Christaki et al., 2017), in the present study Clades 8, 10–11 and 14 dominated (Figure 1C). Among these clades, only Clade 14 was known to reach high abundances related to dinoflagellate blooms in summer in the North-Western English Channel (Chambouvet et al., 2008). Clade 8 has not been reported previously as dominant in the North-Western English Channel (Chambouvet et al., 2008) or in other long term coastal studies (e.g., Käse et al., 2021, their Supplementary Material S1). This clade was clearly dominating within the Group II community (25.5% of the reads), prevailing in 8 out of the 15 samples that showed important peaks (>600 reads). Clade 8 also showed the highest occurrence during all years, being present in up to 97% of the samples (in 2018, Figure 4).

Dinospores were present throughout the sampling period at low abundances (ca. 10^3 cells L^{-1}) and were punctuated by a few transient peaks (ca. 10^5 cells L^{-1}). In a modelling study, the critical carrying capacity that ensures stable coexistence of dinoflagellate host and parasitoid varied from 10^3 to 10^5 L^{-1} (Salomon & Stolte, 2010). The dinospore abundances observed here imply that there is a 'seed population' that could rapidly develop when the hosts reach a certain abundance. However, grazing by heterotrophic dinoflagellates and ciliates of the dinospores is most likely another important factor that keeps dinospore populations under control since their size fits within the range of their prey's size range (e.g., Grattepanche, Breton, et al., 2011; Sherr & Sherr, 2002).

Dinospore and dinoflagellates dynamics

In natural systems, the strength of infection by Syndiniales Group II has been related to the abundance of the dinoflagellate hosts because higher hosts density enhances encounter rates (Park et al., 2004). Consequently, dinoflagellate blooms are associated with the increase of the abundance and therefore prevalence of dinospores (e.g., Alves-de-Souza et al., 2012, 2015; Anderson & Harvey, 2020; Chambouvet et al., 2008; Sehein et al., 2022; Velo-Suárez et al., 2013). Not surprisingly, Syndiniales Group II showed seasonal patterns similar to dinoflagellates, characterized by increases in abundance and richness during summer and autumn, (Figures 2 and 3). The dinoflagellate abundances in this study were drastically lower than most of those previously reported in coastal ecosystems. However, the abundance of dinospores and the proportion of infected cells were comparable (Table 4). Given that each infection produces hundreds of dinospores (Coats & Park, 2002), even a small increase in the number of available hosts would result in a significant increase in the number of dinospores released throughout the parasite–host dynamics (Alves-de-Souza et al., 2015). The strongest infection witnessed during this study (June 2018), corresponded to the highest dinospore and dinoflagellate abundances (*P. minimum*) (Figures 2E and 3C), lasted more than a week and reached a prevalence of 38.5% (Table 3). Prevalence in natural populations of infected dinoflagellates is highly variable and can escalate up to 80% or even higher values during epidemics (Coats et al., 1996) though most commonly reported infection prevalence is much lower (Coats & Bockstahler, 1994; Chambouvet et al., 2008; Salomon et al., 2009; Siano et al., 2011; Alves-de-Souza et al., 2012; Li et al., 2014, Table 4). In this study, the *P. minimum*'s 'rise and fall' coincided with the temporal dynamics of Clade 8 and particularly to a specific ASV (ASV13). Two other dinoflagellates (*Scirpsiella sp*. and *Amphidinium spp*.) showed signs of infections. Although prevalence in natural dinoflagellate populations is usually observed at host densities of the order of 10^5–10^6 L^{-1}, an infection can occur at considerably lower host conditions (Salomon et al., 2009). One feature that can explain our observation is that dinospores are flagellated, allowing them to chase their host and thus increase infection rates. Also, the ability of parasites to have a diversity of hosts could be an adaptive/survival feature when certain hosts become scarce. Our study supports this hypothesis since Syndiniales Group II may have had diversified larger range of hosts in autumn. During the autumn peaks of dinospores, various cells presented signs of infection and metabarcoding data revealed that samples were dominated by a variety of Syndiniales Group II clades and ASVs. However, despite the large number of samples processed in this study—at the exception of the coincidence of *P. minimum* and ASV13 in June 2018,—it was not possible to evaluate the extent of specific versus non-specific infections. Finally, given that the infective parasite cells have an ephemeral life (e.g., Cachon, 1964; Coats & Park, 2002) and should have to rapidly find a host, one may speculate how they manage to do this in such highly complex planktonic communities with low host abundances. Moreover, the production of allelopathic compounds by dinoflagellates as a defence mechanism killing dinospores and preventing infection (Long et al., 2021) could further complicate the chances of survival of these ephemeral swimmers. As such, these dinospores should have their own strategies to increase the chances of finding their hosts (by chemotaxis, for example); an area of research that deserves further investigation.

Community assembly processes of Syndiniales Group II

Including parasitism in planktonic food web models is vital to better understand the dynamics of hosts and parasites and to better appreciate the role of parasitism in the functioning of planktonic ecosystems (e.g., Alves-de-Souza et al., 2015; Montagnes et al., 2008; Salomon & Stolte, 2010). In several studies Syndiniales dynamics have been related to environmental variables such as temperature, salinity, light intensity and nutrient concentrations (Alves-de-Souza et al., 2012, 2015; Käse et al., 2021; Li et al., 2014; Yih & Coats, 2000 and references therein). In this study, a PCA (Figure S2) revealed clear seasonal patterns of environmental variables. However, multivariate analysis such as CCA and dbRDA between environmental parameters and Syndiniales Group II did not show any consistent pattern (data not shown). This is not surprising, since environmental variables such as the ones mentioned above are not expected to have direct influence on Syndiniales Group II, but affect them indirectly through the dynamics of their hosts. Here, as an alternative to multivariate analysis of environmental variables and ASVs abundance, ecological processes regulating the Syndiniales Group II community were explored. This exploratory analysis showed the prevalence of stochastic (i.e., drift) processes in Syndiniales Group II community assembly (Figure 5B). However, June and July were the only months when part of the processes could be inferred as deterministic (Figure 5A). It is hypothesized here, that deterministic processes were related to specific Syndiniales-dinoflagellate interactions observed during these months (see previous section and Table 2).

In conclusion we considered both morphological and metabarcoding data using a relatively large data set (287 samples) which is an approach rarely undertaken. Since metabarcoding data are subjected to PCR biases and are always expressed in relative abundances, and morphological data are absolute abundances but do not provide a precise image of the diversity, it is important to combine these two complementary data sets. Most abundant clades and ASVs affiliated to Syndiniales Group II were observed in consecutive years, providing an indication of the existence of local populations, and featured consistent temporal patterns. Increasing abundance and richness were always observed during the second half of the year. In summer, they were related to dinoflagellate hosts; in autumn, Syndiniales Group II could have more diversified hosts than earlier in the year. This is a case study for coastal ecosystems presenting abundant nutrients but no dinoflagellates blooms. While the dinoflagellate abundances in this study were much lower than most of those previously reported in coastal ecosystems, the number of dinospores and the prevalence were comparable. The low abundance of dinospores in the great majority of samples was consistent with the low host abundance, indicating a stable coexistence punctuated by rare transient peaks. Given all the above, the strategies of dinospores to increase their chances of survival in highly complex planktonic communities and in low host abundances deserves further attention. Overall, the prevalence of stochastic processes renders a priori less predictable the seasonal dynamics of Syndiniales Group II communities to future environmental change.

AUTHOR CONTRIBUTIONS

Urania Christaki: Conceptualization (lead); data curation (equal); formal analysis (equal); funding acquisition (lead); investigation (equal); methodology (equal); resources (equal); software (equal); supervision (lead); validation (lead); visualization (equal); writing – original draft (lead); writing – review and editing (lead). **Dimitra-Ioli Skouroliakou:** Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); resources (equal); software (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (supporting); writing – review and editing (equal). **Ludwig Jardillier:** Conceptualization (equal); funding acquisition (equal); methodology (supporting); writing – review and editing (supporting).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Raw sequencing data have been submitted to the Short Read Archive under BioProject number PRJNA851611. The rest of the data can be obtained on request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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