Composition and patterns of taxa assemblages in the Western Channel assessed by 18S sequencing, microscopy and flow cytometry.

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Abstract: Plankton monitoring by microscopy offers long-term ecological perspective of plankton communities but different detection approaches are biased uniquely. Genetic identification of marine plankton has become standard but is still not used in routine monitoring. This study assessed the diversity of plankton taxa using 18S high throughput sequencing from 2011-2012 from small-volume (~200ml) samples from the Water and Microplankton Sampler (WaMS) deployed on the Continuous Plankton Recorder platform (CPR). The 18S-HTS survey revealed a bias towards heterotrophic taxa, and phototrophs under 10µm within the photosynthetic community. In comparison with phytoplankton microscopic counts from the CPR survey and Western Channel Observatory station L4, only 8-12 taxonomic families were common to all three surveys, with a bias towards larger diatoms and dinoflagellate taxa in microscopy surveys. The WaMS survey detected a contrasting but complementary taxa set to that of microscopic surveys. Additional Quantitative PCR was carried out on the picoeukaryotic pelagophyte, *Aureococcus anophagefferens*, and the nanoeukaryotic potential harmful algae, *Pseudo-nitzschia delicatissima*, from 2011-2013. This confirmed the persistence presence of *A. anophagefferens* in the Western Channel and an elevated abundance of both species in 2011. Species specific seasonality were distinct from those of aggregrate phytoplankton groups.

Keywords: Plankton; Monitoring; Harmful algae; microscopic, genetic, Western Channel

1. Introduction outline

A key goal in marine phytoplankton ecology is to understand the role of community structure the drivers that alter their dynamics and the resulting impact on ecosystem services. Environmental sequencing of plankton in marine systems has revealed extensive unknown diversity, particularly in the nano- and picoplankton cell size range (≤5µM) many of which are uncultivatable [1], capturing the unseen or hard to detect range of oceanic photosynthetic micobes [2]. Phytoplankton sustain the majority food webs through the conversion of inorganic CO_2 to biomass [3]. Biodiversity has been recognised as playing an essential role in ecosystem services and social goals that are intertwined [4, 5]. Monitoring phytoplankton adequatly is key to meeting the goals of Good Environmental Status, as set out in the Marine Strategy Framework Directive (MSFD) legislature or the UK post-Brexit equivalent, including the maintence of

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biodiversity [6]. Although indicators have been developed to measure the impact of anthropogenic pressures such as nutrient and organic matter enrichment or climate change on the biodiversity of phytoplankton [7], only microscopically counted phytoplankton groups are included, such as diatoms and dinoflagellates, or at poor resolution, such as chlorophyll-a. Global marine biodiversity studies such as TARA-oceans and other large scale molecular studies reveal the extensive diversity and abundance of phytoplankton in the nano- and pico-size range (2-20, <2µm respectively) notably haptophytes, pelagophytes and a class of prasinophytes under 2µm called Mamiellophyceae [3, 8]. Taxa numbers were ten-fold higher than estimated by light microscopy [3]. Approximately one-third of eukaryotes could not be identified to any known representative in the genetic public databases indicating the poor state of knowledge of eukaryotic microbes in general [1]. Moreover Phytoplankton between 0.8-5µm were not distributed evenly but even that fraction contained dinoflagellates and diatoms, contrary to popular belief that these two taxa belong to larger phytoplankton size fractions. Softer, flagellated phytoplankton are not captured as their morphology is destroyed upon preservation [9]. There is a mismatch with current routine phytoplankton biodiversity measurements carried out mostly through microscopy-based methods, and actual phytoplankton diversity that may lead to inaccurate or uninformative advice. Comparison of high throughput sequencing (HTS) versus microscopy approaches to measuring phytoplankton diversity revealed biases in both approaches with HTS more likely to pick up rare phytoplankton species and picoplankton but was less succesful at species-level detection overall [8]. Thus a combination of both approaches are key to covering complete phytoplankton assemblages.

So far only microscopically generated phytoplankton data is used in assessments of marine health. A major reason for this is the relative ease in quantifying cells required for ecological assessments or key indicators, such as evenness, the relative abundance of a taxa in its community [10]. Currently there are no large-scale genetic quantification methods for phytoplankton as most identification relies on the ribosomal DNA (rDNA) marker which ranges from one to thousands of copies per cell [11], a key impediment for the inclusion of genetically acquired diverse taxonomic data into standard monitoring programmes. One of the few phytoplankton genera where genetic quantification is useful are for cryptic or tiny phytoplankton species that are difficult to assess at species-level by microscopy. The harmful algae, *Pseudo-nitzschia* (cleve) H. Peragallo are a group of 52 diatom species which can produce the toxin domoic acid causing Amnesiac Shellfish Poisoning in human and marine animals [12]. This genus contains cryptic groups with major conflicts with morphological groups [13] that can only be distinguished to 2-6 morphotypes using light microscopy due to their small or delicate ultrastructural features [14]. Toxin production is often associated with some species or even populations within a species [15]. There is evidence that silica, iron, ocean acidification influences toxin production in some Pseudo-nitszschia species [16]. However they are normally monitored at the genus level routinely, which can lead to inaccurate or data gaps in assessing conditions where there is a risk of toxin production.

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Twelve new species have been formally identified from *Pseudo-nitzschia delicatissima* or *Pseudo-nitzschia pseudodelicatissima* species-complex [17] and references theirin. The potential for non-equivalent comparisons have resulted in contrasting affinities with nutrients and temperature [12, 17]. Only 50% of Psuedo-nitzschia species are toxigenic, and can vary even when isolated from the same location [12]. Toxin production often occurs at a late exponential growth phase [12] so early quantifiable detection of *Pseudo-nitzschia* at a species or strain level is required for the appropriate and accurate management , and prediction of harmful algae [18, 19].

Species-level data is in short supply for phytoplankton less than ten microns in size and are only represented by genus at best in ecological models [20]. Nano- and picophytoplankton (under 10µm) are often most commonly observed by flow-cytometry which can detect cells on gross characteristics, commonly size and pigment, although they represent thousands of species. Studies combining flow-cytometry detection of pico- and nanophytoplankton with high throughput sequencing revealed distinct phenology and habitats [21]. *Aureococcus anophagefferens* is pelagophyte, single-celled picophytoplankton of 2µm in size recorded to grow to bloom level in the east coast of USA and South Africa, in what is known as a brown tide event due to toxins that kill shellfish and the poor light conditions resulting from their growth [22]. This organisms grows well in eutrophic condition in low light conditions with low inorganic nutrients but elevated organic carbon and nitrogen nutrients [22]. It is not routinely measured in many coastal regions, but indirectly measured through flow cytometry as picoeukaryote. Unlike other eukaryotes it contains a unique suite of genes that provides an advantage in dealing with poor light, nutrient stress and competition [22, 23]. In this study provides an opportunity to compare the seasonal occurrence of this organisms against other similarly sized photosynthetic picoeukaryotes where it does not normally grow to high densities.

Two long-term microscopic surveys are routinely carried out in the Western Channel (also known as the English Channel, part of the Celtic Sea). Firstly, the Continuous Plankton Recorder Survey which collects plankton on 270µm mesh using an automated collecting device deployed on Ships of Opportunity [24, 25]. It has been sampling plankton in this region since 1956 but generally larger plankton [25]. The survey captures just over 600 phytoplankton entities over 30 routes in the Atlantic and Pacific oceans. Genetic detection of phytoplankton [26, 27] is possible but presents more challenges as the samples are preserved and stored in buffered formalin which creating cross-linkages between DNA and proteins or other DNA [28, 29], and if not regularly buffered degrades DNA into smaller fragments. Secondly, the Western Channel Observatory (www.westernchannelobservatory.org.uk) has been sampling at station L4 for phytoplankton for at least 20 years, along with other physicochemical variables such as Sea Surface Temperature and nutrients [30]. However, there is a paucity of routine offshore-sampling for nano- and picophytoplankton. Therefore this region was chosen as a testbed for comparing genetic biodiversity with other routine assessments. Genetic data was acquired from small-volume (≤200ml) samples collected from an additional

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automated Water and Microplankton Sampler (WaMS) from 2011-2013 in the payload of the CPR device. The WaMS machine and its preliminary data has been reported [31].

In this study, we address three questions: (1) What genetically-detected species are observed from offshore samples with standard HTS methods used from 2011-2012 and how much phytoplankton is represented in those samples? (2) How comparable are species assemblages captured in the same region between HTS and routinely captured phytoplankton from two different surveys in the same region using microscopic detection over 2011-2012? (3) To assess the extent that single-species seasonal abundance compares with that of aggregated communities to which they belong over 3 years from 2011-2013. Here we use quantitative real-time PCR measurements of two phytoplankton, *Pseudo-nitzschia delicatissima,* with reported varying degrees of toxicity, and *Aureococcus anophagefferens*, a picophytoplankton. We discuss to what extent does genetically acquired phytoplankton match with, and enhance standard monitored phytoplankton.

2. Materials and Methods

2.1: Sampling

The Water and Microplankton Sampler (WaMS) mechanism is described elsewhere [31]. Approximately monthly sampling was deployed within the payload of a Continuous Plankton Recorder (CPR) instrument on a Ship of Opportunity operated by Brittany Ferries from 2011-2013 in the Western Channel between Roscoff, France and Plymouth, UK (see Fig.1). The WaMS pumps 200-500ml of water directly into ten sampling bag pre-programmed sampling stations along a 122 mile transect and processed immediately after collection. Two sampling bags were chosen to sample one area. They were combined and filtered onto a PALL SUPOR 0.2µM pore membrane filter (VWR, USA), placed into ethanol and stored at -80ºC until DNA extraction. Duplicate 2ML samples were preserved in for flow cytometry were preserved in 1% TEM grade Gluteraldehyde (Sigma) flash-frozen and stored at −80 °C for 24 h before thawing and analysing as described [31]. All samples are listed in Table S1. Sampling interruptions occurred in January 2011 due to a sailing break from Brittany Ferries and on other occasions either due to machine error. In March -May 2013 the machine went into repairs and was not available for sampling.

Phytoplankton cell counts were obtained from station L4 phytoplankton time-series [32]. The dataset contained taxa-specific and total functional group abundance (cells mL-1) and biomass (mgC m-3) data collected from the Western Channel Observatory's Station L4 monitoring site (50 15.0'N; 4 13.0'W), situated off the south-west coast of England, United Kingdom (from henceforth called WCO station L4). Samples have been collected weekly (weather permitting) from the 10m depth since October 1992. Paired 200mL water samples were collected using a 10L Niskin bottle attached to a CTD and are fixed in acid Lugol's iodine [33] for enumerating all phytoplankton cells >2µm and neutral formaldehyde for enumerating coccolithophores. Samples are analysed at Plymouth Marine Laboratory according to the Utermöhl counting technique [34] and since 2016, these methods follow the British and European standard document "Water quality – Guidance standard for

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routine microscopic surveys of phytoplankton using inverted microscopy (Utermöhl technique)" (BS EN 15204:2006). Abundance of each taxa (cells mL-1) are calculated according to the number of individuals per unit volume of sub-sample analysed.

2.3 Nutrient and Sea Surface Temperatures

Water samples were taken from WCO station L4. The analyses are reported for Nitrate+Nitrite, Nitrite, Silicate and Phosphate concentrations with details provided in Appendix 1. The dataset begins in 2000 and runs through till mid 2022, and the data is an ongoing time series. In the early years (2000-2007) the samples were taken and frozen to be batch analysed at a later date. Detailed methodology can be found at PML-WCO_nutrient_protocols.doc.doc (westernchannelobservatory.org.uk). Data from 2000-2021 were found at BODC Western Channel Observatory (L4) doi: 10.5285/bc3cf5ce-d18c-1f42-e053-6c86abc02e29 [35] and supplemented with up to date information to February 2022 . Sea surface temperature (SST) data are collected using sensors attached to a sampling rosette, by the R/V Plymouth Quest, at WCO station L4 were downloaded from the Western Channel Observatory data download site, https://www.westernchannelobservatory.org.uk/data/CTD/ and also Table S12. The sampling protocol can be found at www. westernchannelobservatory.org.uk/documents/l4_sampling.pdf. Both datasets extended beyond other phytoplankton measurement period but were included in order to make comparison from a good representative baseline.

2.2: CPR microscopic data

Plankton sampling using the CPR survey collection is described in detail elsewhere [24, 25] This study used samples collected on a Ship of Opportunity operated by Brittany Ferries from 2011-2012 in the Western Channel between Roscoff, France and Plymouth from 2011-2012. Plankton was counted in a semi-quantitative manner using log-transformed data [25], DOI: https://doi.org/10.17031/1808. *Vorticella* sp. and nematocysts were excluded as non-phytoplankton entities whilst Brown cysts, Black Spine cyst, Filamentous algae and phytoplankton colour) were excluded as too ambiguous.

2.3 Water Sample DNA Extraction

Water sampler DNA extraction was completed as follows: The filter was removed with clean forceps and placed into a sterile, 2ml microfuge tube prior to DNA extraction and centrifuged at 13000 x g to collect any remaining cells. Excess ethanol supernatent was removed and the remaining ethanol was evaporated in the fume cupboard. For WaMS samples from February to March 2011, buffer AP1 from DNeasy Plant kit (Qiagen, Valencia, USA) was added using volumes specified in the kit, and DNA extraction proceeded according to the manufacturers protocol and described more fully in Stern et al. (2015).

from thereon was completed by CTAB buffer extraction, followed by phenol-chloroform extraction process adapted from [36] as described by [31] 500µl of CTAB buffer containing 5.6 µg/ml of Proteinase K and 0.2% ß-Mercaptoethanol (Sigma) was added to each sample and incubated for 1h 30 minutes at 55°C. DNA was extracted using equal volumes of

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phenol (Sigma), followed by chloroform/isoamylalcohol mix (Sigma). DNA was precipitated with isopropanol and washed with ice cold 70% ethanol. The pellet was resuspended in 60µl of TE buffer.

2.4 Quantitative real-time PCR-HRM Assay development

Algae culture growth, preparation of standards and DNA extractions see Appendix 2. All quantitative assays were assessed through High Resolution Melt-Curve (HRM) quantitative real time PCR (qRT-PCR)) that melts the PCR product at a specific temperature for species detection and simultaneously carrying out qRT-PCR on DNA. *Pseudo-nitzschia fraudulenta* and *Pseudo-nitzschia delicatissima* assays were based on previously validated study [37]. *Pseudo-nitzschia multiseries* was developed as part of this study [38]. *Aureococcus anophagefferens assay* was based on an earlier study [39]. Table 2 these summarises assays. HRM-qPCR amplifications were performed using a Rotor Gene 6000 (Qiagen, Valencia, USA) in 10µl volumes using a 72 sample rotor using the green channel and three step with HRM option.

For HRM- qRT-PCR assays, reactions were run in duplicate or triplicate for standards (see Table 2) and contained 5µL of SensiFAST HRM Kit (Bioline Reagents Limited, London), 0.4µl of each primers, 2µl (*P. fraudulenta, P. multiseries*) or 4µl (*P. delicatissima*) of DNA in a total reaction volume of 10 µl. Additionally, genomic DNA from their corresponding cultured species were included to determine any differences in outputs from cloned PCR products and to assess copy numbers per cell. Due to a later failure in *P.fraudulenta* CCAP1061/41 (accession number OM350396), an additional *P. fraudulenta* qRT-PCR assay was performed on DNA dervied from *P. fraudulenta* culture IO83-07 to convert ITS copy numbers to known cell concentrations. Negative controls with water instead of DNA were used to ensure results were from their respective samples and not external contamination. The optimised thermal cycling conditions were as follows: 1 cycle of 95°C for 3min for polymerase activation, followed by 40 cycles of denaturation at 95°C for 5s, primer annealing at 60°C for 10s and extension at 72°C 20s. Following the cycling phase, HRM melting phase ranged from 70-90 °C, rising in 0.1 °C increments.

Melt curves were inspected to assess contamination and range of curves. HRM standard reports were downloaded from Corbett Rotor Gene 6000 software (Qiagen) All assays revealed species-melt curves whose peak were within 0.5°C of standards. Fig. A1 shows HRM curves of standards and the corresponding genomic DNA positive controls of standards for *P. multiseries*, *P. fraudulenta* and *P. delicatissima*.

2.4.1 Quantification: qRT-PCR data treatment

Standard curves were generated by qRT-PCR and calculated against their respective DNA copy numbers or with DNA concentrations using Corbett Rotor Gene 6000 (Qiagen, Valencia, USA) Rotor Gene Q software under Cycling A Green channel. A full description is in Appendix 2 showing standard curves for *P. fraudulenta* and *P. multiseries* in Fig. A2,

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P. delicatissima in Fig. A3 and *A. anophagefferens* in Figs. A4-7. Reaction parameters, standard curves and values were extracted from Corbett Rotor Gene 6000 software. Table A1 in Appendix 2 shows qRT-PCR run parameters for *P. fraudulenta* and *P. multiseries* which showed low reaction efficiencies and significant inter-run variability for inter-annual comparisons. Thus only presence is recorded for these species.

The target copy numbers per cell for *Pseudo-nitzschia delicatissima* calculated from the qRT-PCR run containing 2011 samples and found to be between 15-20 copies/cell. For the *Aureococcus anophagefferens* assay standard curves were generated in duplicate or triplicate as described in Appendix 2. To improve temporal coverage, an average was taken of all samples taken at each sampling month as some sampling stations were missed due to mechanical error of the WaMS device. Calculated cell concentrations are shown in supplementary Tables S8 and S9 for *P. delicatissima* and *A. anophagefferens* respectively

2.4.2 Environmental Sample Testing

DNA extractions from WaMS samples were subsequently tested for the presence of *P. fraudulenta*, *P. delicatissima*, *P. multiseries* and *Aureococcus anophagefferens* using the previously optimised protocol. Each HRM-qRT-PCR run included positive control, negative (water), negative (DNA extraction water control) and standard dilution series, run in duplicate or triplicate. A total of 105 different environmental samples were screened from years 2011 (42 from February 2011-February 2012) and 2013 (35 from March-December, 2012). A selection of products from each 2011 and 2012 was checked for species-specificity by DNA sequencing (Source Bioscience (Nottingham, UK) for sequencing according to their protocol. Environmental samples were tested singly except for *A. anophagefferens* runs AA2013pt1 and AA2013pt2, where they were run in triplicate.

2.5: DNA amplification, sequencing and bioinformatic analysis

Twenty nine samples of genomic DNA were shipped to Molecular Research LP (http://www.mrdnalab.com, Shallowater, TX, USA) where a 500-bp portion of the variable V4 region of the nuclear 18S rRNA was amplifed by PCR using the adaptor-linked barcoded eukaryotic primer pair euk516F (5'-GGAGGGCAAGTCTGGT-3') and euk1055R (5'-CGGCCATGCACCACC-3'). Amplification was performed using HotStarTaq Plus Master Mix Kit (QIAGEN) under the following conditions: initial denaturation at 94 ◦C for 3 minutes, followed by 30 cycles of 94 ◦C for 30 seconds, 53 ◦C for 40 seconds, and 72 ◦C for 1 minute, concluding with an elongation step at 72 ◦C for 5 minutes. Amplicons were pooled in equimolar concentrations and purified using Agencourt AMPure beads (Agencourt Bioscience, MA, USA). Amplicon libraries were pyrosequenced using Roche 454 GS-FLX Titanium chemistry (hereafter called HTS for high throughput sequencing).

2.5.1 Sequence processing

Raw sequence data were processed using QIIME v1.9.1 [40]. In the first round of screening (\first pass filtering"), reads were checked for the presence of the forward sequencing primer (euk516F) and a valid barcode and single reads removed (the denoising step). This

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process was carried out separately for reads from Febraury-April 2011 and for April 2011-February 2012. The output files for both of these were combined to c ontain reads from 42 samples in total were processed. Barcoded reads were imported and had forward primers removed (allowing for a maximum of one mismatch) before being demultiplexed and filtered according to their Phred quality scores. Quality criteria were a minimum read length of 200 bp, a maximum read length of 700 bp, no ambiguous bases, a maximum homopolymer length of 6 (as homopolymer reads are a specific problem to 454 GS-FLX technology used at the time). Additional filtering criteria were also applied with a sliding window quality score of 50 to remove poor quality sequences during denoising steps.

Operational Taxonomic Unit (OTU) clustering on the resulting dataset was carried out at the 97% sequence similarity with a word length of 12 and maximum mismatch range of 20-500bp. Taxonomic assignment was performed using UCLUST [41] and the RDP classifier [42] using the pick OTU command, with a minimum bootstrap threshold of 80%. UCLUST compares the overall sequence similarity of a queried representative sequence against a database of reference sequences whilst RDP classifier compares query sequences to reference sequences using 8bp long strings across the entire length of the sequence and estimates a confidence score for the assignment of each taxonomic level based on 100 bootstrap replicates. For both approaches, the Silva 111 eukaryotic rDNA database was used for identification of taxa. OTU tables were generated from which taxa summary plots and alignments were created. Searches were performed using the BLASTn tool [43] to check the identities and search for Chimeric reads that did not show complete alignment with a reference sequence. Chimeras were removed from the alignment using chimera slayer. Sequences were deposited in Genbank (ERP105780)

2.5.3 Phylogenetic analysis

Selected sequences from the partial 18S read alignment output from Qiime or from Sanger sequencing from WaMS samples were taken, checked for identity by Blastn [32].

2.6 Enumeration of phytoplankton and bacteria by flow cytometry

Phytoplankton and bacteria were enumerated using a BD Accuri™ C6 flow cytometer equipped with a 50 mW solid state laser providing blue light at 488nm. Samples were analysed at a nominal flow rate of 100 µL min^{-1} and a core of 20 µm for 5 minutes and 1 minute respectively for phytoplankton and bacteria. The flow rate was calibrated on each sampling day using Beckman Coulter™ Flowset™ fluorospheres at a known concentration. Besides counting the cells, the flow cytometer also measured chlorophyll fluorescence (CHL. >675 nm), phycoerythrin fluorescence (PE. 585+/-20 nm), green fluorescence (530+/-15 nm), side scatter (SSC. light scattered by particles at 90° to the direction of the laser beam) and forward light scatter (FSC. light scattered by particles at narrow angles in the same direction as the laser beam). Measurements of light scatter and fluorescence, data storage in listmode format and subsequent data analysis were made using a CFlow Plus software (Becton Dickinson, Oxford) with log amplification on a seven-decade scale.

Phytoplankton data acquisition was triggered on both chlorophyll fluorescence, and forward light scatter using prior knowledge of the position of *Synechococcus* sp.

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cyanobacteria to set the lower limit of analysis. Density plots of FSC vs. CHL fluorescence, PE vs. CHL fluorescence and SSC vs. CHL fluorescence were used to discriminate *Synechococcus* sp. cyanobacteria, picoeukaryote phytoplankton (0.5-3 μm), coccolithophores, cryptophytes and nanophytoplankton (eukaryotes >3 μm, excluding the coccolithophores and cryptophytes). For quantification of bacteria, 500 μL of preserved sample (0.5% gluteraldehyde final concentration) was mixed with 5 μL 1% Sybr Green I DNA dye (1% solution made from adding 1 μL of x10,000 Sybr Green I concentrate to 99 μL 300 μM potassium citrate buffer) and 45 μL 300 mM potassium citrate buffer. Samples were mixed by hand and then placed in the dark at room temperature for one hour before analysis. Data acquisition for bacteria was triggered on both green fluorescence and forward light scatter. Density plots of CHL fluorescence vs. green fluorescence and SSC vs. green fluorescence were used to quantify total heterotrophic bacteria. The complete flow cytometry dataset from 2011-2016 from which this data was extracted is available at DOI https://doi.mba.ac.uk/data/2955. Only picoeukaryote and nanoeukaryote data was used for further analysis. To increase robustness data from all positions from each cruise was aggregated into mean value.

2.7 Comparisons between microscopy and genetic findings

The WCO L4 dataset had a taxonomic hierarchy derived from World Register of Marine Species taxonomy or WoRMS [44] to accompany the data that included diatoms, dinoflagellates, coccolithophorids, flagellates and other (Amoebozoa, Radiolaria). To update class-level taxonomy of diatoms [45] , classification of Bacillariophyceae were altered to their corresponding classes according to the AlgaeBase database [46]. Comparisons were made at Class, Order, Family and Genus level with UCLUST and RDP assigned taxa observed in WCO L4 data with that identified from WaMS partial 18S reads over 2011-2012 period using as close a date as possible to each other. If the dates did not correspond then two nearest dates from WCO L4 data were used for comparisons. All positions of WaMS data were used for comparisons. Any non-taxonomic assignments were ignored. Next, phytoplankton data from the CPR dataset was classified according to WoRMS again using class-level classification according to AlgaeBase and split into the same hierarchical classifications. Only phytoplankton taxa were considered and both the number of taxa and the actual observed taxa in each category was counted. The RDP classifier and UCLUST classification systems for 18S reads from the 2011-2012 WaMS samples differed from WoRMs, and in those cases the nearest equivalent taxa to WoRMS was used for comparison. If a genus name was given, the family and order could be inferred from WoRMs database.

3. Results

3.1 Nutrient and SST trends

The average baseline SST between 2000-2020 was 12.73°C, with winter average SST°C (ranging from 7.37-13.21°C) and Spring average SST 10.16°C (ranging from 7.35-14.23°C) summer SST average of 15.23°C (ranging from 11.54-19.04°C) and autumn average of 14.83°C (ranging from 11.01-18.39°C). SST had a single annual cycle (Fig. 2, panel 1).

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Between 2011-2013 average temperatures were similar to the baseline at 12.48°C. Seasonal averages of Winter, Spring, Summer and Autumn where phytoplankton measurements were taken in this study were 10.06°C, 9.86°C, 14.98°C, 14.64°C respectively. Overall temperatures were cooler, especially over the summer. In 2011 a cooler winter SST 9.43°C led to warmer than average Spring at 10.33°C followed by average summer and autumn temperatures. All 2012 seasonal average SSTs were near average except for a warmer than average summer SST at 15.02°C. 2013 had near average SST except for spring SST at 8.75°C that was cooler than average.

Nutrient cycles over 21 years (see Fig. 2, panel 2) revealed an overall repeating annual cycle for all the nutrients, peaking over the winter and at their lowest levels during the summer months. There are also point increases during the summer months due to occasional increased river run-off, but the overall annual trends are similar. A 21 year baseline shows levels of nitrate, nitrate and nitrite together, ammonia, silicate and phosphate to be 0.18 μM, 3.27 μM, 0.27 μM, 2.31 μM, 0.26 μM respectively. In the summer (June-August) period, the 21-year baseline nitrite was 0.02 μM along and together with nitrate were both 0.02 μM, ammonia levels were 0.13 μM, silicate levels were 0.25 μM and phosphate levels were 0.06 μM. Summer nutrient depletion levels varied each year occurring from April-June in 2011, extended in April to September in 2012 and June-August in 2013 (Fig 2, panel 3). All year average silicate levels in 2011 were two-thirds lower and up to twice as low comparing just winter silicate levels. Nitrite levels were approximately a third lower in 2011 but nitrite and nitrate levels were average. The most pronounced change in 2011 were winter (Dec-Feb) ammonia levels in that were four times less than the 21- year winter average to the extent that summer ammonia levels were higher. In 2012 summer and winter ammonia levels were at equal levels approximately and summer nitrite and nitrate levels were eleven times higher against the corresponding 21-year average. In 2013 all nutrient levels of the entire year, winter and summer were near their 21 year average levels, although summer nitrite and nitrite levels were lower than 2012 but still five times higher than than the corresponding 21- year average. Components of the nitrogen cycle appear to be altered from 2011-2013 however, the ammonia cycle is less clear than other nutrients and is a more difficult to interpret as part of the time-series was treated differently and is prone to contamination.

3.2 Quantitative real-time PCR of potential harmful algae taxa

Quantitative real-time PCR (qRT-PCR) combined with HRM was developed for four harmful algae species *Pseudo-nitzschia delicatissima*, *Pseudo-nitzschia fraudulenta* and *Pseudo-nitzschia multiseries* and finally *Aureococcus anophagefferens*.

3.2.1 Pseudo-nitzschia assay

HRM curves from plasmid standards and genomic DNA from positive controls revealed a specific peak at 84-84.52°C for *P. fraudulenta* (strain PLY1St.36A), with 84°C observed for standards at the lowest concentration, although non specific peaks were identified outside its range which may be the reason for reduced reaction efficiency. Sequencing the ITS2 region of the genomic DNA control confirmed it was *P. fraudulenta* (LN873237).

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Sequencing *P. fraudulenta* ITS2 amplicons after qRT-PCR from WaMS DNA in samples E4_5.1/WS12 confirmed the product was ITS2 from *P. fraudulenta* (LN873238). *P. multiseries* HRM curve showed a specific peak range of 82.47-82.7. qRT-PCR derived amplicons from the positive *P. multiseries* genomic control (Pm12) confirmed its identity (accession number OP504083) No significant differences in HRM curve range was found between 2011-2012 samples for P. multiseries qRT-PCR however some non-specific amplicons were also observed.

The plasmid standard curves for *P. fraudulenta* and *P. multiseries* were generated for each qRT-PCR HRM run (Fig. S2) with good correlation coefficient value (R^2>0.99) and consistency between standard Ct value between replicates but their efficiency figures were lower than the recommended 90% on the standard curve qRT-PCR results, ranging from 70-72% for *P. fraudulenta* and 66-70% for *P. multiseries* likely reflected in lowered overall reaction efficiency values of the standard curve slope (M: -4.236 to -4.56) which should be near -3.322. Due to their lowered efficiency the results were not deemed to be reliable for subsequent interpretation of species cell numbers.

HRM analysis of *P. delicatissima* revealed a species-specific peak between 82-82.5C (Fig. A1 B, Appendix 2). *P.delicatissima* reaction efficiency (see Table 3) was between 0.8-1.2, 2011 was lower than idea 90% or more efficiency. Sequencing of positive control q qRT-PCR amplicons confirmed the assay detected *P. delicatissima* ITS1 (OM350397), The copy number calculated of 15-20 copies/cell is similar to average copies/cell reported by other studies for this species: 16 and 26 copies/cell [47], 29 copies per cell [48]. As 2011 efficiency was lower for runs that analysed 2012, 2013 samples, a student's t-test was performed on 2011 and 2012 Ct values revealing no statistical difference between runs (p=0.075).

3.2.2 *Aureococcus anophagefferens* assay

The PCR product of Aureococcus assay developed by Popel et al. [39] had a HRM melt curve peak at 82°C (see Fig A1C, Appendix 2) and was specific to Aureococcus upon sequencing products from environmental samples E4_10_11 (1)/WS32, E1_12_11/WS34 and E1_2_12 or WS39 (accesion numbers OP484337-OP484339 respectively). The reaction efficiencies of 150 samples (tested in four runs) ranged were 0.93 for AA2011 run, 0.82 for AA2012 run and 0.78 and 0.61 for AA2013 part 1 and part 2 respectively Three runs (2012, 2013 pt1) were under optimal 90% reaction efficiency and may have underestimated cell abundances (Table 3). Other parameters (M, R, R^2) were similar to each other and were within recommended manufacturers guidelines (Qiagen, UK). Run AA2011 had very similar reaction parameters to those published by Popels et al. 2003 [39]. The potential accuracy of mean detection capability is 1.4 cells on average [39]. The Ct value range was similar to assays AA2011 and AA2012 but for AA2013pt 1 and pt 2 the Ct range was lower than described [39] which may explain the lower reaction efficiencies of those assays and may mean copy numbers of environmental samples are lower than they would be under optimal reaction conditions. The calculated cell numbers recovered for all AA2011-2013 assays are in Tables S9a and averages in Table S9b.

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3.3 Environmental analyis of harmful algae species in the Western channel by qRT-PCR

Pseudo-nitzschia delicatissima, qRT-PCR results revealed elevated abundances in 2011 compared to 2012 and 2013 (Fig. 3A). By contrast microscopy results indicated elevated abundances in 2011 and 2013 and over a longer period from July-October 2013 (Fig. 3B). Both analyses showed reduced presence in 2012, although samples were missing for six out of 36 months, including 3 month of Spring in 2013that may skew qRT-PCR results for this species.. Inspection of station E5, that is most similar in location to WCO station L4, revealed similar pattern to that of average values over all stations (not shown). Of note cell abundances from the qRT-PCR results were an order higher than microscopic abundances. Although quantitative data could not be used for *Pseudo-nitzschia fraudulenta* or *Pseudo-nitzschia multiseries*, their presence and absence (Table 1) shows *P. multiseries* was completely absent using this assay. *P. fraudulenta* was present from February to October but showed a seasonality with elevated frequency of occurence in April and October 2011. The next year it appeared year round in at least one sample from each cruise mostly in mid channel.

The *A.anophagefferens* qRT-PCR assay revealed that average cell numbers for all stations in July 2011 was significantly elevated compared to all other dates over a three year period.This was at a single station (E5_7_11) at $11x10^9$ cells/ml, see Tables S9a and S9b. However, these values were above maximum standard curve values of $4 x10^6$ cells/ml and so not likely to be accurate. Moderately elevated cell concentrations were observed in September, October and December 2011 at 44, 160 and 503 cells/ml respectively. In September and November of 2013 cell concentrations reached maximum levels of 60 and 25 cells/ml, although these 2013 values are likely underestimated due to reduced assay reaction efficiency. No detectable *A. anophagefferens* was observed in 2012 even though standard curve parameters were at acceptable levels. There was little preference for station location occurencein this small dataset. The gaps in sampling may have missed key growth periods.

3.4 Small eukaryotic cells in the Western Channel using Flow cytometry

Over 2011-2013, a comparison was made between different sized phytoplankton measured by microscopy (generally larger than 20µm) and flow cytometry (FC) generally under 20µm (Fig. 4). Picoeukaryotes ranged from 24-52870 cells/ml, with an average of 6500 cells/ml and numerically dominated nanoeukaryotes (8-4670 cells/ml, average 689 cells/ml) by a factor of 10. Picoeukaryotes peaked in late spring in 2011 versus in summer in 2012. A summer-autumn picoeukaryote peak occurred in 2013, although with missing samples there could have been another. Microscopic total phytoplankton counts were of the same order as nanoeukaryotes. The diatoms followed a similar pattern to the total phytoplankton, except in 2013 with an autumn peak instead of the spring/summer total phytoplankton peaks. Unfortunately there was no WaMS flow cytometry data for May 2013 when there was a large spike in microscopic phytoplankton from WCO station L4.

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The numbers of cells measured by FC did not match the maximum *A. anophagefferens* levels measured by a factor of 10^5 likely due to cell concentrations being outside the dynamic quantitative range of the qRT-PCR assay Outside of this genetically quantified *A. anophagefferens* made up a negligible proportion of total picoeukaryotes as measured by FC. In terms of its seasonal pattern, *A. anophagefferens* had a major peak in abundance in July 2011 just after the total picoeukaryotic bloom but after that very little temporal correspondence was observed with picoeukaryotes with this species preferring Autumn and Winter. Only microscopically detected *P. delicatissima* and diatoms (Fig. 3) show similar abundance trends with nanoeukaryotes.

3.5 Genetic taxa trends

After quality filtering and clustering, the total number of reads from 42 combined samples was 49033, ranging from 69-7500, with a mean of 1167.5 and median of 692 reads per sample (see Table 4). Read and OTU distributions were unequal across samples and the first sequencing run showed poorer OTU counts compared to the second. Measures of alpha diversity in Table 4 confirmed this and suggest the planktonic community was incompletely sampled. Calculated chao1 rarefaction curves for each sampling tow failed to reach plateaus (Fig. 5). Due to the bias it is likely rare taxa are not represented in sequencing run 1 and subsequently any community analysis (beta diversity) would be likely be biased.

Using UCLUST approach, Taxa at hierachy level 3 (class level or equivalent) from all major eukaryotic lineages were observed in the Febraury 2011- February 2012 samples, though most groups were relatively rare (Fig. 6). Level 3 was the most complete in terms of genetic taxonomic assignment (also shown in Table S2), however UCLUST taxa assignments were made to level 5 (family/genus level), shown in Tables S3-35. The dominant taxonomic groups over all samples was uncultured eukaryotes at 34% followed by Metazoa at 26% then alveolates at 25% then a significant minority consisting of Stramenopiles at 6% and chloroplastida of 4% (see Table S2). Rare taxa contributed just 5% of total reads, but represented by 19/24 taxa while a majority of the dataset (95%) corresponded to reads shared among five taxa. The summer season had less uncultured eukaryotes and were dominated by Alveolata (samples WS17-22). The proportions of Chloroplastida were absent or minimal in summer and relatively higher in winter, Spring and Autumn of 2011. At taxonomic level L4, the Alveolates in samples WS17-22 were dinoflagellates. Most of the Metazoa were arthropods (see Table S3). Haptophytes mostly appeared from June onwards

Diversity assessments using the RDP classified dataset were similar to UCLUST (see Fig. 7 and Table S6); lower taxa were divided into levels that did not always correspond to taxonomic hierachies- a class or genus could be in the same level. A second caveat are some outdated taxonomic classifications. A third (299) of the 922 Eukaryotic OTUs were unidentifiable and a further 147 were unidentifiable beyong Kingdom level. The majority of other OTUs belonged to SAR supergroup or Opisthokonta. Only a third of the total number of OTUs (262) could be identified at level 3 (ranging from phylum to class) and here the majority of taxa belonged to crustacean arthropods (7%), Mamiellophyceae (9%)

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five alveolate groups namely, Ciliophora, Protalveolata, Dinoflagellata and Cecozoa, totalling 53% and Stramenopiles mainly Diatomea and Bicosoecida at 9%. Sixteen OTUs could be identified to genus or equivalent level for those without official genus names. Of interest, Aureococcus identified by UCLUST was also confirmed by qRT-PCR and sequencing respectively.

3.6 Phylogenetic analysis of partial 18S reads of WaMS samples, WS1-43

Confirmation of UCLUST and RDP results using maximum likelihood method of phylogeny on selected taxa were able to confirm taxa to genera level, although to low confidence considering the short size of the partial 18S reads and the limits of the markers resolution (Fig 8). Only shallow phylogenetic positions were considered to confirm the presence of a taxa as deeper level taxa clusters were unreliable with low bootstrap support. Within the MAST group (Fig. 8A), one other OTU, sister to Biocosoecida could not be placed and the divergent nature of these sister sequences mean that OTUs in the MAST groups are often unclassified. The Haptophytes confirmed the presence of Phaeocystis and an unidentified Prymnesiophyte sister group to Chrysochromulina. Within the dinoflagellates (Fig. 8C), Prorocentrum taxa was split into different clades and one OTU grouped with one clade containing the benthic *Prorocentrum lima*, although with low support. Few OTUs could be identified to genus level with the exception of *Karlodinium*, *Torodinium* and *Protoperidinium*. Three sequences were sister to *Toridinium* and *Karlodinium* and likely to belong to Gymnodiniales. Four other sequences were sister to a *Gymnodinium* species, whilst four others were more deep branching of unknown origin. Phaeocystsis was the only haptophyte that could be identified. Three other reads were sister to two Chrysochromulina species, and could be another of the same species or a related genus.

3.7 WaMS 18S genetic reads versus Microscopy results

WCO L4, CPR WaMS genetic and CPR morphological dataset had significant different standard taxa measured (see Table 5). Table S9 shows a complete comparison of taxa present in 2011-2012 from WCO L4 with genetically detected taxa from WaMS. Due to disparity only photosynthetic categories were compared between datasets (Fig. 9). Of the coccolithophorids observed in WCO L4 six were found in CPR category too and a reasonable comparative match. The best alignment in terms of taxa measured and observed were in the Diatoms and Dinoflagellates categories. The Flagellate and Other category of WCO L4 had fewer taxa and showed the least number of common taxa with CPR and WaMS genetic taxa, the latter being mostly heterotrophic flagellates. Table S5, reveals genetic detection of taxa in WaMS sample was just over 73% were heterotrophic/mixotrophic compared to only 38% of autotrophic/mixotrophic taxa. Ciliophora were measured by WCO L4 than the CPR dataset not surprisingly as they are soft-bodies organisms that would be destroyed on the CPR silk. As expected the CPR and WCO L4 morphological datasets shared the most number of photosynthetic taxa observed (31 out of a combined 100). Only 20 families within photosynthetic category could be identified and between 8-12 were shared with morphological observations. The type of taxa not observed by CPR or WCO L4 morphology included Cercozoa, SAR group,

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Labyrinthulomycetes, Fungi, Kathablepharidae, Picozoa and Excavata. A surprisingly large number of metazoa were identified considering the small volume of samples including Echninodermata, Cnidaria, Mollusca, Urochordata and Maxillopoda. Within the most comparable photosynthetic category species under 10 microns were not represented in the morphological datasets such as Pegalophyceae (*Aureococcus*), Chrysochromulina and Imantionia of Prymnesiophyceae, Geminigera and Teleaulax of Cryptophyceae and Mamiellophyceae of Chloroplastida. Dinoflagellelates had the most shared taxa (5 families) and 4 genera.

4. Discussion

This study has revealed genetic approaches detect different communities of phytoplankton to microscopic methods in the main. Only 38% of the genetically detected taxa from WaMS were autotrophic or mixotrophic of which 8-12 taxa were also identified by microscopy at the family level. Additionally, the absolute abundance of *Pseudo-nitzschia delicatissima* detected by qRT-PCR differed from it's equivalent morphospecies level microscopic counts. Genetically detected phytoplankton were mostly in the pico- to nanoplankton size range and those that have acquired phototrophy such as ciliates. However there is low taxonomic resolution with partial 18S marker, most resolved to family-order level, confirming conclusion from microscopic and genetic surveys with freshwater plankton [8]. The 18S-V4 region although most popular due to its elevated representation in public databases (Lopesdos-Santos et al. 2021), did not perform well in distinguishing dinoflagellates or haptophyte likely due to the short marker region (300 of the 550bp) available for analysis, however it indicated the presence of broader taxa assemblages that can inform more precise monitoring. Interestingly, most taxa found in January to May 2011 of WaMS in our earlier study [31] were present year round that would confirm a consistent seed of species that can grow under the right conditions. All supergroups in this study were also found in the Eastern (English) Channel in other 18S amplicon sequencing surveys, such as chlorophytes [49].

Thus, each of these methods have specific advantages. Phytoplankton microscopy results show a high degree of resolution with most taxa over nanoplankton size range being identified to species, especially for diatom and dinoflagellate species. The differences in species assemblages detected by microscopy and genetic approaches lead the path to a complementary approach to routine monitoring our seas covering breadth and depth. The variety of ciliates and radiolaria captured would enhance trophic connections and knowledge of carbon flux in summer and late winter [50]. Most of the organisms detected genetically in WaMS samples are not included in any official assessment of marine plankton health [7]. However, there are hurdles to overcome using nucleic acid high-throughput sequencing, including quantitative bias such as over-representation of 18S reads for alveolates [1] or metazoa, and the lack of resolution in some taxa. Approaches that over-come these includingsuch as blocking oligonucleotides for to remove metazoa signal [51] and the use of improved primer cocktails [52] or better DNA markers. The use of long-read high throughput methods have a real chance of providing high-resolution genomic and taxonomic diversity assessments at the species or even strain level [53]. With

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the inclusion of prokaryotic genetic diversity surveys, a massive increase in detection of trophic networks could be achieved. Even presence data can have a powerful effect on building ecological models, such as those the prediction subnetworks of plankton organisms significantly associated with carbon export using metagenomic TARA ocean data [54].

A key benefit of genetic survey was the additional recovery of a wide variety of hetertrophs which are key to nutrient cycling, many of which are associated with phytoplankton such as *Fragilariopsis* and the tintinnid ciliate, *Salpingella* [55]. Eleven members of uncultured and incompletely characterised MArine STramenopiles group or MAST groups that represent a substantial portion of the abundance and diversity of the heterotrophic eukaryotic plankton community. These are globally abundant bacteriovores, symbionts and parasites [56, 57] and many are likely intertwined with phytoplankton. There are about 18 MAST clades that are shown to be influenced by temperature, nitrite and the abundance of the cyanobaceteria, Synechococcus. Continuous monitoring of heterotrophic organisms alongside phytoplankton could be used to develop much-needed biodiversity or food web indicators of Pelagic habitats in official assessments of marine health that are unassessed due to lack of data [58]. Many MAST-3, 4, 7, 11 as well as several phytoplankton species, many of them dinoflagellates were positively associated with carbon export [54].

DNA Sequencing versus Microscopy and Flow Cytometry

It would be expected that surveys recorded using the same method would be more similar and CPR and WCO L4 datasets showed more overlap with each other than with WaMS, which has smaller sampling volume and a different detection method. Sample volume is clearly important, only 50ml of WaMS samples was used for DNA extractions compared to 200ml of WCO L4 water and $3m^3$ of filtered water on CPR silks. However although the WaMS was deployed on the CPR, the taxa found were more similar to those found in WCO L4. Although the CPR captures organisms 20µm and below, the 240 micron mesh-size of the CPR is designed to capture larger phytoplankton and zooplankton although can capture plankton as small as 2µm as the mesh gets clogged [59]. For genetic approaches to be used routinely, a period of comparison and standardisation will be necessary to bring about the most effective monitoring strategy to cover all taxa [60]. This would include harmonisation of taxonomic reference guides. Both WCO L4 and CPR survey relied mostly on WoRMS databases, whereas the genetic databases of RDP and SILVA. At the species level this was fine, but this broke down at higher order levels. Software that easily converts one set of reference taxa to another is vital for more comparative studies.

Total phytoplankton counts by microscopy are of a similar order to that of nanoeukaryotic abundance in WaMS samples by FC, indicating its utility in supplementing the measurements of smaller phytoplankton aggregrate groups. In 2011 they show similar seasonal pattern, later years there were sampling interruptions that make direct comparison are difficult especially in 2013. Range of picoeukaryotes and nanoeukaryotes in WaMS samples were less than that found at WCO station L4 [61]. Tarran and Bruun [61]

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study that overlapped this one, reported a clear seasonal cycle with nanoeukaryotes but not with picoeukarytes, but is not clear with this three-year dataset. The seasonal profile of pico- and nanoeukaryotes from WaMS, with two peaks in 2011 and 2012 differed to that from L4 station with a single early summer peak from 2007-2013. This may be due to the Atlantic influence on WaMS samples with lower nutrient levels, and demonstrates how open water conditions change the phytoplankton community dynamics.

Detection of potentially harmful algae species by qRT-PCR by WaMS

This study validated two out of four assays that identified the presence and abundance two species, one of which cannot be identified through microscopy. However, there are clear challenges to qRT-PCR method. Two assays failed due to inter-run variability, low reaction efficiency in the case of *P. fraudulenta* or non-specificity in the case of *P. multiseries*. Other aspects that hampered consistent detection of species were a) low volume of water collected that resulted in low cell abundance often below the dynamic range of standards used and b) inconsistent sampling performance of WaMS due to machine error. So far no automated water sampling system on a moving system has been achieved on a routine basis. The CPR survey tows at 20-30 knots and the speed of the boat is extremely challenging sampling environment and these problems need to be overcome. Nevertheless, even the presence data of four different *Pseudo-nitzschia* species showed variable patterns of occurrence showing different growth patterns even within the same environment. Such information can inform better predictions, as many can only access less-resolved taxa.

Both *P. delicatsisima* and *A. anophagefferens* internal controls appeared to have worked well, although the reaction efficiency for the *P. delicatissima* and *A. anophagefferens* varied over runs, likely down to reagent variation. However, the development and succesful use of such assays will be beneficial for official assessments of ocean health..There is a noted knowledge gap for harmful algal bloomsfor many reasons including the difficulty in finding common characteristics [7]. Thus, the use of species-specific data on harmful algal bloom species as specific indicators of physico-chemical conditions or on likely toxicity status may be more informative.

It is notable that there were exceptionally high levels of *A. anophagefferens* in summer of 2011 which ws distinct from FC detected picoeukarotes of the same size that peaked in May 2011. Nor did it match any other phytoplankton group. This species has been reported to prefer low light, low inorganic nitrogenous and phosphorus conditions, but higher organic carbon and nitrogenous nutrient conditions [22, 62] with a suite of genes that can adapt to low light and elevated organic carbon or nitrogen sources. In 2011 it is notable that ammonia levels were unusually relatively elevated in summer. This might suggest utilisation of ammonia by *A. anophagefferens* but equally likely another phytoplankter in that year. Earlier studies shown that in the winter, inorganic nitrate is the primary N source in the Western Channel taken up in the Spring but regenerated N sustains summer phytoplankton, although its uptake was not related to known phytoplankton phylotypes or cultures [63] and historical data from the Western channel shows a relationship with dissolved organic nitrogen and nitrate at nearby WCO station E1 [30]. It is hard to explain

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the elevated summer growth of this species in 2011 and could be sourced from organic N sources from an earlier spring picoplantkon bloom. This species has it's own distinct ecological preference and likely present at low levels all year round but may grow quickly in response to the right environmental condtions. Further investigation is needed to improve our understanding of nutrient cycling in this organism that might benefit the development of indicators of eutrophication.

The microscopy results for *P. delicatissima* from WCO L4 station differed from those measured by qRT-PCR, the former showing a summer and autumn peak in 2011 and 2013 whilst genetically-detected *P. delicatissima* only occurred in 2011. Their abundance levels differed too. *P. delicatissima* has been reported to be one of the most abundant diatoms [64] and here its microscopically detected seasonality was more similar to total diatoms at WCO station L4. In 2011 it's abundance peaked in March from genetic WaMS detection and in June from microscopic counts. There are several confounding factors that may contribute to mismatch in microscopic versus genetically detected patterns, including lack of correspondence of sampling dates, missing WaMS sampling or measurements and differences in sampling methods. Light microscopic measurements of *P.delicatissima* is insufficient to distinguish between six similar species belonging to *P. delicatissima* complex and *P. pseudodelicatissima* complex [65, 66] so, it is possible a species complex was detected in 2013 but not in WaMS sample. Additionally, the use of DNA may not represent the current growth as it can detect dead *P. delicatissima* signals. The genetic detection of *P. delicatissima* without microscopically detected *P.delicatissima* complex has been reported earlier [67]. A study of *Pseudo-nitzschia* spp. measured on the French coast of the Western Channel [68] revealed that the timing, abundance and toxity of *Pseudo-nitzschia* spp. was highly influenced by local conditions, although temperature, salinity and light all positively influenced *Pseudo-nitzshchia* growth and are drivers of many phytoplankton taxa. Temperature and nutrients were reported to influence diatom assemblages at WCO station L4 [63]. *P. delicatissima* is reported to prefer use ammonium as a N source in the absence of nitrate [69], however ammonium causes physiological stress on these cells and can trigger domoic acid production [70] but Dissolved Organic Matter benefits *Pseudo-nitzschia* growth. Silicate and phosphate cause physiological stress [70] and were shown to be associated with domoic acid toxin production in Pseudo-nitzschia captured from WCO station L4 samples, although not in *P. delicatissima* cells [71]. This might indicate there are inherently non-toxic varieties of this species. Another study found that only 31% of *Pseudo-nitzschia* spp. variability can be explained by environmental variables [72]. The biological pathway is also important such as bacterial interactions as reviewed by [71] and indeed domoic acid can remains in the water to be consumed by copepods [73], some of whom can themselves can neutralise the toxin in their bodies [74]. There is a need for holistic biological and physicochemical assessments of this genus to better understand environmental drivers of toxin production. The genetic assays developed [37] and tested here will likely improve monitoring to elucidate conditions that lead to toxin versus non-toxin producing *Pseudo-nitzschia* spp.. A predictive model based on molecuar data for *Alexandrium minutum* has recently been achieved [75] showing the utility of species-specific harmful algae monitoring that could be translated into indicators for policy.

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5. Conclusions

Genetically-measured taxa composition measures different communities and complements microscopic observations. The species-level detection of morphologically indistinct taxa often show different profiles to aggregate community that provided insights into their habitat preferences and promise as indicators.

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Figures

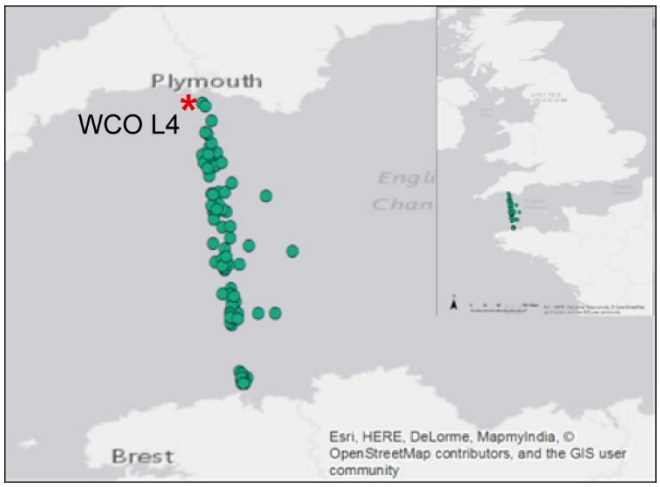

Figure 1: WaMS sampling positions in the Western Channel from 2011-2013. Asterix shows the approximate location of WCO station L4.

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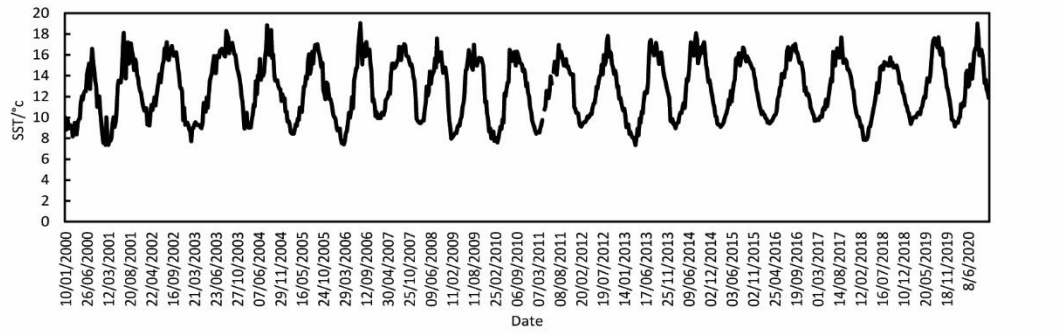
1



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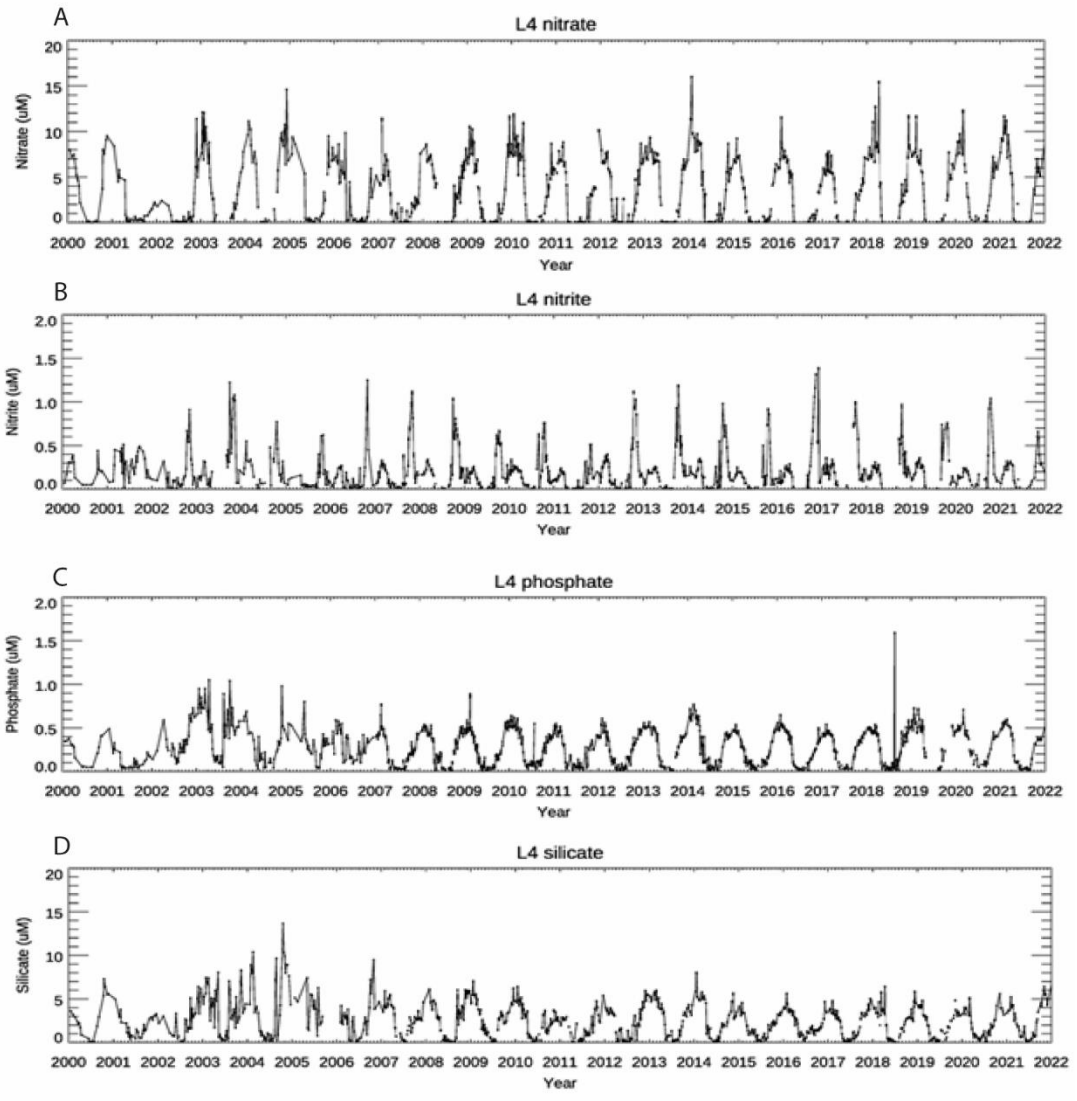

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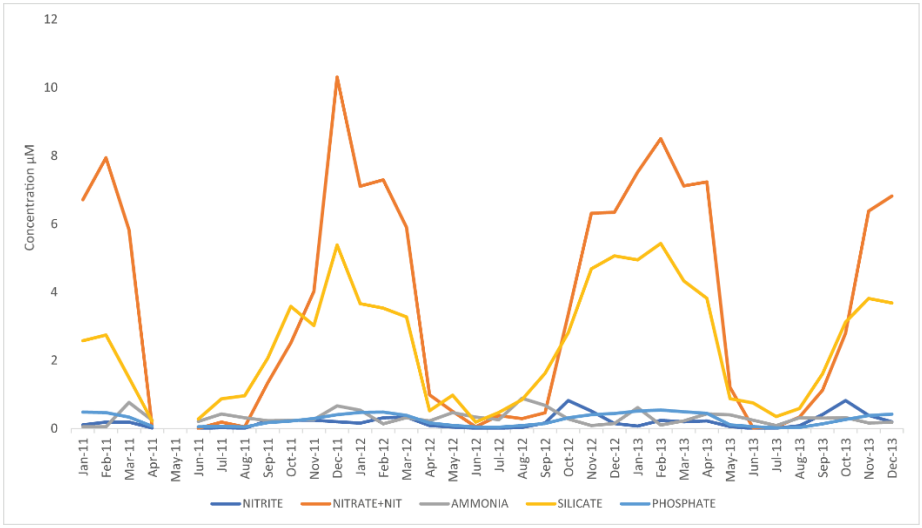

Figure 2: Panel 1 Sea surface temperatures from 2000-2020. Panel (2) Weekly measurements of Nitrate (A), Nitrite (B), Phosphate (C) and Silica concentrations from WCO L4 station from 2000-2022. Panel (3) Nutrient levels in period 2011-2013.

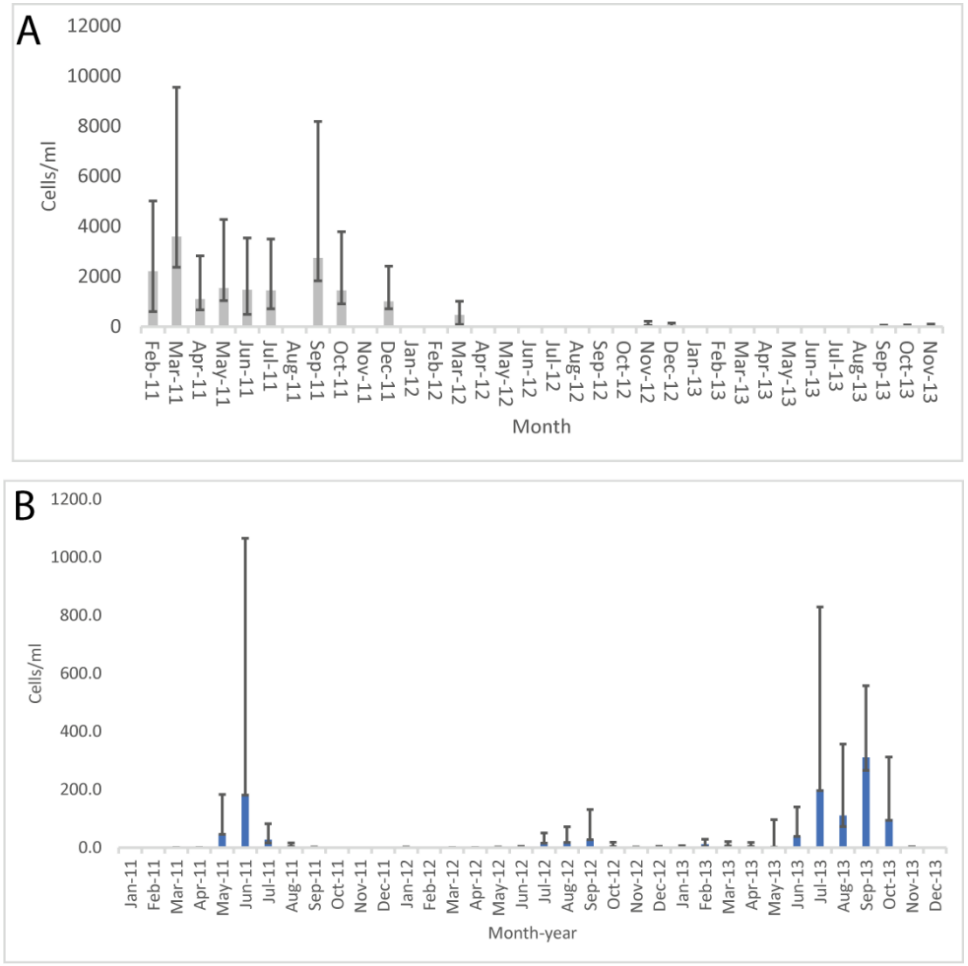

Figure 3 Comparison of *P.delicatissima* cell counts (A) measured by qPCR averaged over all stations versus light microscopy measured at Western English Channel station L4 (B)

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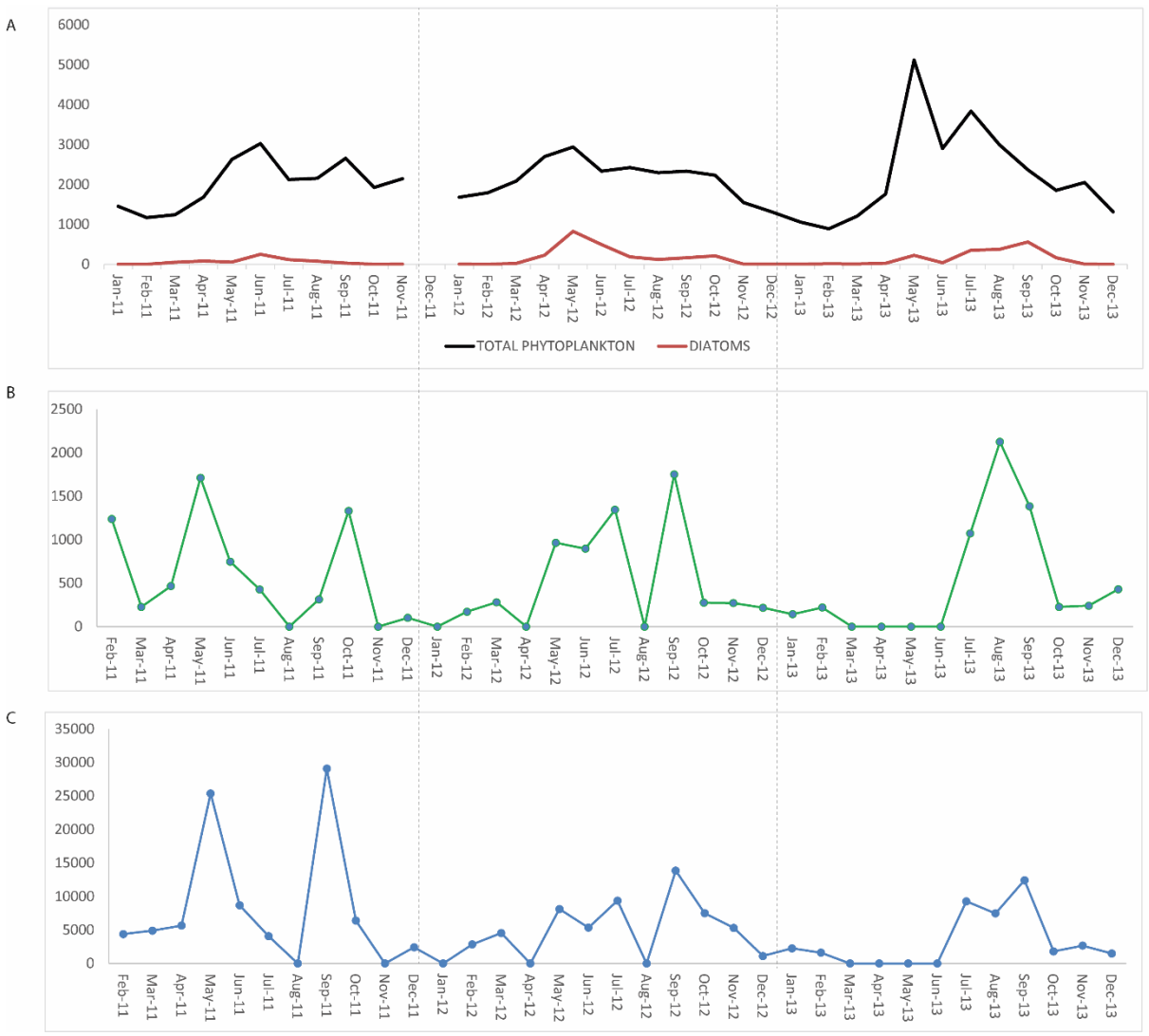

Figure 4: Monthly averaged seasonal cell abundance of Phytoplankton microscopic cell counts (cells/ml) from WCO L4 versus flow cytometric measurements of aggregated photosynthetic Nanoeukaryotes that are 10 microns or less (B) and Picoeukaryotes that are 3 microns or less (C) from the WaMS 2011-2013 (cells/ml)

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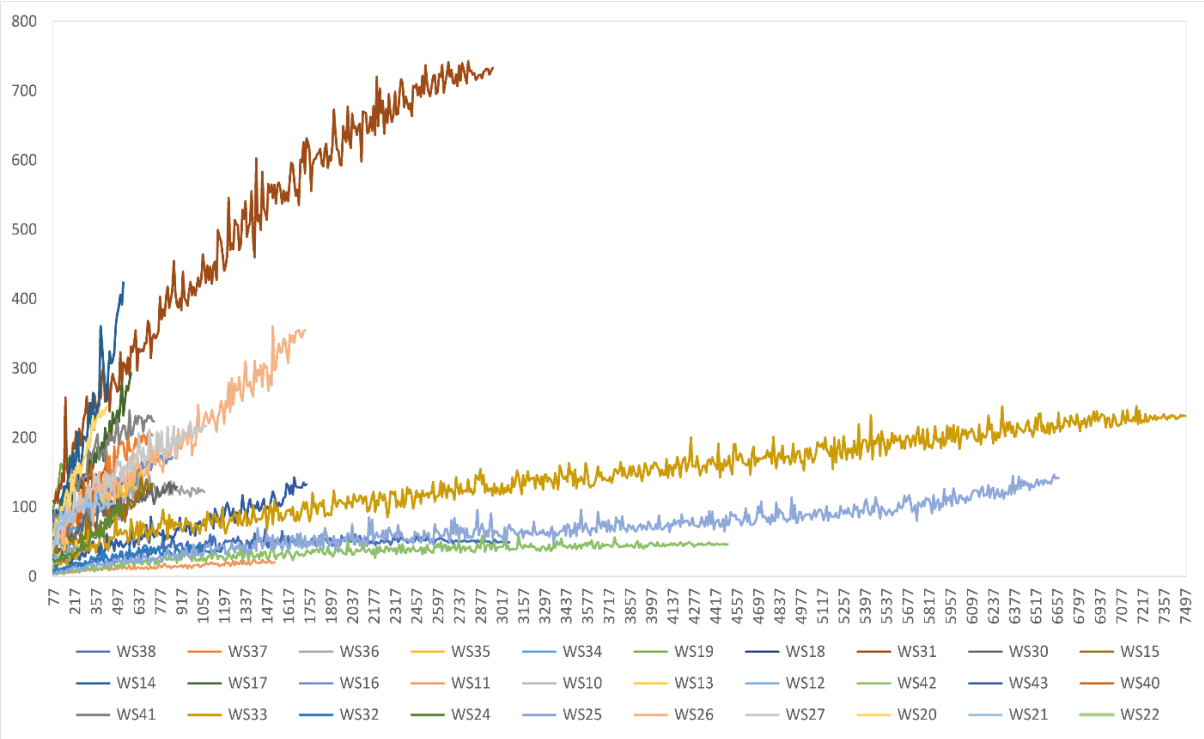

Figure 5: Chao1 indices of alpha diversity of sequence reads WS1-WS42 corresponding to WaMS samples from 2011-2012. Sequence reads are shown on vertical axis and Chao1 diversity values on the horizontal axis.

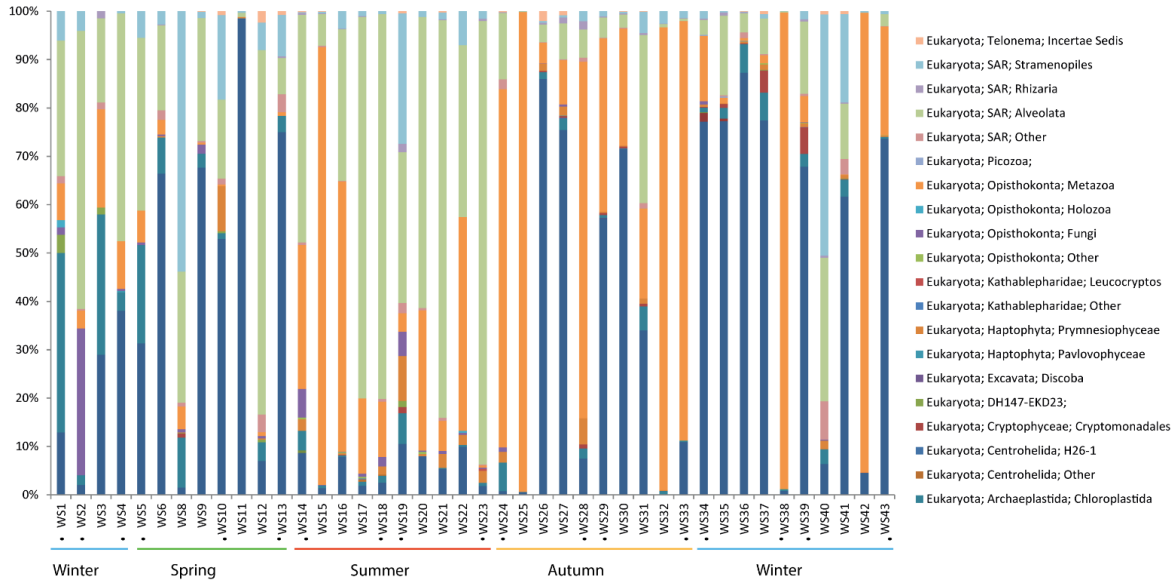

Figure 6: Taxa summary chart of WaMS samples from 2011-2012 partial 18S rDNA sequencing reads at level L3. Dots next to each sample indicate coastal samples. Coloured lines indicates season the sample was taken

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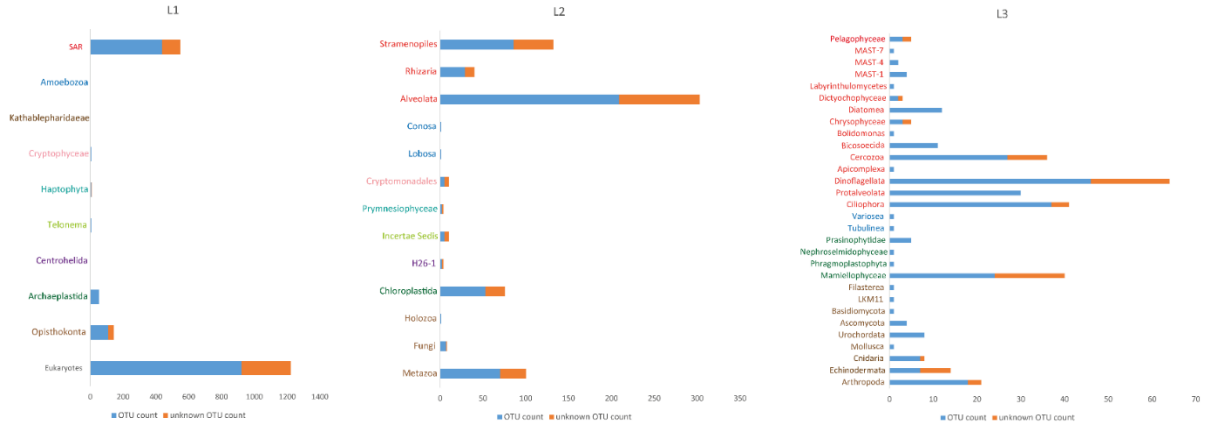
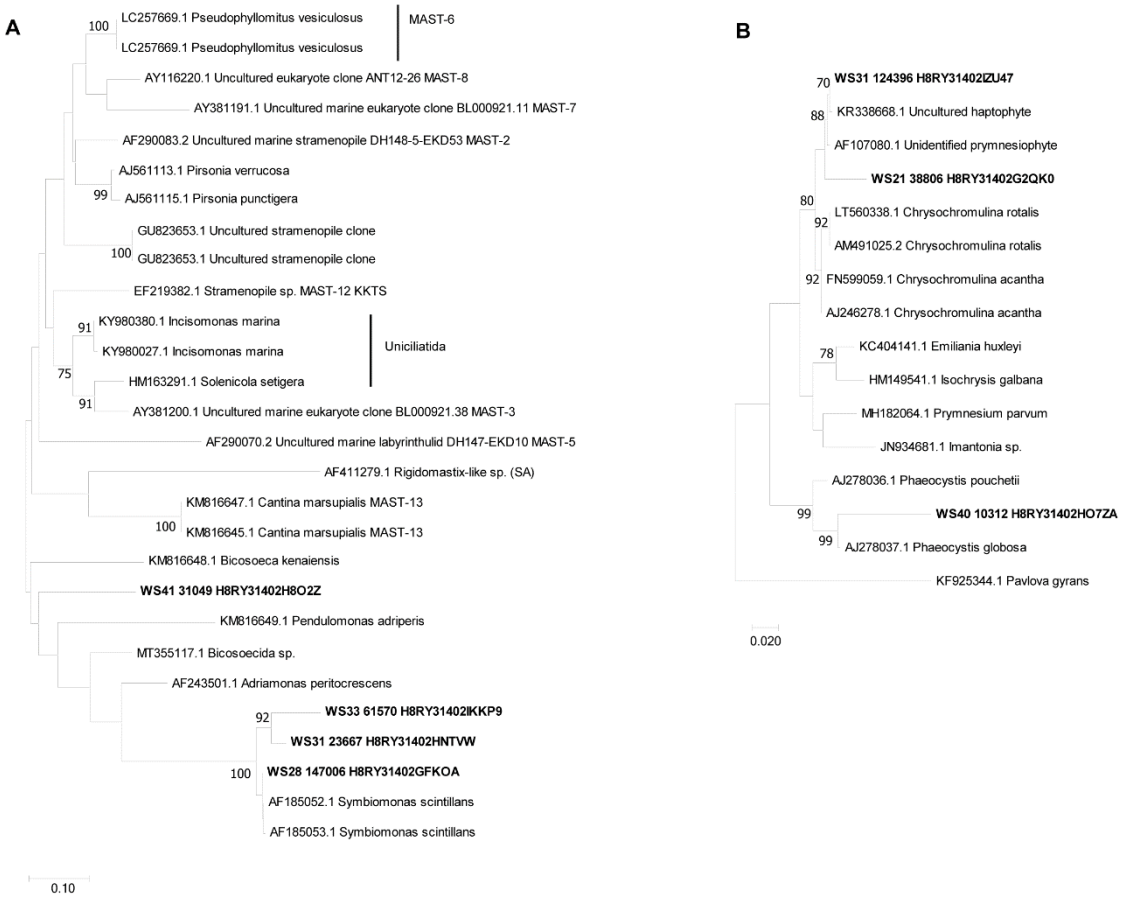


Figure 7: RDP classified OTU with an 80% or more confidence score at three levels L1 (Kingdom), L2 (Phyla), L3 (Phyla to class level). Blue bars are number of OTUs within taxa categories and orange bars show OTUs that have not been further classified. Horizontal axis shows number of OTUs. Type colours of taxa relate to the kingdom such as red for taxa belonging to SAR, brown for Opisthokonta, Green for Archaeplastida and blue for Amoebozoa.



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C

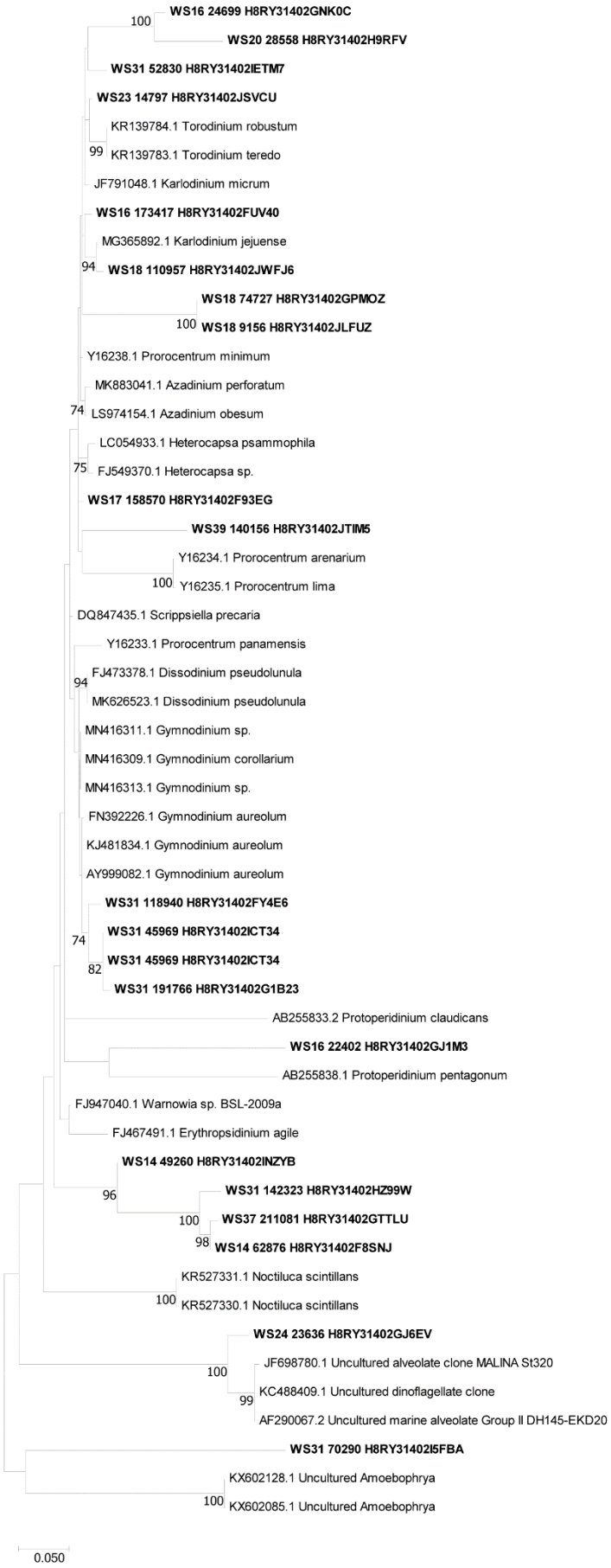

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Figure 8. Maximum Likelihood phylogeny of OTUs belonging to MAST group (A), haptophytes (B) and dinoflagellates (C). Bootstrap support of 75 are shown by branches. Bold type indicates OTUs from this study.

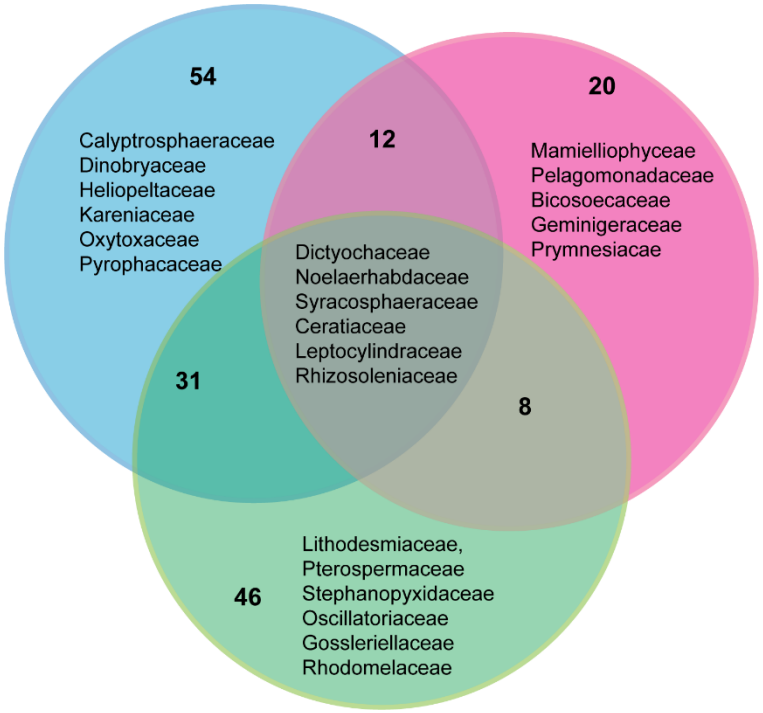

Figure 9: Venn-diagram showing the overlap in taxa observed in the English Channel in 2011-2012 by WaMS (pink), CPR microscopy (Green) and L4 microscopy (Blue). Examples of taxa observed in each sector are also listed but not exhaustive.

Tables

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Table 1: Summary of Samples taken in the English Channel from 2011-2013 using the WaMS deployed within the CPR sampling platform and tests carried out on them. All CPR tow samples will have microscopic plankton semi-quantitative abundance. Empty positions indicate no samples were available to analyse. No samples were taken in August and November 2011, April 2012, March, April and October 2013. Positions E1-E5 denote sample positions taken progressively from Roscoff, France to Plymouth, UK. Blue type, sample positions not collected so inferred from timing of previous deployments. Pn: Position, Y: Year, M: month, Lat: Latitude, Lon: Longitude, In situ T: In situ temperature, FC: Flow cytometry. AA: *A. anophagefferens*, PD: *P.delicatissima*, PF *P.fraudulenta*, PM: *P.multiseries*

									qRT-PCR test			
CPR tow	**Pn**	**Y**	**M**	**Lat**	**Lon**	**T.**	**FC**	**Seq sample**	***AA***	***PD***	***PF***	***PM***
344PR	E1	2011	2	48.03	-3.83	9.56	Yes	WS1	Yes	Yes	Yes	Yes
		2011	2									
		2011	2									
		2011	2									
	E5	2011	2	49.94	-4.12	9.31	Yes	WS2	Yes	Yes	Yes	Yes
345PR		2011	3									
	E2	2011	3	49.28	-4.02	9.75	Yes	WS3	Yes	Yes	Yes	Yes
		2011	3									
		2011	3									
	E5	2011	3	49.78	-4.12	9.78	Yes	WS4	Yes	Yes	Yes	Yes
346PR	E1	2011	4	48.80	-3.96	10.5	Yes	WS5	Yes	Yes	Yes	Yes
	E2	2011	4	49.08	-4.01	10.25	Yes	WS6	Yes	Yes	Yes	Yes
	E3	2011	4	49.37	-4.04	10.08	Yes	WS7	Yes	Yes	Yes	Yes
	E4	2011	4	49.67	-4.11	10.17	Yes	WS8	Yes	Yes	Yes	Yes
	E5	2011	4	49.97	-4.17	10.18	Yes	WS9	Yes	Yes	Yes	Yes
347PR	E1	2011	5	48.82	-3.93	N/A	Yes	N/A	Yes	Yes	Yes	Yes
	E2	2011	5	49.11	-3.98	11.95	Yes	WS10	Yes	Yes	Yes	Yes
	E3	2011	5	49.40	-3.97	12.4	Yes	WS11	Yes	Yes	Yes	Yes
	E4	2011	5	49.68	-4.03	12.56	Yes	WS12	Yes	Yes	Yes	Yes
	E5	2011	5	49.95	-4.09	12.38	Yes	WS13	Yes	Yes	Yes	Yes
348PR	E1	2011	6	48.78	-3.96	13.7	yes	WS14	Yes	Yes	Yes	Yes
	E2	2011	6	49.10	-4.02	12.9	yes	WS15	Yes	Yes	Yes	Yes
	E3	2011	6	49.42	-4.10	13.9	yes	WS16	Yes	Yes	Yes	Yes
	E4	2011	6	49.68	-4.10	13.9	yes	WS17	Yes	Yes	Yes	Yes
	E5	2011	6	49.96	-4.13	13.9	yes	WS18	Yes	Yes	Yes	Yes
349PR	E1	2011	7	48.83	-3.96	14.5	yes	WS19	Yes	Yes	Yes	Yes
	E2	2011	7	49.16	-4.02	14.4	yes	WS20	Yes	Yes	Yes	Yes
	E3	2011	7	49.41	-4.04	15.3	yes	WS21	Yes	Yes	Yes	Yes
	E4	2011	7	49.70	-4.09	15.7	yes	WS22	Yes	Yes	Yes	Yes
	E5	2011	7	49.97	-4.13	15.7	yes	WS23	Yes	Yes	Yes	Yes
351PR	E1	2011	9	48.81	-3.93	N/A	yes	WS24	Yes	Yes	Yes	Yes
	E2	2011	9	49.15	-3.78	N/A	yes	WS25	Yes	Yes	Yes	Yes

	E3	2011	9	49.48	-3.69	N/A	yes	WS26	Yes	Yes	Yes	Yes
	E4	2011	9	49.77	-3.83	N/A	yes	WS27	Yes	Yes	Yes	Yes
	E5	2011	9	50.01	-3.97	N/A	yes	WS28	Yes	Yes	Yes	Yes
352PR	E1	2011	10	48.81	-3.95	14.7	yes	WS29	Yes	Yes	Yes	Yes
	E2	2011	10	49.13	-4.00	14.9	yes	WS30	Yes	Yes	Yes	Yes
	E3	2011	10	49.39	-4.05	15.0	yes	WS31	Yes	Yes	Yes	Yes
	E4	2011	10	49.79	-4.08	14.9	yes	WS32	Yes	Yes	Yes	Yes
	E5	2011	10	49.93	-4.10	14.7	yes	WS33	Yes	Yes	Yes	Yes
354PR	E1	2011	12	48.79	-3.96	13.0	yes	WS34	Yes	Yes	Yes	Yes
	E2	2011	12	49.15	-4.01	12.7	yes	WS35	Yes	Yes	Yes	Yes
	E3	2011	12	49.41	-4.03	12.5	yes	WS36	Yes	Yes	Yes	Yes
	E4	2011	12	49.70	-4.06	12.3	yes	WS37	Yes	Yes	Yes	Yes
	E5	2011	12	50.00	-4.09	12.0	yes	WS38	Yes	Yes	Yes	Yes
355PR	E1	2012	2	48.31	-3.95	10.3	yes	WS39	Yes	Yes	Yes	Yes
	E2	2012	2	49.12	-4	10.6	yes	WS40	Yes	Yes	Yes	Yes
	E3	2012	2	49.44	-4.06	10.3	yes	WS41	Yes	Yes	Yes	Yes
	E4	2012	2	49.72	-4.12	10.2	yes	WS42	Yes	Yes	Yes	Yes
	E5	2012	2	49.95	-4.16	9.7	yes	WS43	Yes	Yes	Yes	Yes
356PR	E1	2012	3	48.82	-3.96	10.40	yes		Yes	Yes		
	E2	2012	3	49.15	-3.98	10.70	yes		Yes	Yes		
	E3	2012	3	49.44	-4.06	10.10	yes		Yes	Yes		
	E4	2012	3	49.72	-4.12	10.30	yes		Yes	Yes		
	E5	2012	3	49.95	-4.16	10.10	yes		Yes	Yes		
358PR	E1	2012	5	48.81	-3.96	11.9	yes		Yes	Yes		
	E2	2012	5	49.25	-4.02	11.6	yes		Yes	Yes		
	E3	2012	5	49.61	-4.06	11.6	yes		Yes	Yes		
	E4	2012	5	49.95	-4.07	12.2	yes		Yes	Yes		
	E5	2012	5	50.27	-4.17	11.4	yes		Yes	Yes		
359PR	E1	2012	6	48.80	-3.96	13.41	yes		Yes	Yes		
	E2	2012	6	49.20	-4.02	13.22	yes		Yes	Yes		
	E3	2012	6	49.62	-4.13	13.6	yes		Yes	Yes		
	E4	2012	6	49.94	-4.15	13.8	yes		Yes	Yes		
	E5	2012	6	50.25	-4.16	14.2	yes		Yes	Yes		
360PR	E1	2012	7	48.80	-3.96	14.65	yes		Yes	Yes		
	E2	2012	7	49.25	-4.06	14.90	yes		Yes	Yes		
	E3	2012	7	49.65	-4.11	15.05	yes		Yes	Yes		
	E4	2012	7	49.99	-4.14	14.87	yes		Yes	Yes		
		2012	7									
361PR	E1	2012	8	48.80	-3.96		yes		Yes	Yes		
	E2	2012	8	49.26	-4.05		yes		Yes	Yes		
	E3	2012	8	49.66	-4.09		yes		Yes	Yes		
	E4	2012	8	50.00	-4.13		yes		Yes	Yes		
	E5	2012	8	50.32	-4.18		yes		Yes	Yes		

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362PR		2012	9	48.8	-3.96		Yes					
		2012	9	49.26	-4.05		Yes					
		2012	9	49.66	-4.09		Yes					
		2012	9	50.00	-4.13		Yes					
		2012	9	50.33	-4.18		Yes					
364PR	E1	2012	10	48.84	-3.97	14.40	yes		Yes	Yes		
	E2	2012	10	49.21	-3.99	14.5	yes		Yes	Yes		
	E3	2012	10	49.55	-4.02	14.5	yes		Yes	Yes		
	E4	2012	10	49.9	-4.13	14.3	yes		Yes	Yes		
	E5	2012	10	50.25	-4.15	14.5	yes		Yes	Yes		
Additional	E1	2012	10	48.84	-3.97	14.40	yes		Yes	Yes		
	E2	2012	10	49.21	-3.99	14.5	yes		Yes	Yes		
	E3	2012	10	49.55	-4.02	14.5	yes		Yes	Yes		
	E4	2012	10	49.9	-4.13	14.3	yes		Yes	Yes		
	E5	2012	10	50.25	-4.15	14.5	yes		Yes	Yes		
365PR	E1	2012	11	48.83	-3.96	13	yes		Yes	Yes		
	E2	2012	11	49.22	-4.01	13.5	yes		Yes	Yes		
	E3	2012	11	49.6	-4.05	13.6	yes		Yes	Yes		
	E4	2012	11	49.92	-4.09	13.2	yes		Yes	Yes		
		2012	11				yes					
366PR	E1	2012	12	48.8	-3.96	11.3	yes		Yes	Yes		
	E2	2012	12	49.19	-4.01	12	yes		Yes	Yes		
	E3	2012	12	49.52	-4.11	11.8	yes		Yes	Yes		
	E4	2012	12	49.88	-4.13	11.6	yes		Yes	Yes		
	E5	2012	12				yes					
367PR	E1	2013	1	48.78	-3.96		yes		Yes	Yes		
	E2	2013	1	49.15	-4.02		yes		Yes	Yes		
	E3	2013	1	49.49	-4.05		yes		Yes	Yes		
	E4	2013	1	49.84	-4.01		yes		Yes	Yes		
	E5	2013	1	50.17	-4.12		yes		Yes	Yes		
368PR	E1	2013	2	48.8	-3.96		yes		Yes	Yes		
	E2	2013	2	49.13	-4.02		yes		Yes	Yes		
	E3	2013	2	49.4	-4.06		yes		Yes	Yes		
	E4	2013	2	49.7	-4.09		yes		Yes	Yes		
	E5	2013	2	50.05	-4.12		yes		Yes	Yes		
371PR		2013	5	48.78	-3.94							
		2013	5	49.15	-3.87							
		2013	5	49.51	-3.92							
	E4	2013	5	49.79	-4.05				Yes	Yes		
	E5	2013	5	50.1	-4.14				Yes	Yes		
372PR	E1	2013	6	48.78	-3.94				Yes	Yes		
	E2	2013	6	49.15	-3.87				Yes	Yes		
	E3	2013	6	49.51	-3.92				Yes	Yes		

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	E4	2013	6	49.79	-4.05				Yes	Yes		
	E5	2013	6	50.1	-4.14				Yes	Yes		
374PR	E1	2013	7	48.80049	-3.95		yes		Yes	Yes		
	E2	2013	7	49.18	-4.02		yes		Yes	Yes		
	E3	2013	7	49.55	-4.10		yes		Yes	Yes		
	E4	2013	7	49.90	-4.12		yes		Yes	Yes		
	E5	2013	7	50.22	-4.17		yes		Yes	Yes		
375PR	E1	2013	8	48.77	-3.95		yes		Yes	Yes		
	E2	2013	8	49.23	-4.03		yes		Yes	Yes		
	E3	2013	8	49.64	-4.09		yes		Yes	Yes		
	E4	2013	8	49.98	-4.11		yes		Yes	Yes		
	E5	2013	8	50.28	-4.16		yes		Yes	Yes		
376PR	E1	2013	9	48.78	-3.94				Yes	Yes		
	E2	2013	9	49.15	-3.87		yes		Yes	Yes		
	E3	2013	9	49.51	-3.92		yes		Yes	Yes		
	E4	2013	9	49.79	-4.05		yes		Yes	Yes		
	E5	2013	9	50.1	-4.14		yes		Yes	Yes		
378PR	E1	2013	11	48.78	-3.95		yes		Yes	Yes		
	E2	2013	11	49.12	-3.98		yes		Yes	Yes		
	E3	2013	11	49.45	-4.04		yes		Yes	Yes		
	E4	2013	11	49.78	-4.11		yes		Yes	Yes		
	E5	2013	11	50.11	-4.15		yes		Yes	Yes		

Table 2: qRT-PCR assays developed, species, primers, years tested

Assay	Reference	Marker	Primers	Size of product/bp	Standard range copies/µl
Pseudo-nitzschia fraudulenta	Andre et al. (2011)[37]: PN5.8S F-HRM, QPfrau R-HRM	ITS1	PN5.8SF-HRM 5′ CAGCGGTGGA TGTCTAGGTTC -3' QPfrauR-HRM 5′ CCGCTGCTAG AGCGGTCAGA G 3′	225	3.18 x10^8 -3.17 x 10^6
Pseudo-nitzschia multiseries	PMulsF (this study), PN5.8S R-HRM (Andre et al.	ITS2	PMulsF-HRM 5′ CTAGACTACT GTAGTCAAAC TTAACCGGCA AC 3′	201	5.75 x 10^8-5.75 x 10^4

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	2011)[37]		PN5.8SR-HRM 5′ GAACCTAGAC ATCCACCGCT G 3′		
Pseudo-nitzschia delicatissima	QPdelRa2F (this study), PN5.8SR-HRM (Andre et al. 2011)[37]	ITS	QPdelRa2F GTGCAATACT TTGTTGGGTTT CG PN5.8SR-HRM 5′ GAACCTAGAC ATCCACCGCT G 3′	182	2.5x 10^2 to 2.5 x 10^7
Aureococcus anophageffe rens	Aa1685f, Popels et al. 2003, [39] Euk B (Medlin et al. 1988)[76]	18S	Aa1685f ACCTCCGGAC TGGGGTT, EukB	118	10^2 -10^6

Table 3: Reaction efficiency of qRT-PCR assays in this study PD (*P. delicatissima*), AA (*Aureoccocus anophagefferens*) for assay run 2011,2012 and 2013. E=efficiency, M=slope, B=offset or y-intercept, R, R^2 values indicate fit to equation

	PD 2011	**PD 2012**	**PD 2013**	**AA 2011**	**AA 2012**	**AA 2013 pt1**	**AA 2013 pt2**
R	0.999	0.999	0.996	0.994	0.994	0.998	0.984
R^2	0.985	0.997	0.992	0.987	0.9888	0.995	0.969
M	-3.935	-2.900	-3.266	-3.502	-3.858	-3.979	-3.223
B	36.361	28.489	34.76	43.483	43.900	38.268	29.398
E	0.796	1.212	1.023	0.9302	0.8163	0.7837	0.6076

Table 4: OTU table count of reads from WaMS sample between 2011-2012. Y: year, M: month, SR: sequence run (run 1 was carried out first and reported in Stern et al. 2015), #: number, PDM: Phlogenetic distance whole tree mean, C1M: Chao I mean, SM: Shannon mean, +/-M: above/below mean

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Y	M	SR	#/sample	# OTU	+/- M	Above/below median	PDM	C1M	SM
2011	2	1	WS1	132	-1035.452	-560	4.867548	36.0	24.100
2011	2	1	WS2	346	-821.452	-346	1.6509	22.0	9.300
2011	3	1	WS3	69	-1098.452	-623	4.633776	93.1	24.500
2011	3	1	WS4	263	-904.452	-429	1.586147	9.5	7.200
2011	4	1	WS5	182	-985.452	-510	2.120118	39.8	18.600
2011	4	1	WS6	655	-512.452	-37	3.218702	29.3	14.900
2011	4	1	WS8	930	-237.452	238	1.08946	15.7	9.500
2011	4	1	WS9	924	-243.452	232	0.78551	9.9	6.400
2011	5	1	WS10	257	-910.452	-435	0.68059	22.0	12.400
2011	5	1	WS11	1535	367.548	843	0.154481	2.7	2.500
2011	5	1	WS12	386	-781.452	-306	1.175051	23.2	14.100
2011	5	1	WS13	268	-899.452	-424	2.10681	27.6	15.100
2011	6	2	WS14	544	-623.452	-148	1.46735	62.5	23.900
2011	6	2	WS15	729	-438.452	37	1.561149	23.6	13.600
2011	6	2	WS16	861	-306.452	169	2.271352	56.7	25.300
2011	6	2	WS17	592	-575.452	-100	2.49747	43.4	20.900
2011	6	2	WS18	358	-809.452	-334	3.344375	74.0	25.200
2011	7	2	WS19	237	-930.452	-455	3.779641	105.0	35.700
2011	7	2	WS20	427	-740.452	-265	1.589128	32.9	27.300
2011	7	2	WS21	484	-683.452	-208	1.889013	48.0	17.100
2011	7	2	WS22	242	-925.452	-450	2.368124	57.5	18.400
2011	7	2	WS23	445	-722.452	-247	0.71281	38.0	15.000
2011	9	2	WS24	540	-627.452	-152	0.650258	22.1	12.500
2011	9	2	WS25	6674	5506.548	5982	0.319075	4.5	2.900
2011	9	2	WS26	1734	566.548	1042	1.622526	29.2	11.400
2011	9	2	WS27	1080	-87.452	388	1.786829	32.9	14.200
2011	9	2	WS28	240	-927.452	-452	3.088336	79.0	24.000
2011	10	2	WS29	928	-239.452	236	2.057396	39.3	16.000
2011	10	2	WS30	893	-274.452	201	0.256501	36.7	12.100
2011	10	2	WS31	2966	1798.548	2274	2.601002	89.1	31.000
2011	10	2	WS32	1175	7.548	483	0.316267	7.1	4.300
2011	10	2	WS33	7500	6332.548	6808	0.431583	12.9	6.500
2011	12	2	WS34	451	-716.452	-241	0.772125	26.0	11.700
2011	12	2	WS35	766	-401.452	74	1.965868	51.0	13.100
2011	12	2	WS36	1072	-95.452	380	1.2185	20.4	9.800
2011	12	2	WS37	736	-431.452	44	2.471535	25.9	13.400
2011	12	2	WS38	3072	1904.548	2380	0.046794	4.0	3.200
2012	2	2	WS39	902	-265.452	210	2.747683	83.8	18.600
2012	2	2	WS40	455	-712.452	-237	0.916246	23.0	13.500
2012	2	2	WS41	738	-429.452	46	1.699598	66.8	19.200
2012	2	2	WS42	4505	3337.548	3813	0	4.4	3.500

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2012	2	2	WS43	1740	572.548	1048	0.67496	10.8	7.200
			Total	**49033**					

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Appendix 2: Quantitative real-time PCR methods and validation, Appendix 1: Nutrient analysis Methods from WCO station L4. Tables S1: WaMS sample information, S2 Taxa summary table of reads assigned by UCLUST from WaMS samples from Febraury 2011-February 2012 at taxonomic hierarchy L3, Table S3: Taxa summary table of reads from WaMS samples from Febraury 2011-February 2012 at taxonomic hierarchy L4, Supplementary Table S4:Taxa summary table of reads from WaMS samples from Febraury 2011-February 2012 at taxonomic hierarchy L5; Table S5: Taxa summary table of reads from WaMS samples from Febraury 2011-February 2012 at taxonomic hierarchy L6; Table S6: RDP classified Taxa levels and their associated counts from partial 18S reads of samples WS1-42 from WaMS samples; Table S7: Summary methods used in phylogenetic analysis of sequences produced from WaMS samples; Table S8: Calculated *Pseudo-nitzschia delicatissima* cell numbers derived from qRT-PCR Ct values for samples with detectable Ct values; Table S9: Calculated *Aureococcus anophagefferens* cell numbers derived from qRT-PCR Ct values for samples with detectable Ct values; Table S10: WCO L4 Phytoplankton microscopic taxa set from 2011-2012 and each taxa's nearest match to WaMS genetically-generated taxa. Grey type shows species absent from entire L4 microscopic dataset; Table S11: CPR Phytoplankton taxa measured and observed (in grey font) from CPR area D3, Western Channel dervied from 2011-2012 dataset at https://doi.org/10.17031/1808 and additional taxa. Table S12: Sea Surface Temperature data from L4 from 2000-2020.

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