University of Plymouth

PEARL **https://pearl.plymouth.ac.uk**

Faculty of Science and Engineering School of Biological and Marine Sciences

2024-02-01

The silent majority: Pico- and nanoplankton as ecosystem health indicators for marine policy

McQuatters-Gollop, A

https://pearl.plymouth.ac.uk/handle/10026.1/22475

10.1016/j.ecolind.2024.111650
Ecological Indicators
Elsevier BV

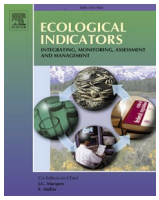
All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.

Ecological Indicators 159 (2024) 111650

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind



Original Articles

The silent majority: Pico- and nanoplankton as ecosystem health indicators for marine policy

Abigail McQuatters-Gollop [a,*], Rowena F. Stern [b], Angus Atkinson [c], Mike Best [d], Eileen Bresnan [e], Veronique Creach [f], Michelle Devlin [f], Matthew Holland [a], Clare Ostle [b], Katrin Schmidt [g], Lawrence Sheppard [b], Glen Tarran [c], E. Malcolm S. Woodward [c], Paul Tett [h]

[a] School of Biological and Marine Sciences, University of Plymouth, Drake Circus, Plymouth PL4 8AA, UK
[b] The Marine Biological Association (MBA), The Laboratory, Citadel Hill, Plymouth PL1 2PB, UK
[c] Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth PL1 3DH, UK
[d] Environment Agency. Kingfisher House, Goldhay Way, Peterborough PE2 5ZR, UK
[e] Marine Scotland Science, Marine Laboratory, Victoria Road, Aberdeen AB11 9DB, UK
[f] Centre for Environment, Fisheries and Aquaculture Science (Cefas), Pakefield Road, Lowestoft NR330HT, UK
[g] School of Geography, Earth and Environmental Sciences, University of Plymouth, Plymouth, UK
[h] Scottish Association for Marine Science, Scottish Marine Institute, Oban PA37 1QA, UK

ARTICLE INFO

Keywords:
Picoplankton
Biodiversity
Marine strategy framework directive
Pelagic habitats
Ecosystem approach
Synechococcus

ABSTRACT

A healthy marine ecosystem is a fully functioning system, able to supply ecosystem services whilst still maintaining resilience to human-induced environmental change. Monitoring and managing the health of resilient marine ecosystems requires indicators that can assess their biodiversity state and food web functioning. Plankton are crucial components of pelagic habitats, occupying the base of the pelagic food web. Larger plankton have long been used to monitor ecosystem productivity and biodiversity due to their identification via traditional light microscopy. In contrast, the regular monitoring of pico- and nanoplankton (<20 μm; hereafter called "tiny plankton") only started with the development of flow cytometry techniques, which has limited their inclusion as ecosystem health indicators.

Four UK plankton surveys have sampled and identified these tiny plankton for up to 14 years, providing an opportunity to test their suitability as indicators of ecosystem state. We investigated six groups of tiny plankton, including heterotrophic nanoeukaryotes, photosynthetic nanoeukaryotes, photosynthetic picoeukaryotes, and *Synechococcus* cyanobacteria, and two groups of heterotrophic bacteria. Flow cytometry and light microscopy data from an inshore Western English Channel station revealed that 99.98 % of plankton abundance and 71 % of plankton biomass was derived from tiny plankton cells too small to be quantified accurately under a light microscope and thus not adequately considered in assessments of pelagic habitats.

Different UK marine and coastal regions showed consistency in peak abundances of these tiny plankton. We used a novel wavelet coherence method to identify time-based relationships between tiny plankton and environmental variables linked to human pressures. Relationships were found between nitrogenous nutrients and all tiny plankton groups, most commonly at sub-annual to annual time scales. Photosynthetic picoeukaryotes, heterotrophic nanoeukaryotes, and HNA-bacteria were associated with high sea surface temperatures. Given the here established relationship between tiny plankton and environmental variables, and their importance in the full plankton assemblage, we recommend that, alongside existing microplankton lifeforms, tiny plankton groups can be used as plankton lifeforms, either individually or in combination, to inform biodiversity indicators that meet policy obligations under the EU Marine Strategy Framework Directive (MSFD), (Oslo-Paris Convention) OSPAR strategies, and the UK Marine Strategy.

* Corresponding author.
E-mail address: abigail.mcquatters-gollop@plymouth.ac.uk (A. McQuatters-Gollop).

https://doi.org/10.1016/j.ecolind.2024.111650
Received 22 November 2023; Received in revised form 22 January 2024; Accepted 23 January 2024
Available online 1 February 2024
1470-160X/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

Recent policy initiatives, such as the United Nations Sustainable Development Goals and the Kunming-Montreal Global Biodiversity Framework explicitly recognise the role that marine biodiversity plays in delivering critical ecosystem services (Scharlemann et al., 2020). Rather than compartmentalising the ecosystem, they take a holistic approach to management, considering all species and habitats, including plankton. The European Union Marine Strategy Framework Directive (MSFD), and the UK's post-Brexit equivalent, the UK Marine Strategy (UKMS), are more explicit and require assessment of plankton biodiversity indicators against targets representing Good Environmental Status (Borja et al., 2010; Ferreira et al., 2011; McQuatters-Gollop et al., 2022). The state of plankton biodiversity is indicative of the state of the wider pelagic habitat, with plankton forming the base of the marine food web and consequently supporting global food provision (Capuzzo et al., 2018; Fenchel, 1988). More specifically, phytoplankton biodiversity supports multiple ecosystem services including carbon and nutrient cycling and oxygen production (Naselli-Flores and Padisák, 2022), and can contribute around 50 % of global primary production (Falkowski and Raven, 2007). Most nutrients are recycled in the upper pelagic zone through respiration and consumption, and their concentrations vary according to the regional food web. Understanding how plankton communities respond to changes in anthropogenic pressures, such as climate change and nutrient loading, is therefore key to managing and protecting the marine environment sustainably and preserving essential ecosystem services.

Plankton size is a fundamental trait directly connected to their physiology that controls growth and interaction with their environment (Marañón, 2015). Plankton size structure is a key determinant in how efficiently energy is transferred from primary producers up to planktivorous fish (Atkinson et al., 2021; Mehner et al., 2018). In contrast to microplankton (>20–200 μm) that seasonally dominate coastal systems, pico- and nano-plankton (<20 μm) dominate oligotrophic, open water and nutrient-poor regions with complex food webs and limited carbon export to higher trophic levels (Agawin et al., 2000; Chavez et al., 2011). This domination by tiny plankton has been encountered in large stratifying areas of the Northwest European shelf, where the tiny phytoplankton can dominate in summer. Increased stratification and reduced summer nutrient supply in the Western English Channel, as seen under climate warming, favour the dominance of the photosynthetic prokaryote (bacteria) *Synechococcus*, whose small size and lack of essential fatty acids has been linked to a 50 % summer decline in copepod abundance over the last 60 years (Schmidt et al., 2020). Such studies reveal the important effect that cell size has on marine productivity.

Globally, many plankton time-series datasets exist, especially in the Northeast Atlantic (O'Brien et al., 2017). Many of these underpin national and international policy and decision making (McQuatters-Gollop et al., 2022; Ostle et al., 2021). However, the majority of these time-series focus on the abundance of larger photosynthetic microphytoplankton (predominantly > 20–200 μm in size), microzooplankton (predominantly 20–200 μm in size), or bulk indicators such as chlorophyll to evaluate measures of ocean primary productivity and plankton community structure (Devlin et al., 2009; Devlin et al., 2007; Holland et al., 2023b; McQuatters-Gollop et al., 2019; O'Brien et al., 2017). These long-term, light microscope-based time-series are important to understand change in ecosystem structure and functioning and to inform policy mechanisms (Bedford et al., 2020), but the emphasis on larger plankton can neglect or under-emphasise an important portion of the plankton community, which includes the photosynthetic picoplankton (defined here as < 3 μm) and the nanoplankton (2–20 μm). These groups can be 3 orders of magnitude more abundant than microphytoplankton, depending on oceanographic conditions (Agawin et al., 2000). Furthermore, they can make up the majority of photosynthetic biomass during the productive season (Pedrotti et al., 2017). Marine microbes are largely responsible for the redistribution of oceanic carbon, converting an estimated > 95 % of recalcitrant dissolved organic matter into forms digestible by marine organisms (Turner, 2015). This conversion is indirectly performed by heterotrophic organisms - zooplankton, microzooplankton (single eukaryotic microbial cells) and bacteria. The rate of heterotrophy in bacteria is estimated to be 15 % and is 30–70 % for microzooplankton (20–200 μm in size). Together these rates can be larger than the heterotrophic consumption rate in mesozooplankton over 200 μm, estimated at 20–35 % (Turner, 2015). Picoplankton that are consumed by zooplankton also contribute to carbon export through excretion and sinking of marine snow, which would otherwise remain mainly at the surface due to the small size of picoplankton (Turner, 2015). Plankton < 20 μm (henceforth called "tiny plankton") comprise a wide variety of functional roles but, with a few notable exceptions (González-García et al., 2023), have rarely been part of any long-term continuous monitoring program. Their presence can contribute to some indicators (e.g. chlorophyll), but their small/soft bodied forms are often difficult to enumerate using light microscope counts of Lugol's or formalin-preserved cells.

While there are very few long-term (i.e. > 30 years) data series that include the tiny plankton, there has been an increase in the collection of tiny plankton data over the last 20 years as routine use of flow cytometry in marine biological time-series surveys has provided a technique that identifies and quantifies these tiny plankton (Li and Dickie, 2001; Zubkov and Burkill, 2006). Until recently, policy makers had no information about what changes in plankton communities mean in terms of biodiversity assessments and ecosystem services and the types of management measures to implement. To address this gap, a plankton indicator has been developed to inform on the state of plankton diversity under MSFD and UKMS (McQuatters-Gollop et al., 2019; McQuatters-Gollop et al., 2022; McQuatters-Gollop et al., 2023; OSPAR, 2017). This indicator groups plankton taxa by functional traits, such as size, trophy, and motility, creating 'lifeforms' (McQuatters-Gollop et al., 2019; Ostle et al., 2021; Tett et al., 2007). Changes in lifeforms can then be linked to changes in environmental pressures such as climate change or nutrient loading (Bedford et al., 2020; Holland et al., 2023b). This flexible method can use data from disparate plankton surveys, regardless of sampling method, level of taxonomic identification, and enumeration procedure (McQuatters-Gollop et al., 2019). The use of lifeforms has contributed to a successful integration of various plankton datasets from across the UK and northern Europe to better inform the biodiversity assessments under both the MSFD and UKMS (McQuatters-Gollop et al., 2019; McQuatters-Gollop et al., 2022; OSPAR, 2017) and has identified shelf-wide and more regional trends in plankton lifeforms (Bedford et al., 2020). The lifeform indicator approach, so far, only includes lifeforms based on light microscopy, but the indicator's flexible nature means that lifeforms can be created based on routinely measured tiny taxa (e.g. pico- and nano-plankton) providing a valuable dataset to interrogate and understand changes at the base of the food web.

In this study we use tiny and microplankton data from four UK time-series using several sampling methods and counted by flow cytometry and light microscopy to assess how tiny plankton can be used as indicators of GES in temperate marine seas. Our specific aims were (1) to demonstrate that tiny plankton are important marine pelagic components in terms of abundance and biomass; (2) to test seasonal variability and long-term trends in tiny plankton abundance in relation to environmental variables; (3) to propose a set of tiny plankton lifeforms that could be assessed routinely at UK monitoring sites; and (4) to consider how these tiny plankton data could be integrated into current UK and OSPAR assessments of marine ecosystem health.

2. Methods

2.1. Plankton lifeforms and traits

An indicator of change based on the abundances of plankton functional types, or lifeforms, is used in the implementation of the MSFD and

UKMS for assessing biodiversity (Holland et al., 2023a; McQuatters-Gollop et al., 2019; McQuatters-Gollop et al., 2023). We define **life-form** operationally as

> the set of traits that identifies a functional group of plankters, thus the set of species (observed to possess these traits) that are supposed to respond in similar ways to ecohydrodynamic conditions and which are similar in their biogeochemical and trophic interactions.

This approach is derived from Margalef (1978) (see also Wyatt (2014)), who grouped phytoplanktonic lifeforms by traits related to survival in specific hydrodynamic conditions, and distinguished diatoms and dinoflagellates on this basis. As with the use of plankton functional traits in models (Le Quere et al., 2005), the approach aggregates the organisms responsible for pelagic habitat structure and function into a small number of packages. It has practical advantages in that it allows the use of plankton data identified at different taxonomic resolutions, which has suited the UK's integrated but diverse plankton monitoring programme. However, it is currently only applied to zooplankton and larger phytoplankton (>5 μm, but mostly > 20 μm).

2.2. Monitoring and grouping the tiny plankton

Size is an important descriptor for the categorisation of plankton. The categorisation of planktonic organisms as belonging to the pico-plankton (0.2—2 μm), nanoplankton (2—20 μm) or microplankton (20—200 μm) derives from Sieburth et al. (1978), likely based on what passed through certain sizes of pore in membrane filters or the mesh apertures in nets. This is inexact, because plankton shapes diverge widely from those of spheres. In this paper, size group boundaries are understood as fuzzy, with the upper picoplankton size boundary set at 3 μm, as in Tarran and Bruun (2015), and noting that some studies set the boundary at 5 μm (De Vargas et al., 2015). For routine light microscopy purposes, 4 to 5 μm is the lower size-threshold of what can be reliably identified and counted as phytoplankton. Other optical methods, such as flow cytometry (Tarran and Bruun, 2015), can identify and count phytoplankton below this limit, and sort into aggregate groups consisting of many different plankton taxa. Flow cytometry makes multiple measurements on cells using their optical light scattering and fluorescence properties (cell pigments for photosynthetic cells or specific, introduced dyes for non-photosynthetic cells) at rates up to 1000 s of cells per second which can be used to discriminate and enumerate the major components of pico-and nano-plankton communities within minutes (Zubkov and Burkill, 2006). Thus, heterotrophic bacteria, photosynthetic prokaryotes (cyanobacteria), and different size classes of photosynthetic and heterotrophic eukaryotes can be distinguished through the differences in light scattering and fluorescence properties.

Nanoplankton (generally 2–20 μm in size) include a diverse array of single-celled photosynthetic and non-photosynthetic eukaryotic plankton as well as parasitic plankton (Vaulot et al., 2008). The photosynthetic varieties, called photosynthetic nanoeukaryotes, contain mostly flagellated algal cells, some coccoid forms, and some diatoms. This intermediate-sized group is found in open ocean and coastal waters but thrives in nutrient-rich waters and can make up 70 % of the photosynthetic biomass (Pedrotti et al., 2017). Many photosynthetic eukaryotic nanoplankton species are mixotrophic, grazing on bacteria in the same way that heterotrophic non-photosynthetic eukaryotic plankton do but with the added advantage of also being able to obtain energy through photosynthesis (Hartmann et al., 2012; Zubkov and Tarran, 2008).

The picoplankton (<2 μm) consists of viruses, heterotrophic prokaryotes and archaea, photosynthetic prokaryotes (cyanobacteria), and eukaryotic single cells. Prokaryotic groups include *Synechococcus*, a genus of photosynthetic single-celled cyanobacterium, high nucleic acid (HNA) bacteria-and low nucleic acid (LNA)-bacteria (the latter two named by Li, et al., (1995) based on the relative amount of DNA using fluorescent DNA labels). LNA-bacteria were originally thought to be inactive although that has subsequently been disproved (Hu et al., 2022). These latter two groups are prokaryotes that have different amounts of nucleic acid content in their cells but represent a diverse array of heterotrophic species. LNA-bacteria can make up 20–90 % of freshwater and marine bacterial diversity, and are the smallest bacteria, with low growth rates and lower in biovolume than HNA-bacteria in marine systems but with the ability to increase their surface area to increase nutrient uptake (Hu et al., 2022). Picoplanktonic eukaryotes include tiny coccoid and flagellated cells in what is now known as the supergroup Stramenopiles (e.g. diatoms and dinoflagellates), and within the supergroup Archaeplastida, the Chlorophyceae (e.g. green algae) (Adl et al., 2019). In this study we only consider two subcategories of picoplankton, photosynthetic picoeukaryotes and *Synechococcus,* because these are the only groups for which long-term, quantitative monitoring data exist.

We extended the lifeform approach and applied it by grouping tiny plankton taxa identified by flow cytometry and microscopy based on common traits of cell size, trophic mode of nutrition, and flow cytometry fluorescence colour (Table 2). We used tiny plankton group definitions in line with Thyssen et al. (2022) and BODC vocabulary standards (https://vocab.nerc.ac.uk/collection/F02/current/). We also identified common habitat characteristics and main predators of similarly described groups from the literature. To examine change in tiny plankton over time and how these relate to environmental variables, we concentrated on groups with consistent time-series datasets > 5 years in length in UK waters.

2.3. Tiny plankton data

Flow cytometry pico- and nano-plankton data were acquired from the L4 station at the Plymouth Marine Laboratory (PML) Western Channel Observatory (www.westernchannelobservatory.org.uk, referred to as L4), Marine Directorate of the Scottish Government's Scottish Coastal Observatory monitoring site at Stonehaven (SCObs SH), and the Marine Biological Association's Water and Microplankton Sampler (CPR WaMS), an automated sampler fitted within the payload of the Continuous Plankton Recorder, which is towed in the Western Channel between Roscoff, France, and Plymouth, UK (Stern et al., 2023; Stern et al., 2015). Four pico- and nano-plankton datasets collected from these programs were identified as having adequate time-series (>5 years) for analysis and consistently-identified functional groups within UK waters (Fig. 1; S1). All of these samples were analysed by a single analyst using a common methodology for flow cytometry (see below), either live or from preserved samples, as described in Tarran and Bruun (2015). Additionally, data for light microscopically counted photosynthetic nanoeukaryotes and heterotrophic nanoeukaryotes were obtained from the Scottish Association of Marine Science Lorn Pelagic Observatory (LPO).

All four datasets were used to identify the tiny plankton lifeforms and investigate monthly, seasonal, and interannual changes in tiny plankton (Fig. 2). L4 was the longest dataset assembled and had the most complete sampling coverage with supporting environmental data and so was also used to quantify the role of tiny plankton in plankton community abundance and biomass and to investigate coherence with environmental pressures (Table 1, Fig. 2).

2.4. Estimating tiny plankton abundance and biomass

To provide an estimate of the proportion of tiny plankton, data from 2007 to 2020 at Plymouth's L4 station were used to analyse the abundance and biomass across the size spectrum from bacteria to fish larvae (Fig. 3). During this period, plankton were analysed using flow cytometry, light microscope analysis of settled water samples, and light microscope analysis of net catches, making it the most complete plankton dataset across the size spectrum available in this study and in the UK.

The flow cytometric methods (see section 2.2) used surface values

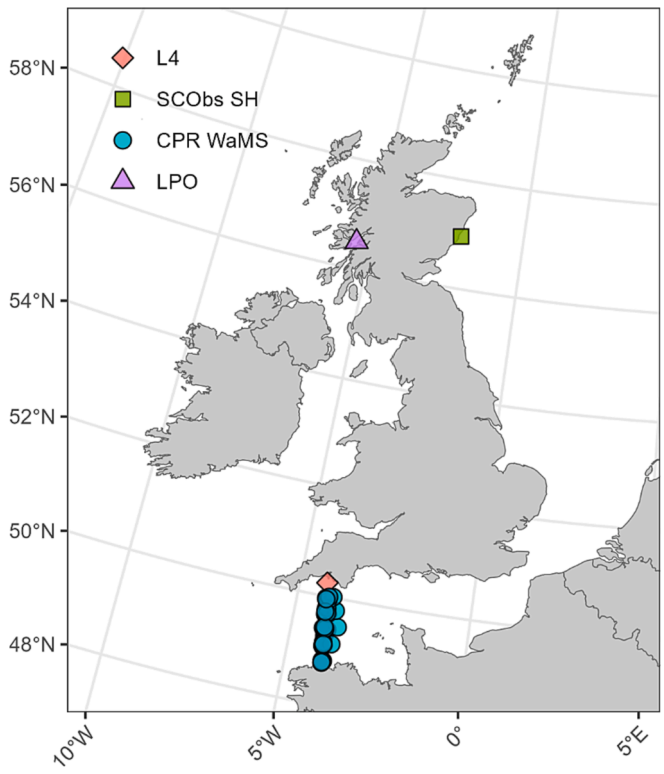


Fig. 1. Sampling locations of pico- and nano-plankton time-series datasets in the UK and nearby waters that were analysed for this study.

from the most recent dataset stored at BODC (Tarran and May, 2019, 2020, 2021) and used seasonally averaged, L4-specific estimates of cell biovolume based on filtration through filters of variable pore size. Cell dimensions were then converted to carbon values using a carbon conversion factor of 0.22 pgC μm^3 (Booth, 1988). Bacteria (i.e. HNA- and LNA- bacteria) were the only taxon for which L4-specific measurements were not used; instead, conversion factors from Heywood et al. (2006) were applied.

For the microplankton functional groups enumerated by light microscopy at L4 (Fig. 3), the most recent dataset available from BODC (Widdicombe and Harbour, 2021) of samples settled with the Utermöhl method was used (Utermöhl, 1958). To enumerate abundance, around 300 individual taxa at 613 sampling timepoints were used as described in Widdicombe et al. (2010) with L4-specific dimensions approximated to simple geometric shapes to estimate volume per cell, from which carbon mass was calculated from standard equations of Menden-Deuer and Lessard (2000). Zooplankton samples were collected using 631 timepoints of replicate 0–50 m tows with a 200 μm WP2 net, analysed as described in Atkinson et al. (2015). Seasonal, L4-specific measurements of the length of the component taxa were then converted to carbon as described in McEvoy et al. (2022).

All the component abundance and biomass values of the taxa from the L4 site were summed into the component functional groups as defined by their lifeform allocations (McQuatters-Gollop et al., 2019) and into the tiny plankton groups used in this paper. Long-term monthly mean values were obtained for each lifeform and then averaged to provide the annual means (Fig. 3).

2.5. Seasonal, intra- and inter- annual changes in tiny plankton in UK waters

The pico- and nano-plankton data were aggregated to the tiny plankton groups listed in Table 2 by the individual data providers within each of the four sampling programmes. Where multiple years of data were available, monthly means were calculated for the top 10 m of vertical profiles, to improve comparability between sampling sites (Fig. 4). These monthly means were used to calculate seasonal means for the duration of each time-series (Fig. 4).

2.6. Links between tiny plankton and environmental variables in the Western Channel

With approximately weekly sampling spanning a 14-year period, the L4 dataset is the longest and most comprehensive dataset available for a more detailed analysis. Various statistical methods have previously been used to discriminate long-term trends from seasonal and shorter-term variability in environmental time-series. These include Generalised Additive Models (GAM) (Zarauz et al., 2008) and spectral analysis by

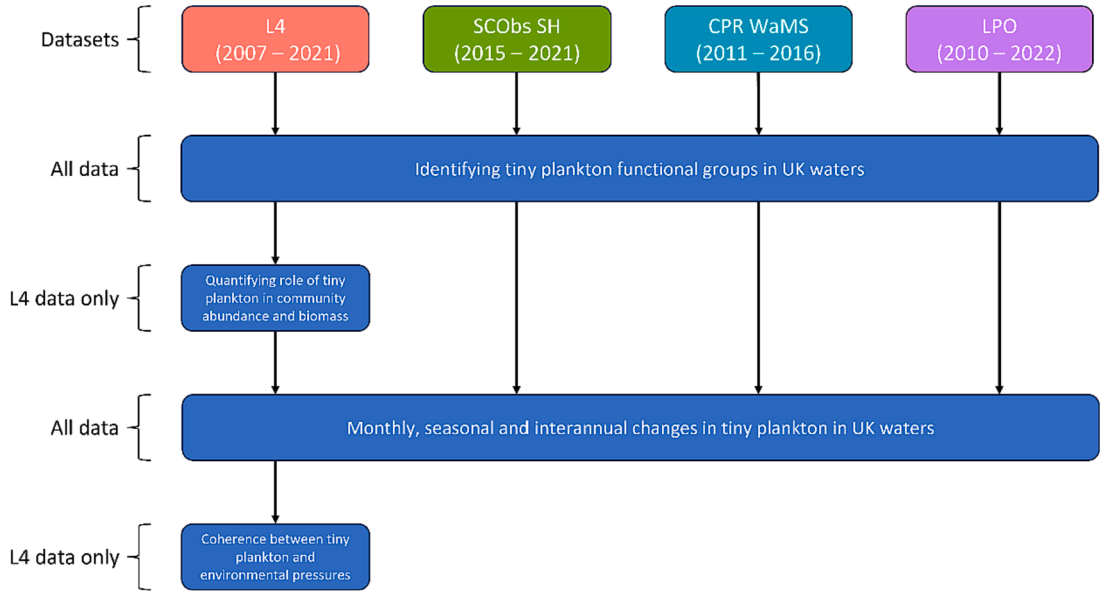


Fig. 2. The use of each dataset for the four types of tiny plankton analysis varies depending on the research question and the type of data (Table 1). Where the L4 dataset has been used for each of the questions, the other three datasets were used to identify the tiny plankton lifeforms and monthly, seasonal and interannual changes in tiny plankton.

Table 1
A summary of UK pico- and nano-plankton datasets. All datasets were collected via flow cytometry, apart from photosynthetic nanoeukaryotes at LPO, which were recorded via microscopy. N/A- data not available. L4 = Plymouth Marine Laboratory (PML) station L4, LPO = Lorn Pelagic Observatory station, SCObs SH = Marine Directorate of the Scottish Government's Scottish Coastal Observatory monitoring site at Stonehaven, and CPR WaMS = the Marine Biological Association's Continuous Plankton Recorder Water and Microplankton Sampler.

Group	L4	LPO	CPR WaMS	SCObs SH
Sampling depth	0 and 10 m (discrete)	1 *m*	10 *m*	0–10 m (integrated)
Sampling area	Western Channel	Western Scotland	Western Channel	Eastern Scotland
Heterotrophic nanoeukaryotes	2007–2021	2010–2022 (microscopy)	N/A	N/A
Photosynthetic nanoeukaryotes	2007–2021	2010–2022 (microscopy)	2011–2016	2015–2021
Photosynthetic picoeukaryotes	2007–2021	N/A	2011–2016	2015–2021
Synechococcus	2007–2021	N/A	2011–2016	2015–2021
LNA-bacteria	2007–2021	N/A	N/A	2015–2021
HNA-bacteria	2007–2021	N/A	N/A	2015–2021

fitting Fourier series (Defriez et al., 2016). However, these methods assume a stationary time-series and do not account for strong seasonal variability. The more recently developed method of wavelet analysis estimates the spectral characteristic of an environmental signal as a function of time. The method has been used to explore variability in seasonal chlorophyll patterns across ecosystems (Winder and Cloern, 2010) and to examine spatial synchrony of plankton dynamics (Sheppard et al., 2019). Here, we apply this method to the tiny plankton abundance time-series and extend it to examine the relationship between the timescales of variability in these time-series and those of the matching environmental variables of temperature and nutrients.

For L4 data we linearly interpolated a daily sequence between the available weekly plankton, sea surface temperature (SST), and nutrient time-series data using the interp1 function in Matlab. Then, a timescale-specific analysis of fluctuations, carried out over timescales longer than the weekly sampling interval, was performed.

All L4 tiny plankton and environmental variable time-series included a strong seasonal variability. A continuous wavelet transformation was applied to the daily data to disaggregate variability by timescale (Addison, 2017). The wavelet transformation allows the following of fluctuations at a particular timescale so both intra-annual and multi-annual fluctuations in the data can be examined. The timescales examined using the wavelet-transformed data ranged from one cycle every 14 days to one cycle every 10 years, in increments of 5 percent. The Morlet wavelet is a complex plane wave oscillation localised by a Gaussian envelope, see (Sheppard et al., 2019; Sheppard et al., 2017) for detailed method description. The central frequency of the mother wavelet was 1; this means that at all scales, the width parameter of the envelope was equal to the timescale of the oscillation. This transformation yields a magnitude and phase for all times and timescales of oscillation in the data.

The tendency of wavelet components was drawn from two variables to maintain a particular phase difference at a particular timescale; this is known as the phase coherence of the variables. Variables incorporating correlated fluctuations at a particular timescale will have high coherence with near-zero phase shift; alternatively, high coherence with a large phase shift indicates a lagged or negative correlation. Note that the fewer cycles of oscillation that are available at a particular timescale, the more likely the unrelated data will exhibit an arbitrary phase relationship over the length of the time-series which can manifest as bias in observed coherence values and lower statistical power at the longest timescales.

Artificial surrogate data were used to test for significant timescale-specific coherence between two time-series. This method ranks the observed coherence relative to a distribution of artificial coherence values generated under the null hypothesis of no association, taking account of the spectral characteristics of the data and its autocorrelation (Schreiber and Schmitz, 2000) using the methods presented in Sheppard et al. (Sheppard et al., 2019; Sheppard et al., 2017) in Matlab (alternatively see the R package wsyn (Reuman et al., 2021)).

The tendency towards highly-ranked coherence in each of the three timescale bands, compared to surrogate data, was then further examined. The mean rank found in a band of timescales can be compared to the distribution of possibilities consistent with the null hypothesis, and thus a p-value is obtained for the band (Sheppard et al., 2019; Sheppard et al., 2017). Timescale bands of 14 days to six months (short timescale), six months to 2 years (bracketing the annual fluctuation), and 2 years to 10 years (long timescale) were tested.

Having determined the pairs of time-series for which wavelet coherence was significant, the typical phase difference between each pair in each timescale band was evaluated (Fig. S3). First, the circular mean (over time) phase difference between wavelet components was evaluated for each timescale. Then the circular mean (over timescales) of these values was evaluated inside each band; this is called the "typical phase difference". If the coherence was deemed significant in a band (ranked higher than 95 percent of surrogates in the band, as above) then the transform status of the time-series, either in-phase or in antiphase, was reported for this band (Table 3, Tables S1–S3). If the typical phase difference had a magnitude less than pi/2 rad the transforms are in-phase; in this range of timescales the maxima in one time-series tend to coincide with the maxima in the other time-series. If the transforms were in phase across a range of timescales this means the time-series tend towards positive correlation; henceforth we will refer to an in-phase relationship in a particular timescale band as a positive relationship between the variables at those timescales. If the typical phase difference had a magnitude greater than pi/2 rad the transforms are in antiphase; in this range of timescales the maxima in one time-series tend to coincide with the minima in the other time-series. If the transforms are in antiphase across a range of timescales this means the time-series tend towards negative correlation; henceforth we will refer to an anti-phase relationship in a particular timescale band as a negative relationship between the variables at those timescales.

The data were limited especially in testing coherence of cycles of multi-year blocks. This was an exploratory analysis of a large number of tests, which meant that 5 % of relationships would have returned a p-value < 0.05 purely by chance. A decision was made, however, to exclude Bonferroni correction at $p < 0.05$ significance threshold, as that only selects the strongest coherence relationship with such a large dataset, and we avoid the loss of relatively strong ecologically important relationships at this exploratory stage. We therefore do not refer to the relationships as significant, but instead use the p value to estimate the strength of the identified relationships.

3. Results

3.1. Tiny plankton datasets in UK waters

Six groups of tiny plankton routinely quantified using mainly flow cytometry were identified for this study based on their year-long high abundances and strong repeatable annual cycles (Table 2). Table 2 broadly states which types of taxa might be represented in these groups based on the systematic classification of Adl et al. (2019) but is not

Table 2
Groups identified in this study by flow cytometry (FC) or microscopy, along with their standard definitions according to the British Oceanographic Data Centre (BODC), mapped to lifeform traits based on: cell size: picoplankton=<3μm, nanoplankton = 2–20 μm; flow cytometry fluorescence: OF = orange fluorescence (from phytoerythrin), RF = red fluorescence (from chlorophyll), GF = green fluorescence after cell staining; trophy: A = autotroph, M = mixotroph (can consume prey and photosynthesize), H = Heterotroph, saprotroph or osmotroph (consuming organic nutrients or prey); main predators: H + M = heterotrophic (phagotrophic) and mixotrophic plankton, NANF = nanoflagellates, MICR = microplankton (ciliates, dinoflagellates); and preferred habitat.

Taxa	Size group	Size (μm)	BODC FC category	Definition of FC group (BODC)	Systematic lineage	Lifeform assignment trait	Trophy: A/M/H	Main predators	Preferred habitat	References
Heterotrophic nanoeukaryotes	nano	<20	None	Nanophytoplankters without pigment fluorescence	*Eukaryota: Stramenopiles (Bicosoecida, Placidida Pirsonia, uncultured MArine STramenopiles e.g, MAST-3) Alveolata: (Dinoflagellata, Colpodellida, Perkinsidae) Rhizaria: (Cercozoa, Vampyrellida) Opisthokonta: (Choanoflagellata) Nucletmyceta: (Fungi, Cryptomycota) Euglenid*	Size	H	H + M, NANF, MICR	ubiquitous, can be variable depending on plankton dynamics. Higher contribution in upwelling regions.	(Adl et al., 2019; Buck et al., 1996; Kang and Kang, 2023; Robinson et al., 2006; Sanders et al., 2000; Schoenle et al., 2019; Vázquez-Domínguez et al., 2008)
Photosynthetic nanoeukaryotes	nano	<20	F0200005	Red only fluorescing eukaryote nanophytoplankters	*Eukaryota: Stramenopiles (MAST, Bacillariophyceae) Alveolata (Dinoflagellata, Ciliophora), OHaptophyta (Prymnesiophyceae) Archaeplastida (Prasinophyceae) Cercozoa (Chlorarachnea) Excludes coccolithophores and cryptomonads*	size, RF	A, M	H + M, MICR	ubiquitous, largest contribution when nutrients low	(Adl et al., 2019; Cavalier-Smith, 2018; Hirakawa et al., 2011; Yung et al., 2022)
Photosynthetic picoeukaryotes	pico	< 3	F0200004	Red only fluorescing eukaryote picophytoplankton	*Eukaryota: Stramenopiles, Prymnesiophyceae, Chlorophyceae*	size, RF	A + M	H + M NANF, viruses	ubiquitous, largest contribution when nutrients low	(Buck et al., 1996; Kirkham et al., 2011; Not et al., 2004; Robinson et al., 2006; Yung et al., 2022)
Synechococcus	pico	< 3	F0200003	Orange fluorescing prokaryote picophytoplankton	*Bacteria (Cyanobacteria, Cyanophyceae)*	size, OF	A	H + M NANF, viruses	well-illuminated, low-nutrient, stratified waters above 6 °C	(Buck et al., 1996; Cavalier-Smith, 2018; Flombaum et al., 2013; Flombaum et al., 2020; Robinson et al., 2006; Schmidt et al., 2020; Ting et al., 2002; Visintini et al., 2021; Yung et al., 2022)
LNA ('low-nucleic-acid' bacteria)	pico	< 3	F0200011	Stained by green emitting fluorescent dye that binds to nucleic acid. Low fluorescence intensity and sideward light scatter (SSC) properties	*Bacteria: Alphaproteobacteria SAR11, Gammaproteobacteria SAR86, Betaproteobacteria, Acidimicrobiia, Bacteroidia, Firmicutes, Fibrobacter*	size, GF-	H	H + M NANF	ubiquitous, oligotrophic systems, open ocean	(Buck et al., 1996; Hu et al., 2020; Hu et al., 2022; Longnecker et al., 2005; Mary et al., 2006; Robinson et al., 2006)
HNA ('high-nucleic-acid' bacteria)	*pico*	*< 3*	*F0200010*	*Stained by green emitting fluorescent dye that binds to nucleic acid. High fluorescence intensity and sideward light scatter (SSC) properties*	*Bacteria: diverse lineages, alpha- and gammaproteobacteria, Acidimicrobiia, Bacteroidia, Acinobacteria. Some taxonomic overlap with LNA-bacteria*	*size, GF+*	*H*	*H + M, NANF*	*ubiquitous, can occur in low nutrient systems*	*(Buck et al., 1996; Hu et al., 2020; Hu et al., 2023; Longnecker et al., 2005; Robinson et al., 2006)*

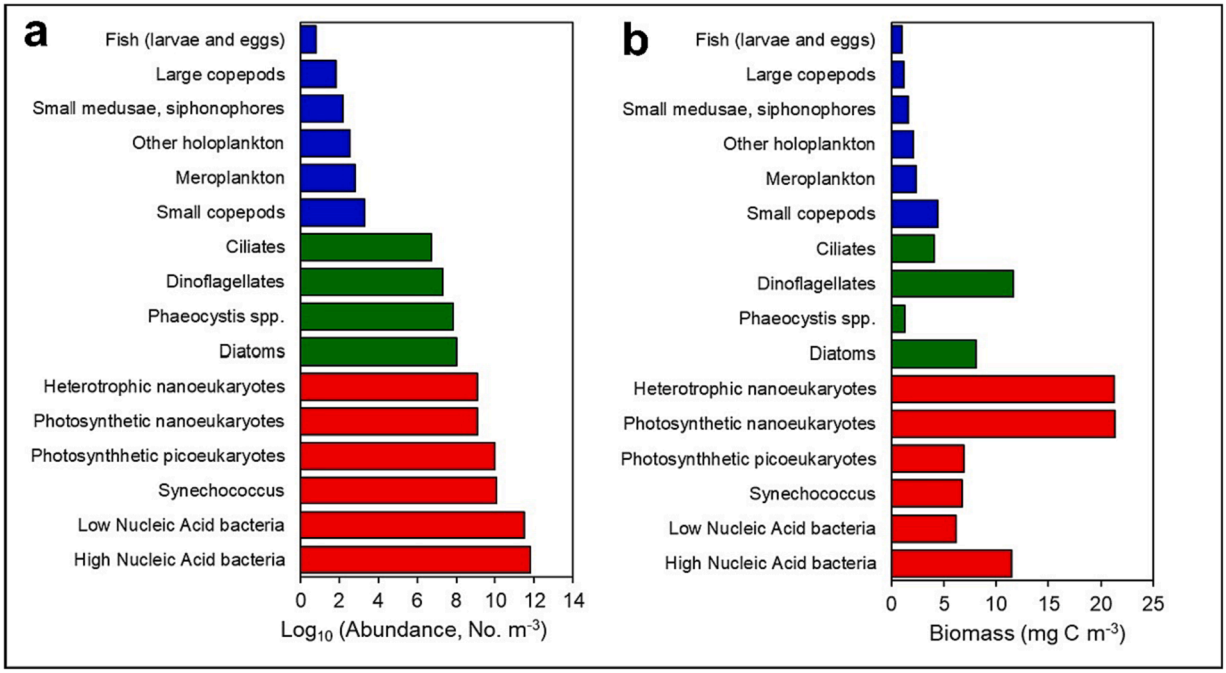


Fig. 3. Plankton functional group abundance (a) and biomass (b) from 2007 to 2020 at station L4 in the Western Channel. Red bars represent taxa best quantified by flow cytometry (Table 1), green bars by light microscope analysis of settled water samples, and blue bars by microscope analysis of net catches. Taxa represented in red are not routinely used as policy indicators due to lack of time-series datasets. Only the major biomass contributing functional groups of the others (green and blue) are illustrated for clarity. Functional groups are ranked according to mean abundance within each sampling method. Biomass values for functional groups are based on conversions from linear dimensions measured at L4; see section 2.4 for more detail of the methods. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

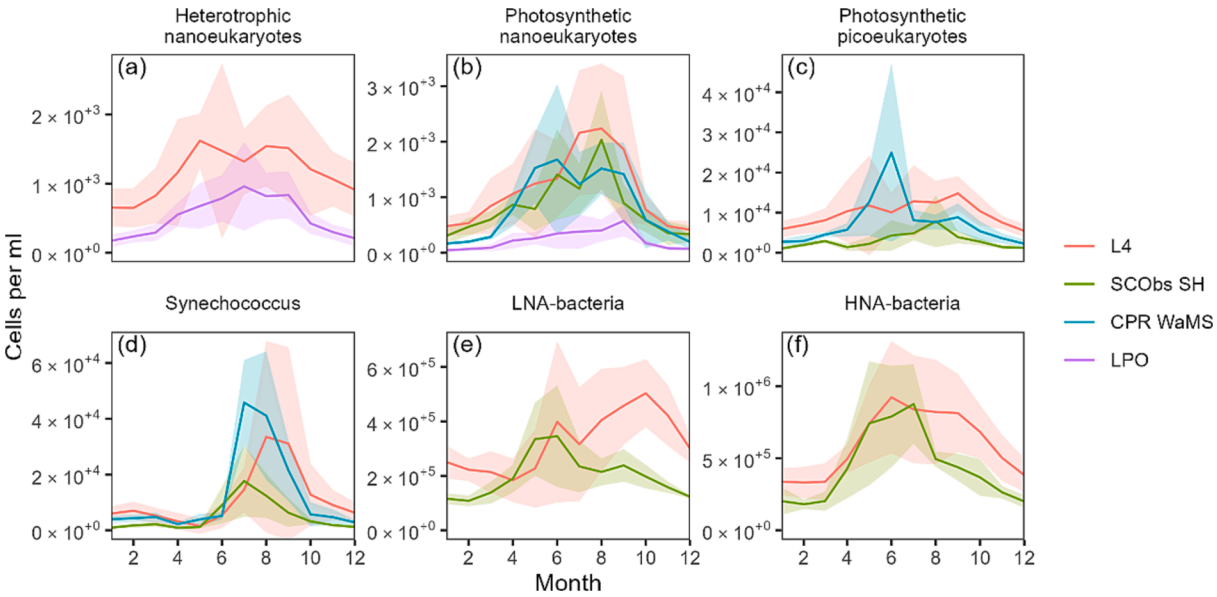


Fig. 4. Seasonal mean total counts (cells per mL) over the complete time-series available for station L4 from 2007 to 2021 (red), SCObs SH (green) from 2015 to 2018, the Western Channel (CPR WaMS, blue) from 2011 to 2016, and LPO (purple) from 2010 to 2017. a) Heterotrophic nanoeukaryotes, b) Photosynthetic nanoeukaryotes, c) Photosynthetic picoeukaryotes d) *Synechococcus*, e) LNA-bacteria, and f) HNA-bacteria at. All abundances were derived from flow cytometry, except those at LPO, which were microscopically counted. The shaded area around each mean represents one standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

exhaustive. In fact, the genus *Synechococcus* is a species complex with 20 different genetic clades (Sohm et al., 2016). The other groups comprise multiple species.

3.2. Tiny plankton abundance and biomass in the Western Channel (station L4)

Abundance and biomass differed across all plankton groups, ranging in size from bacteria to fish larvae, between 2007 and 2020 at station L4 in the Western Channel, UK (Fig. 3) with abundance and biomass for the

Table 3
Summary of coherence relationships with $p > 0.05$: on sub-annual (<1 year), annual (variation peaking at frequency of 1/year), or multi-annual scales based on Western Channel Observatory L4 time series data.

Plankton group	Sub-annual (seasonal)	Annual	Multi-annual
Heterotrophic nanoeukaryotes		SST (+ve) Nitrate (-ve) Silicate (-ve) Phosphate (-ve)	Nitrite (-ve)
Photosynthetic nanoeukaryotes	Nitrite (-ve) Phosphate (-ve)	Nitrite (-ve) Nitrate (-ve) Silicate (-ve) Phosphate (-ve)	Silicate (+ve)
Photosynthetic picoeukaryotes	SST (-ve)	SST (+ve) Nitrate (-ve)	
Synechococcus	Nitrite (-ve)		
LNA- bacteria	Nitrite (-ve)		
HNA- bacteria	SST (-ve)	SST (+ve) Nitrate (-ve)	

plankton groups increasing from the larger size plankton (fish larvae and eggs) to the very tiny plankton. Based on the combined abundance and biomass conversion factors described in section 2.4, the nano- and pico-plankton together (i.e. those groups derived from flow cytometry data and indicated in red in Fig. 3) comprise 99.98 % of abundance and 71 % of biomass of all plankton.

These percentage calculations are highly dependent on both the abundance and biomass conversion factors used for each of the microscopic and flow cytometry approaches. To provide an independent assessment of the veracity of these figures, the data in Fig. 3 were used to construct normalised biomass size spectra (NBSS; Mehner et al., 2018, Atkinson et al., 2021). We also constructed the equivalent NBSS derived only from light microscope counts and microscope-based cell dimensions (which included only the microscope-based nanoplankton, without the flow cytometry data). The inclusion of the flow cytometry data and flow cytometry-based carbon conversions yielded a median NBSS slope value of −1.113, while excluding them yielded a much shallower (less negative) slope of −0.964. The former is close to the overall median value (-1.131) from a *meta*-analysis of aquatic environments (Atkinson et al., 2021), while the value without flow cytometry data is substantially less, and does not fit with theoretically-derived size-based models of energy transfer (Quinones et al., 2003). This finding supports the need for flow cytometry data to provide a holistic view of plankton which fully captures their size structure. Although fixed volume to carbon conversions provide only crude estimates of carbon content, the fact that the slope of the size spectrum is close to the global median shows that the overall balance of pico- and nano-size cells compared to the larger plankton is within the expected range at the L4 site.

The most abundant groups from all lifeforms collected at the long-term L4 site were the heterotrophic bacteria (HNA-, LNA- bacteria), *Synechococcus*, photosynthetic picoeukaryotes, photosynthetic nanoeukaryotes and heterotrophic nanoeukaryotes. The latter's average abundance (and biomass by virtue of using the same carbon conversion factor) was almost identical to that of photosynthetic nanoeukaryotes. The biomass results were similar with heterotrophic (HNA- and LNA-) bacteria and photosynthetic nanoeukaryotes comprising the highest biomass groups. In summary, tiny cells (<20 μm) were both more abundant and higher in biomass than microphytoplankton, zooplankton, and fish, revealing the importance of these flow cytometry-derived groups, even in inshore waters such as those at the Plymouth L4 site.

3.3. Seasonal, intra- and inter- annual changes in tiny plankton in UK waters

There are broad similarities in the seasonality of six tiny plankton groups across all four sites spanning an eight degree range of latitudes (Fig. 4). Photosynthetic nanoeukaryotes and photosynthetic picoeukaryotes showed an elevated abundance from spring to autumn with maximum levels in the summer. The abundances of microscopically determined photosynthetic nanoeukaryotes at the LPO station were much lower than others, even at the other Scottish site, SCObs SH, whilst less disparity was observed in heterotrophic nanoeukaryote counts. Methodological differences may account for this discrepancy. Different dynamics in photosynthetic nanoeukaryotes were observed across sites, possibly resulting from net response of different nano-sized plankton communities to abiotic and biological drivers. However, all sites showed elevated abundances of the nanoplankton groups from spring to autumn indicating common responses to broad-level drivers. *Synechococcus* had a narrower bloom period, with a single peak in abundance that started in June and peaked in July/August across three time-series, indicating a single summer bloom. Both LNA- and HNA-peaked in June at both SCObs SH and L4 although at different abundance levels. LNA-bacteria had a second autumn peak at L4 that may relate to the specific conditions at that site.

Interannual variability in pico- and nano-plankton abundance can clearly be seen across all time-series datasets (Fig. 5, Fig. 6). The seasonal cycle is evident throughout, with some months showing anomalous peaks in abundance at separate sites. For example, in 2018 there were high abundances of all three groups of tiny plankton recorded at Stonehaven (SCObs SH), while L4 only showed a peak in *Synechococcus* but not in photosynthetic nanoeukaryotes or photosynthetic picoeukaryotes. In 2016 there were elevated abundances of photosynthetic nanoeukaryotes recorded at the Lorn Pelagic Observatory (LPO) and in the CPR WaMS samples collected in the Western Channel. All groups showed an annual periodicity that may relate to seasonal drivers. Notably, heterotrophic nanoeukaryote abundances were still important in winter months at all sites. In the Western Channel, there are instances where group abundances between the two Channel time-series, L4 and CPR WaMS, vary (e.g. *Synechococcus* in 2013). This suggests spatial patchiness between the geographical ranges of these two sampling sites – L4 is a single station while CPR WaMS spans the English Channel. Where photosynthetic nanoeukaryotic data from light microscopy are available for both LPO and SCObs SH, there is remarkable consistency in their abundance patterns, although LPO had reduced cell counts compared to SCObs SH. A further exploration of seasonality between *Synechococcus*, photosynthetic picoeukaryotes, and photosynthetic nanoeukaryotes at L4 (Fig. S2) showed years where peak abundance of either *Synechococcus* or photosynthetic picoeukaryotes was at their highest. In those years, we observed only *Synechococcus* or photosynthetic picoeukaryotes dominated, but not both.

The seasonality of HNA- and LNA-bacteria both had a clear summer peak in abundance. HNA-bacteria (mean HNA-bacteria across both sites = 5.17x 10^5e cells/ml) are distinguished by greater mean monthly abundances than those of LNA-bacteria (mean LNA-bacteria across both sites = 2.62x10^5e cells/ml) at both stations. HNA-bacteria had 39 % greater mean annual abundance at L4 than at ScOBS SH (Fig. 4).

3.4. Links between tiny plankton and environmental variables in the Western Channel

The six tiny plankton groups (photosynthetic nanoeukaryotes, heterotrophic nanoeukaryotes, photosynthetic picoeukaryotes, *Synechococcus*, and LNA- and HNA-bacteria) and six environmental variables at station L4 (SST, phosphate, silicate, nitrate, nitrite, and ammonia) were tested for timescale-specific relationships using wavelet analysis (Table 3). The high frequency (weekly samples) and long temporal resolution (~14 years) of the data revealed relationships between some

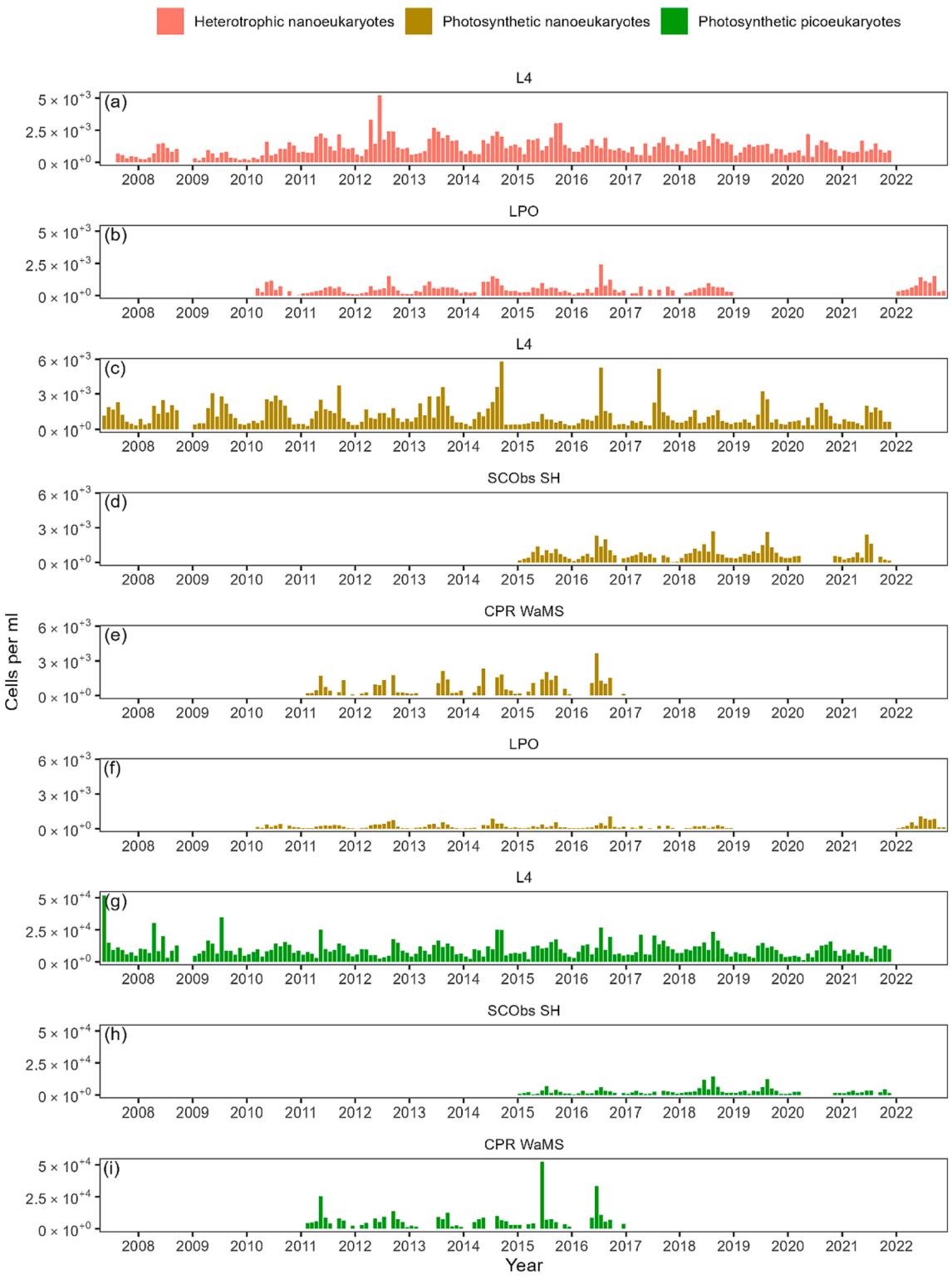


Fig. 5. Monthly mean abundances of eukaryotic tiny plankton groups (where available) for (a-b) heterotrophic nanoeukaryotes, (c-f) photosynthetic nanoeukaryotes, and (g-i) photosynthetic picoeukaryotes from L4, LPO, SCObs SH and CPR-WaMS between 2006 and 2022. Labels on the x axis mark the start of each year. Note – Y-axis scales are consistent across datasets for each tiny plankton group, but differ between groups to display the variability in each time-series more clearly.

plankton groups and environmental variables at sub-annual (seasonal), annual and multi-annual timescales (Table 3; Fig. S1), identified by highly ranked wavelet coherence compared to surrogates.

At sub-annual timescales, six plankton-environmental relationships with $p < 0.05$ were identified; three included negative relationships with nitrite and two with SST. Of the tests at annual timescales, twelve relationships with $p < 0.05$ were identified for nitrate (4), SST (3), phosphate (2), silicate (2), and nitrite (1), although negative relationships with nutrients at this timescale probably only indicate that the growing season coincides with summer when nutrients are low. Only two relationships, between heterotrophic nanoeukaryotes and nitrite, and photosynthetic nanoeukaryotes and silicate, were identified with p

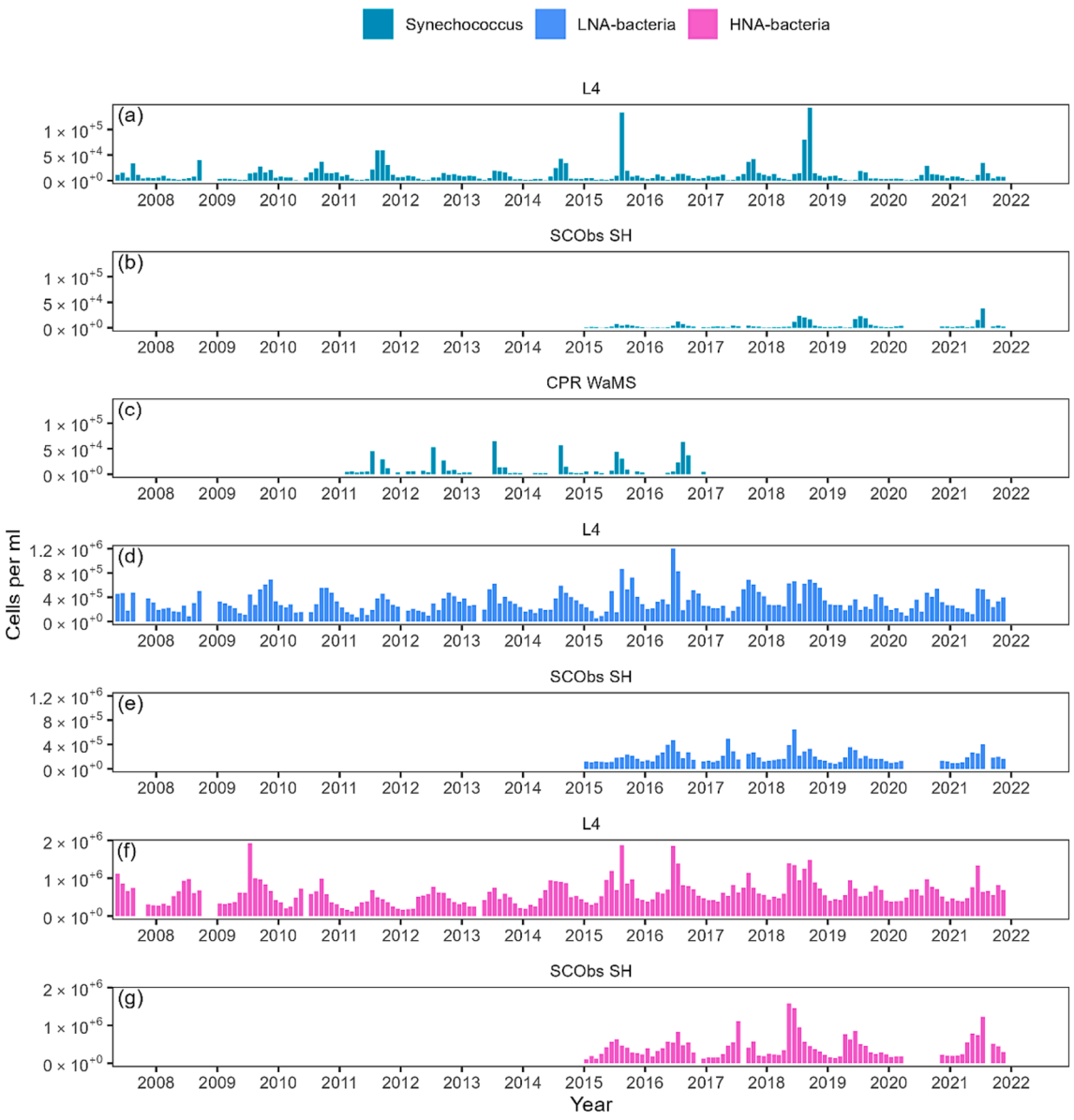


Fig. 6. Monthly mean abundance of bacterial tiny plankton groups (where available) for (a-c) *Synechococcus*, (d-e) LNA-bacteria, and (f-g) HNA-bacteria between 2006 and 2022. Labels on the x axis mark the start of each year. Note – Y-axis scales are consistent across datasets for each tiny plankton group, but differ between groups to display the variability in each time-series more clearly.

< 0.05 over longer (multi-year) timescales, although data availability is most limited for this timescale.

The results of photosynthetic picoeukaryote abundance and SST wavelet analysis are shown here as an example (Fig. 7; Full pairwise significance tests of results are in Tables S1–S3). The high magnitude of the wavelet transformation at the annual timescale for both picoeukaryote abundance and SST indicated strong seasonal variability (Fig. 7C, D). The coherence testing revealed a positive relationship between the fluctuations at the annual timescale, and a negative relationship between the rapid intra-annual fluctuations (Fig. 7E).

Components of the nitrogen cycle were found to have coherence with all six tiny plankton groups. Negative relationships were found between photosynthetic nanoeukaryotes, *Synechococcus*, and LNA-bacteria and nitrite at sub-annual timescales. Photosynthetic nanoeukaryotes and heterotrophic nanoeukaryotes had the highest number of coherent relationships with nutrients across all timescales, almost all of which were negative. Silicate was the only environmental variable to show a positive multi-annual relationship with photosynthetic nanoeukaryotes; many taxa in this group, such as small diatoms and small silicoflagellates, contain silica. Nitrite showed a negative multi-annual relationship with heterotrophic nanoeukaryotes. A possible cause would be heterotrophic decomposition of dissolved organic nitrogen sources.

Photosynthetic picoeukaryotes, heterotrophic nanoeukaryotes, and HNA-bacteria were the only groups that showed direct relationships with SST; these were negative at sub-annual timescales, but positive at annual timescales, likely because growth is higher in summer than winter (Fig. 7). *Synechococcus* showed a negative relationship with nitrite but no relationship with SST, despite its persistent summer abundance peak evident in Figs. 3 and 4. However, even the relationship between *Synechococcus* and nitrite was only at a sub-annual timescale.

It should be noted that the phase relationships found between tiny plankton and SST and tiny plankton and nutrients are complicated, with sub-annual timescale negative relationships with SST indicating a positive association with colder water, and various phase relationships at the annual scale reflecting the relative timing of the annual peak in each variable.

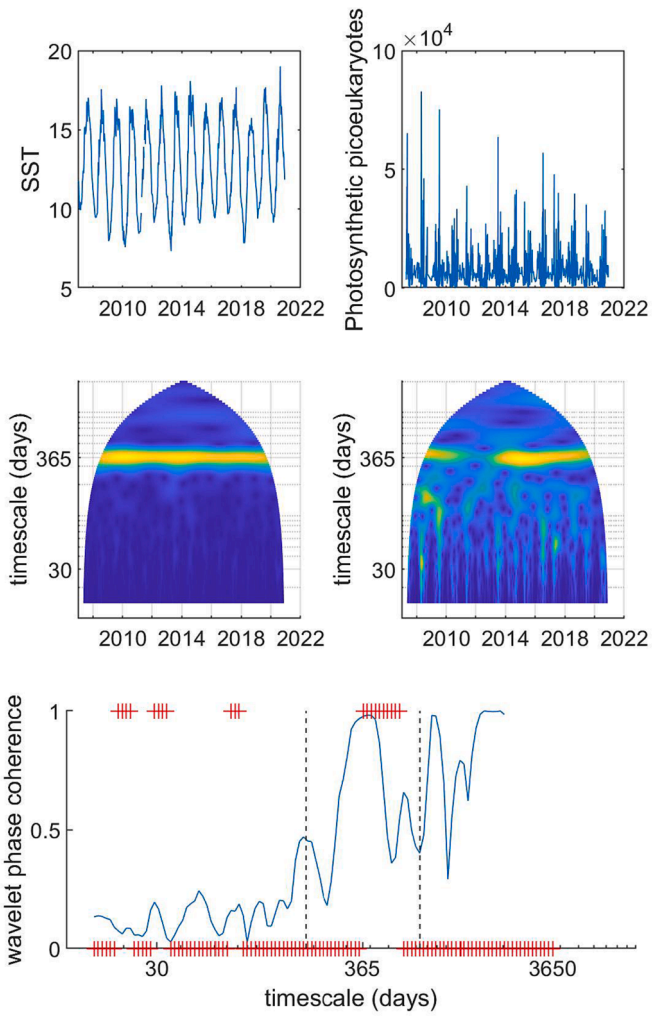


Fig. 7. Example of wavelet analysis performed on the SST and photosynthetic picoeukaryote time-series at station L4 showing time-scale relationships between these variables. The top two panels show the (interpolated) time-series; the middle two panels show the amplitude of their wavelet transforms, as a function of time (x axis) and timescale of variation (y axis; measured in days) to show the frequency of occurrence of both these variables together for SST (left) and picoeukaryotes (right). Yellow represents high amplitude and blue represents low amplitude. The middle panels show the most common frequency of these variables is 365 days (annually). The bottom panel shows their wavelet phase coherence as a function of timescale. The red cross at 1 indicates coherence ranked > 95 % of null surrogates ($p < 0.05$); red cross at 0 indicate > 95 % significance. This shows "in phase" coherence at 365 days at amplitude 1, with amplitude 0 showing no timescale relationship at all between these variables. See Tables S1-S3 for complete results. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

This paper provides the first regional description of the tiny plankton community in UK coastal and marine ecosystems, addressing four key questions relating to their potential use in marine ecosystem status assessments: Are nano- and pico- plankton important components of the plankton? Can the seasonality and variability of tiny plankton groups connect to environmental variables? Which nano- and pico- plankton lifeforms can be defined and assessed routinely? How can tiny plankton data be integrated into the current assessment process for marine ecosystem health?

4.1. Importance of the tiny nano- and pico-plankton (tiny plankton)

The outcomes of the Plymouth L4 analysis demonstrate the importance of the tiny plankton at the Plymouth L4 station (Fig. 3); in particular, the six tiny plankton groups identified in this paper. On average these groups contributed 71 % of the planktonic biomass between 2007 and 2020. While few other time-series can match both the duration and the full-size spectrum coverage of the L4 series, numerous papers (Clark et al., 2022; Schmidt et al., 2020; Uitz et al., 2010) document the importance of tiny plankton in temperate coastal and open waters. Bolaños et al. (2020) used flow cytometry to show phytoplankton < 20 μm in diameter were the main contributors to phytoplankton biomass in the Western North Atlantic and Tett et al. (1988) estimated that photosynthetic nanoeukaryotes represented 74 % of chlorophyll and 69 % of carbon fixation at the Lorn observatory in spring and summer. Estimated rates of bacterial heterotrophy are considerable, at 15 % (Turner, 2015), whilst ciliates smaller than 30 μm consume 72 % picoplankton and 28 % nanoplankton (Rassoulzadegan et al., 1988). There are phenologically-dependent trophic relationships between picoplankton and nanoplankton; ocean dynamics determine if heterotrophic nanoeukaryotes are controlled by their prey or by predators in the Atlantic (Vázquez-Domínguez et al., 2008).

The role of tiny plankton, previously thought to be of major significance mostly in oligotrophic regimes (Agawin et al., 2000; Hartmann et al., 2012; Morris et al., 2002), is becoming increasingly important at the coastal L4 station as the ocean warms. Changes are already evident, moving from a diatom-dominated regime to domination by smaller, non-diatom, cells (Widdicombe et al., 2010). These changes have seen pico-sized plankton dominating in the summer stratified NW European shelf waters, as they can utilise the much lower summer nutrient concentrations in surface waters (Schmidt et al., 2020). With increasing zooplankton diversity (reviewed by Harris (2010)) and reductions in total copepod abundances over the last 50 years (Schmidt et al., 2020), we hypothesise that food webs will alter (see Fig. 8), increasing in their complexity with consequences for the efficiency of energy transfer to higher trophic levels, and to the biodiversity of predators adapting to different feeding strategies.

4.2. Seasonality and variability of tiny plankton groups and relationship to environmental variables

The annual cycles of the six tiny plankton groups examined here were largely consistent across latitudes with some seasonal regularity. Abundances were mostly measured using flow cytometry to distinguish between size and pigment groups. Our exploratory wavelet coherence analysis without Bonferroni corrections showed 20 associations with environmental variables. However, with only 14 years of data, the capacity for multi-year comparisons was limited, so these results are only indicative of trends. We recommend further testing of specific relationships with additional data, particularly long-term seasonal patterns that may be masked when aggregated at annual or multi-annual timescales for improved ecological interpretations. We also show that our results are in line with similar studies on tiny plankton in the Atlantic, detailed below, and from seasonal abundance at L4 reported by Tarran and Bruun (2015).

Most of the coherence results involve short-term correlations with SST and nitrogenous nutrients. Smaller cells take up nutrients more efficiently, and their growth rate is higher than that of microplankton (Marañón, 2015). Negative annual relationships found between plankton groups and nitrogenous nutrients are likely caused by the depletion of nitrate levels that peak in winter and become depleted after the spring bloom at station L4, and then are biologically regenerated into nitrogen, sustaining summer phytoplankton (Ward et al., 2011). *Synechococcus* and photosynthetic picoeukaryotes had similar summer peak seasonal timing. However, these groups differed in their timescale of response to nitrogenous nutrients and showed opposing trends in

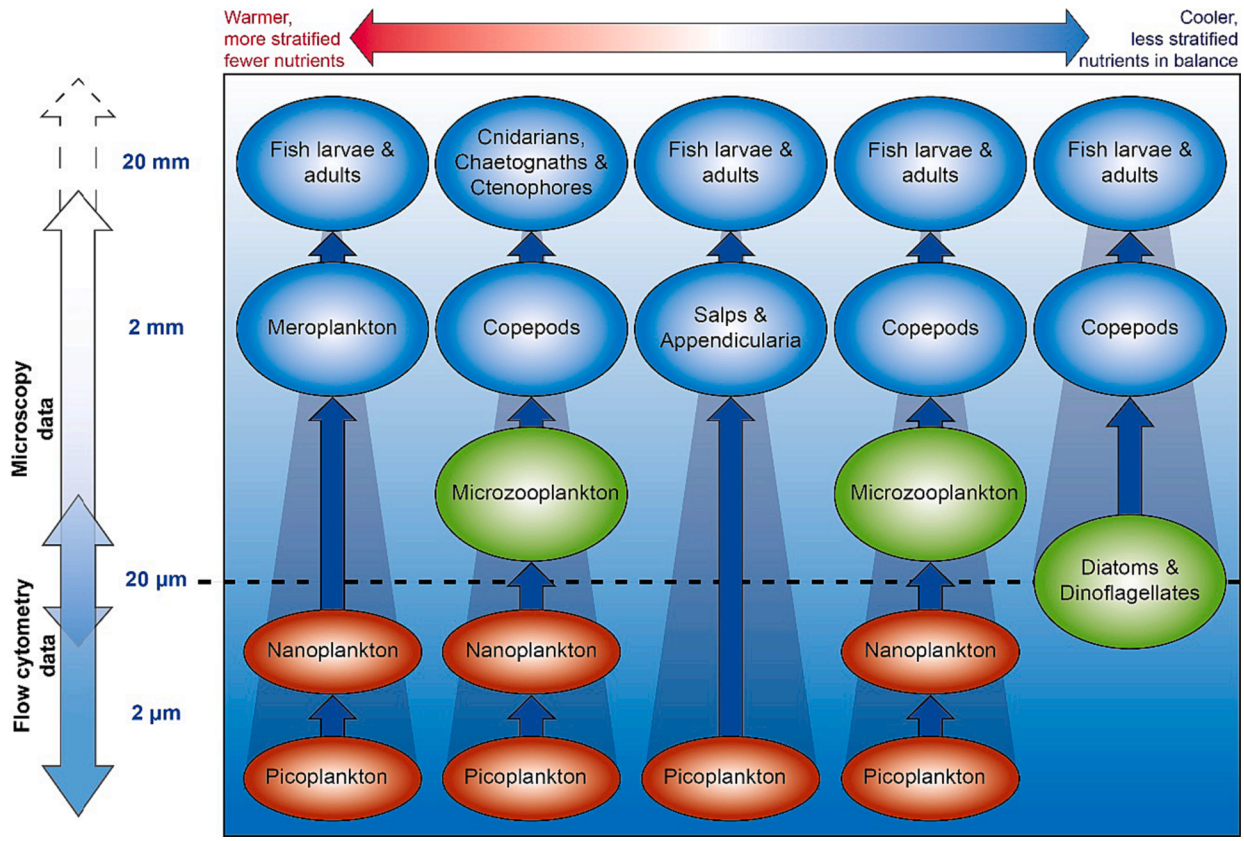


Fig. 8. Simplified schematic illustrating some examples of food chains from primary producers to fish that may emerge from warming, increase in stratification, and nutrient stress/imbalance. This schematic purposefully simplifies the complexity of real food webs which may involve, for example, varying predator and prey sizes, mixotrophy and multiple alternative pathways. The classic "textbook" food chain from diatoms to copepods to fish is illustrated on the right, and various works (e.g. (Capuzzo et al., 2018; Holland et al., 2023b; Jaspers et al., 2023; Legendre and Rassoulzadegan, 1995; Schmidt et al., 2020)) suggests that we are moving away from this to alternative pathways towards the left of the figure. The grey funnels signify that in these alternative food chains, the larger number of trophic steps and/or the base of the food web being smaller sized, sometimes lower quality food would yield lower efficiencies of energy transfer to higher trophic levels. Bubble colours are as in Fig. 3, showing the importance of flow cytometry-derived data (red bubbles) to understand these alternative food chains. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

when one or the other was at its maximum abundance (Fig. S3), also observed by Schmidt et al. (2020). This points to competition for nutrients or exploitation of different nutrient niches, with *Synechococcus* having superior abilities to operate in oligotrophic conditions of low light at the deep chlorophyll-*a* maximum, exploiting the higher concentrations of limiting nutrients like iron and nitrate (Sohm et al., 2016). Despite its peak summer abundance, no association was found between SST and *Synechococcus* here, which is in contrast to other work (El Hag and Fogg, 1986). This genus has 20 ecotypes and northern ecotypes show different responses than SST- influenced tropical to subtropical ecotypes (Bolaños et al., 2020; Sohm et al., 2016). Photosynthetic picoeukaryote abundance had a positive timescale relationship to SST reflecting better conditions for growth and feeding over the year. At sub-annual levels photosynthetic picoeukaryote abundance had a negative timescale relationship to SST that may reflect responses to different sub-annual-scale events or conditions, such as nutrient availability due to SST-induced summer stratification. Schmidt et al. (2020) reported picoeukaryotes increase in abundance especially at the onset of spring to early summer as growth conditions and nutrient availability improves but decline from mid-summer as nutrients become depleted and *Synechococcus's* physiological advantages allow them to outcompete photosynthetic picoeukaryotes.

We found LNA-bacteria were less abundant than HNA-bacteria and revealed different coherence to environmental drivers at different timescales. Small and slow-growing LNA-bacteria (that include the most numerous SAR11 bacteria) are reportedly less abundant that HNA-bacteria in marine coastal waters, and negatively correlated with chlorophyll-*a* (Hu et al., 2022). This supports the lower LNA abundances we observed, although relationships with dissolved nitrogen are likely complex and dependent on variable conditions (Vázquez-Domínguez et al., 2008). Of significance, photosynthetic picoeukaryotes and HNA-bacteria were positively coherent to SST but negatively to nitrate at an annual level. However, at sub-annual timescales both these groups were negatively associated with SST. Heterotrophic nanoeukaryote groups shared this association at the annual timescale with nitrate and SST. This suggests complex, interdependent dynamics at play. Heterotrophic nanoeukaryotes contain bacterivores that are estimated to ingest 17 % of available bacterial prey in meso- and oligotrophic Atlantic regions (Zubkov et al., 1998). At summer peak SST, picoplankton dominate plankton at L4 (Schmidt et al., 2020), strengthening the microbial loop (Marañón, 2015), and leading to increased bacteriovory of heterotrophic nanoeukaryotes on HNA-bacteria. Clark et al. (2022) may provide insights into relationships we found with dissolved nitrogen, noting short-term nitrite is the result of competitive resource sharing from short-term autotrophic production and heterotrophic breakdown of dissolved organic nitrogen. Ultimately nitrification influences nutrient limitation patterns for primary production (Clark et al., 2022). Human-influenced ocean warming, stratification, and nutrient enrichment also affect the microbial nitrogen cycle and also the levels of oxygenation resulting from anaerobic bacterial metabolism (Hutchins and Capone, 2022).

4.3. Which nano- and pico-plankton lifeforms can be defined and assessed routinely?

Lifeforms are constructed by using a set of common traits that identifies a functional group of plankton taxa that respond in a similar way to ecohydrodynamic conditions and are similar in their biogeochemical and trophic interactions (Tett et al., 2008). For assessment purposes (as indicators), lifeforms should also be practical, easy to quantify, and able to inform management actions and priorities. The abundances of these six tiny plankton groups can be measured reliably and show timescale sensitivity to environmental variables related to human pressures, which supports the development of tiny plankton lifeforms for biodiversity assessments.

Tett et al. (2022) distinguished recognition traits used to identify an organism as belonging to a particular taxon; assignment traits used to allocate a taxon to a lifeform; adaptive traits that allow organisms to live in particular environments; and biogeochemical traits and trophic traits that relate to organisms' roles in ecosystems. The last three may be grouped as functional traits and help identify the reasons behind lifeform changes. Characteristics used in flow cytometry, such as red fluorescence and size, assign tiny plankters to lifeforms such as nanophytoplankter at the same time as the instrument recognises an object as a plankter, while the red fluorescence (which distinguishes most nanophytoplankters from cryptomonads) also points to the organism's functional role as an autotroph or mixotroph. More generally, the size, fluorescing pigment type, and nucleic acid content form functional traits relating to niche adaption and trophic and biogeochemical roles. Photosynthetic pigment fluorescence distinguishes the photoautotrophs (some of which may be mixotrophs) from organisms with other nutritional modes, whilst size is certainly a functional trait in relation to niche and trophic role. The third criterion is the most relevant to management. If the abundance of a particular lifeform, which forms the basis of the indicator, changes in the overall abundance or seasonal cycle of that lifeform and is causally linked to an anthropogenic pressure, implying consequences for ecosystem services, then mitigation of the associated pressure can be taken (Graves et al., 2023). The six lifeforms of tiny plankton that we have distinguished here meet these criteria and should therefore be considered in pelagic biodiversity assessment for policy. However, further research to elicit causal links to pressures or final ecosystem services should be conducted as time-series of tiny plankton are extended.

4.4. Recommendations for how tiny plankton data can be integrated into the current assessment processes for marine ecosystem health

Our examination of the seasonal and interannual patterns of tiny plankton across four UK time-series datasets revealed tiny plankton groups are highly abundant and show consistent pattern of responses to environment drivers with time-scale associations to measurable, anthropogenic pressures. These findings increase our understanding of the dynamics of tiny plankton groups, which we found to be a dominant factor in our understanding of overall plankton biomass and abundance in UK waters. The links to environmental pressures demonstrated through time-series wavelet analysis suggest the potential of tiny plankton lifeforms to provide additional, critical information within the pelagic habitats biodiversity indicators. We therefore recommend the six tiny plankton groups explored here for inclusion in future pelagic habitats biodiversity assessments for policy, such as those required under the MSFD and UKMS (Holland et al., 2023a; McQuatters-Gollop et al., 2019; McQuatters-Gollop et al., 2023).

In addition to using lifeform time-series to understand the effects of pressures on the pelagic environment, lifeforms can also be examined in pairs to reveal further information about changes in different aspects of plankton community functioning such as energy flows, benthic-pelagic coupling, and food web structure (McQuatters-Gollop et al., 2019; Tett et al., 2008). Of the tiny plankton groups assessed within this study, we recommend the relative ratios of *Synechococcus* and picoeukaryotes (as demonstrated by Schmidt et al. (2020)), and of *Synechococcus* and photosynthetic nanoeukaryotes (to demonstrate size structure changes) as candidates for lifeform pairs. Both can inform climate-change consequences to ecosystem services, notably tendency towards oligotrophy with increasing SST and/or reduced vertical mixing.

Our timescale relationship results show complex, potentially co-dependent relationships between dissolved nitrogenous and phosphate-containing nutrients, heterotrophic bacteria, *Synechococcus*, photosynthetic pico- and nano-eukaryotes and heterotrophic nano-eukaryotes, especially in nutrient depleted conditions. The response of these tiny plankton lifeforms is of relevance to management as nitrogen is introduced directly through land run-off or is an indirect consequence of climate change-induced ocean warming and stratification, along with oceanic cycling. Ratios of lifeforms with different trophic traits, such as heterotrophic nanoeukaryotes paired with photosynthetic pico- and nano-eukaryotes, may inform energy transfer balance and ecosystem state on productivity and food webs. Differences in the utilisation of dissolved nitrogen could also be a measure of primary production and food web energy transfer. Elevated abundance of *Synechococcus* would lead to a dominance of nutritionally poor food because they lack in polyunsaturated fatty acids (PUFA) (Schmidt et al., 2020), while other picoplankton, such as Pinguiophytes, do contain PUFA (Sang et al., 2012). The poor nutritional quality of *Synechococcus* could thus impair the efficiency of energy transfer to planktivorous fish (Schmidt et al., 2020) (Fig. 8). We recommend further exploration of the co-dependent relationships between heterotrophic nanoeukaryotes, photosynthetic nanoeukaryotes, HNA-bacteria, and LNA-bacteria and nutrients in other UK sites as data become available.

Considering the differences in response times between tiny plankton and other lifeforms, tiny plankton lifeforms may be paired with existing microplankton lifeforms to understand how sub-annual timescale responses link to larger plankton community dynamics (McQuatters-Gollop et al., 2019; Tett et al., 2008). For example, an ecologically meaningful pair could be large phytoplankton and total picoplankton (photosynthetic picoeukaryotes + *Synechococcus)*, as the former are the optimal size for grazing by copepods while there is little evidence of grazing on the latter (Djeghri et al., 2018). Another potential lifeform pair could be dinoflagellates and total tiny plankton which could offer information about predator/prey relationships and controls over the summer dinoflagellate community.

5. Conclusions

Tiny plankton can make up 71 % of plankton biomass but their small size means that they are under-represented in traditional analyses of time series with light microscopy. We have identified six potentially informative tiny plankton lifeforms to support ecosystem state assessments using a novel wavelet-based coherence method to measure timescale relationships. These tiny lifeforms can be monitored routinely with flow cytometry and show consistent relationships with nutrients and sea surface temperature, both of which are linked to human pressures. Heterotrophic nanoeukaryotes, photosynthetic nanoeukaryotes, photosynthetic picoeukaryotes, *Synechococcus*, and HNA- and LNA-bacteria all show promise as lifeforms to diagnose the changing function of pelagic food webs. We also show the need for long-term monitoring of tiny plankton across multiple sites to establish pressure-state relationships to make robust evidence-based decisions.

CRediT authorship contribution statement

Abigail McQuatters-Gollop: Writing – original draft, Conceptualization. **Rowena F. Stern:** Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization. **Angus Atkinson:** Writing – original draft, Methodology, Formal analysis, Conceptualization. **Mike Best:** Writing – review & editing, Investigation, Funding

acquisition, Conceptualization. **Eileen Bresnan:** Writing – review & editing, Investigation. **Veronique Creach:** Writing – review & editing, Investigation. **Michelle Devlin:** Writing – original draft, Investigation, Conceptualization. **Matthew Holland:** Writing – review & editing, Visualization, Investigation, Formal analysis. **Clare Ostle:** Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Katrin Schmidt:** Writing – review & editing, Investigation. **Lawrence Sheppard:** Writing – review & editing, Formal analysis, Conceptualization. **Glen Tarran:** Writing – review & editing, Investigation. **E. Malcolm S. Woodward:** Investigation, Writing – review & editing. **Paul Tett:** Writing – original draft, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data created during this research is openly available from The University of Plymouth PEARL repository at https://doi.org/10.24382/0xgk-qf56.

Acknowledgements

This project was part-funded by the Department for Environment, Food and Rural Affairs (Defra) as part of the marine arm of the Natural Capital and Ecosystem Assessment (NCEA) programme (NC34 Pelagic program-"PelCap"), and partly by the Defra PICO & PICO2 projects. The marine NCEA programme is leading the way in supporting Government ambition to integrate natural capital approaches into decision making for the marine environment. Find out more at https://www.gov.uk/government/publications/natural-capital-and-ecosystem-assessment-programme . AM-G would like to thank the United Kingdom National Environmental Research Council (UKRI-NERC) for support through the NERC Knowledge Exchange Fellowship Scheme (NE/R002738/1) and Exploring the Frontiers of Environmental Science research grant NE/X012360/1 (AMG, RFS, LS, CO). CPR-WaMS work also received funding from the EU Horizons 2020 project AtlantOS under grant agreement No 633211.The CPR- WaMS and Western channel Observatory station L4 are funded through NERC Climate Linked Atlantic Sector Science (CLASS) grant numbers NE/R015953/1 and NE/R015953/1, respectively. We greatly thank the sampling teams and crews of RV Plymouth Quest, RV MBA Sepia and Brittany Ferries, the CPR team for samples and data for CPR-WaMS, without which this work could not happen. The Scottish Coastal Observatory monitoring programme at Stonehaven is funded by the Scottish Government Annual Delivery Plan line 244.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2024.111650.

References

Addison, P.S., 2017. The illustrated wavelet transform handbook: introductory theory and applications in science, engineering, medicine and finance. CRC Press.

Adl, S.M., Bass, D., Lane, C.E., Lukeš, J., Schoch, C.L., Smirnov, A., Agatha, S., Berney, C., Brown, M.W., Burki, F., 2019. Revisions to the classification, nomenclature, and diversity of eukaryotes. J. Eukaryot. Microbiol. 66, 4–119.

Agawin, N.S., Duarte, C.M., Agustí, S., 2000. Nutrient and temperature control of the contribution of picoplankton to phytoplankton biomass and production. Limnol. Oceanogr. 45, 591–600.

Atkinson, A., Harmer, R.A., Widdicombe, C.E., McEvoy, A.J., Smyth, T.J., Cummings, D. G., Somerfield, P.J., Maud, J.L., McConville, K., 2015. Questioning the role of phenology shifts and trophic mismatching in a planktonic food web. Prog. Oceanogr. 137, 498–512.

Atkinson, A., Lilley, M.K.S., Hirst, A.G., McEvoy, A.J., Tarran, G.A., Widdicombe, C., Fileman, E.S., Woodward, E.M.S., Schmidt, K., Smyth, T.J., Somerfield, P.J., 2021. Increasing nutrient stress reduces the efficiency of energy transfer through planktonic size spectra. Limnol. Oceanogr. 66, 422–437. https://doi.org/10.1002/lno.11613.

Bedford, J., Ostle, C., Johns, D.G., Atkinson, A., Best, M., Bresnan, E., Machairopoulou, M., Graves, C.A., Devlin, M., Milligan, A., Pitois, S., Mellor, A., Tett, P., McQuatters-Gollop, A., 2020. Lifeform indicators reveal large-scale shifts in plankton across the North-West European shelf. Glob. Chang. Biol. 26, 3482–3497. https://doi.org/10.1111/gcb.15066.

Bolaños, L.M., Karp-Boss, L., Choi, C.J., Worden, A.Z., Graff, J.R., Haëntjens, N., Chase, A.P., Della Penna, A., Gaube, P., Morison, F., 2020. Small phytoplankton dominate western North Atlantic biomass. ISME J. 14, 1663–1674.

Booth, B., 1988. Size classes and major taxonomic groups of phytoplankton at two locations in the subarctic Pacific Ocean in May and August, 1984. Mar. Biol. 97, 275–286.

Borja, Á., Elliott, M., Carstensen, J., Heiskanen, A.-S., van de Bund, W., 2010. Marine management – Towards an integrated implementation of the European Marine Strategy Framework and the Water Framework Directives. Mar. Pollut. Bull. 60, 2175–2186. https://doi.org/10.1016/j.marpolbul.2010.09.026.

Buck, K., Chavez, F., Campbell, L., 1996. Basin-wide distributions of living carbon components and the inverted trophic pyramid of the central gyre of the North Atlantic Ocean, summer 1993. Aquat. Microb. Ecol. 10, 283–298.

Capuzzo, E., Lynam, C.P., Barry, J., Stephens, D., Forster, R.M., Greenwood, N., McQuatters-Gollop, A., Silva, T., van Leeuwen, S.M., Engelhard, G.H., 2018. A decline in primary production in the North Sea over 25 years, associated with reductions in zooplankton abundance and fish stock recruitment. Glob. Chang. Biol. 24, e352–e364.

Cavalier-Smith, T., 2018. Kingdom Chromista and its eight phyla: a new synthesis emphasising periplastid protein targeting, cytoskeletal and periplastid evolution, and ancient divergences. Protoplasma 255, 297–357.

Chavez, F.P., Messié, M., Pennington, J.T., 2011. Marine Primary Production in Relation to Climate Variability and Change. Ann. Rev. Mar. Sci. 3, 227–260. https://doi.org/10.1146/annurev.marine.010908.163917.

Clark, D.R., Rees, A.P., Ferrera, C.M., Al-Moosawi, L., Somerfield, P.J., Harris, C., Quartly, G.D., Goult, S., Tarran, G., Lessin, G., 2022. Nitrite regeneration in the oligotrophic Atlantic Ocean. Biogeosciences 19, 1355–1376.

De Vargas, C., Audic, S., Henry, N., Decelle, J., Mahé, F., Logares, R., Lara, E., Berney, C., Le Bescot, N., Probert, I., 2015. Eukaryotic plankton diversity in the sunlit ocean. Science 348, 1261605.

Defriez, E.J., Sheppard, L.W., Reid, P.C., Reuman, D.C., 2016. Climate change-related regime shifts have altered spatial synchrony of plankton dynamics in the North Sea. Glob. Chang. Biol. 22, 2069–2080.

Devlin, M., Best, M., Coates, D., Bresnan, E., O'Boyle, S., Park, R., Silke, J., Cusack, C., Skeats, J., 2007. Establishing boundary classes for the classification of UK marine waters using phytoplankton communities. Mar. Pollut. Bull. 55, 91–103.

Devlin, M., Barry, J., Painting, S., Best, M., 2009. Extending the phytoplankton tool kit for the UK Water Framework Directive: indicators of phytoplankton community structure. Hydrobiologia 633, 151–168.

Djeghri, N., Atkinson, A., Fileman, E.S., Harmer, R.A., Widdicombe, C.E., McEvoy, A.J., Cornwell, L., Mayor, D.J., 2018. High prey-predator size ratios and unselective feeding in copepods: a seasonal comparison of five species with contrasting feeding modes. Prog. Oceanogr. 165, 63–74.

El Hag, A., Fogg, G., 1986. The distribution of coccoid blue-green algae (Cyanobacteria) in the Menai Straits and the Irish Sea. Br. Phycol. J. 21, 45–54.

Falkowski, P.G., Raven, J.A., 2007. Aquatic photosynthesis. Princeton University Press, Princeton, USA.

Fenchel, T., 1988. Marine plankton food chains. Annu. Rev. Ecol. Syst. 19, 19–38. https://doi.org/10.1146/annurev.es.19.110188.000315.

Ferreira, J.G., Andersen, J.H., Borja, A., Bricker, S.B., Camp, J., Cardoso da Silva, M., Garcés, E., Heiskanen, A.-S., Humborg, C., Ignatiades, L., Lancelot, C., Menesguen, A., Tett, P., Hoepffner, N., Claussen, U., 2011. Overview of eutrophication indicators to assess environmental status within the European Marine Strategy Framework Directive. Estuar. Coast. Shelf Sci. 93, 117–131. https://doi.org/10.1016/j.ecss.2011.03.014.

Flombaum, P., Gallegos, J.L., Gordillo, R.A., Rincón, J., Zabala, L.L., Jiao, N., Karl, D.M., Li, W.K., Lomas, M.W., Veneziano, D., 2013. Present and future global distributions of the marine Cyanobacteria Prochlorococcus and Synechococcus. Proc. Natl. Acad. Sci. 110, 9824–9829.

Flombaum, P., Wang, W.-L., Primeau, F.W., Martiny, A.C., 2020. Global picophytoplankton niche partitioning predicts overall positive response to ocean warming. Nat. Geosci. 13, 116–120.

González-García, C., Agustí, S., Aiken, J., Bertrand, A., Farias, G.B., Bode, A., Carré, C., Gonçalves-Araujo, R., Harbour, D.S., Huete-Ortega, M., 2023. Basin-scale variability in phytoplankton size-abundance spectra across the Atlantic Ocean. Prog. Oceanogr. 217, 103104.

Graves, C.A., Best, M., Atkinson, A., Bear, B., Bresnan, E., Holland, M., Johns, D.G., Machairopoulou, M., McQuatters-Gollop, A., Mellor, A., Ostle, C., Paxman, K., Pitois, S., Tett, P., Devlin, M., 2023. At what scale should we assess the health of pelagic habitats? Trade-offs between small-scale manageable pressures and the need for regional upscaling. Ecol. Ind. 154, 110571 https://doi.org/10.1016/j.ecolind.2023.110571.

Harris, R., 2010. The L4 time-series: the first 20 years. J. Plankton Res. 32, 577–583.

Hartmann, M., Grob, C., Tarran, G.A., Martin, A.P., Burkill, P.H., Scanlan, D.J., Zubkov, M.V., 2012. Mixotrophic basis of Atlantic oligotrophic ecosystems. Proc. Natl. Acad. Sci. 109, 5756–5760.

Heywood, J., Zubkov, M., Tarran, G., Holligan, P., 2006. Inter-annual stability of prokaryoplankton standing stocks in oligotrophic gyre and equatorial provinces of the Atlantic Ocean. Deep-Sea Res. II 53, 405.
Hirakawa, Y., Howe, A., James, E.R., Keeling, P.J., 2011. Morphological diversity between culture strains of a chlorarachniophyte, Lotharella Globosa. Plos One 6, e23193.
Holland, M., Louchart, A., Artigas, L.F., McQuatters-Gollop, A., 2023a. PH1/FW5 Changes in phytoplankton and zooplankton communities, in: OSPAR Commission (Ed.), The 2023 Quality Status Report for the Northeast Atlantic. OSPAR, London.
Holland, M.M., Louchart, A., Artigas, L.F., Ostle, C., Atkinson, A., Rombouts, I., Graves, C.A., Devlin, M., Heyden, B., Machairopoulou, M., Bresnan, E., Schilder, J., Jakobsen, H.H., Lloyd-Hartley, H., Tett, P., Best, M., Goberville, E., McQuatters-Gollop, A., 2023b. Major declines in NE Atlantic plankton contrast with more stable populations in the rapidly warming North Sea. Sci. Total Environ. 898, 165505 https://doi.org/10.1016/j.scitotenv.2023.165505.
Hu, C., Chen, X., Yu, L., Xu, D., Jiao, N., 2020. Elevated Contribution of Low Nucleic Acid Prokaryotes and Viral Lysis to the Prokaryotic Community Along the Nutrient Gradient From an Estuary to Open Ocean Transect. Front. Microbiol. 11, 612053 https://doi.org/10.3389/fmicb.2020.612053.
Hu, W., Zheng, N., Zhang, Y., Bartlam, M., Wang, Y., 2023. Spatiotemporal dynamics of high and low nucleic acid-content bacterial communities in Chinese coastal seawater: assembly process, co-occurrence relationship and the ecological functions. Front. Microbiol., 14.
Hu, W., Zhang, H., Lin, X., Liu, R., Bartlam, M., Wang, Y., 2022. Characteristics, Biodiversity, and Cultivation Strategy of Low Nucleic Acid Content Bacteria. Front. Microbiol. 13, 900669.
Hutchins, D.A., Capone, D.G., 2022. The marine nitrogen cycle: new developments and global change. Nat. Rev. Microbiol. 20, 401–414.
Jaspers, C., Hopcroft, R.R., Kiørboe, T., Lombard, F., López-Urrutia, Á., Everett, J.D., Richardson, A.J., 2023. Gelatinous larvacean zooplankton can enhance trophic transfer and carbon sequestration. Trends Ecol. Evol.
Kang, Y., Kang, C.-K., 2023. Coupling and decoupling of marine stramenopiles and cyanobacteria in eutrophic coastal waters of Korea. Sci. Total Environ. 893, 164927.
Kirkham, A.R., Jardillier, L.E., Holland, R., Zubkov, M.V., Scanlan, D.J., 2011. Analysis of photosynthetic picoeukaryote community structure along an extended Ellett Line transect in the northern North Atlantic reveals a dominance of novel prymnesiophyte and prasinophyte phylotypes. Deep Sea Res. Part I 58, 733–744.
Legendre, L., Rassoulzadegan, F., 1995. Plankton and nutrient dynamics in marine waters. Ophelia 41, 153–172.
Li, W., Dickie, P., 2001. Monitoring phytoplankton, bacterioplankton, and virioplankton in a coastal inlet (Bedford Basin) by flow cytometry. Cytometry: J. Int. Soc. Anal. Cytol. 44, 236–246.
Li, W., Jellett, J., Dickie, P., 1995. DNA distributions in planktonic bacteria stained with TOTO or TO-PRO. Limnol. Oceanogr. 40, 1485–1495.
Longnecker, K., Sherr, B.F., Sherr, E., 2005. Activity and phylogenetic diversity of bacterial cells with high and low nucleic acid content and electron transport system activity in an upwelling ecosystem. Appl. Environ. Microbiol. 71, 7737–7749.
Marañón, E., 2015. Cell size as a key determinant of phytoplankton metabolism and community structure. Ann. Rev. Mar. Sci. 7, 241–264.
Margalef, R., 1978. Life-forms of phytoplankton as survival alternatives in an unstable environment. Oceanol. Acta 1, 493–509.
Mary, I., Heywood, J., Fuchs, B., Amann, R., Tarran, G., Burkill, P., Zubkov, M., 2006. SAR11 dominance among metabolically active low nucleic acid bacterioplankton in surface waters along an Atlantic meridional transect. Aquat. Microb. Ecol. 45, 107–113.
McEvoy, A., Beesley, A., Atkinson, A., 2022. Subset of zooplankton abundance and biomass time series from net hauls at site L4 off Plymouth, UK between 1988–2020. NERC EDS British Oceanographic Data Centre NOC. https://doi.org/10.5285/d7fb6ce3-7bc9-307b-e053-6c86abc0671b.
McQuatters-Gollop, A., Atkinson, A., Aubert, A., Bedford, J., Best, M., Bresnan, E., Cook, K., Devlin, M., Gowen, R., Johns, D.G., Machairopoulou, M., Mellor, A., Ostle, C., Scherer, C., Tett, P., 2019. Plankton lifeforms as a biodiversity indicator for regional-scale assessment of pelagic habitats for policy. Ecol. Ind. 101, 913–925.
McQuatters-Gollop, A., Holland, M., Louchart, A., Artigas, L.F., 2023. Pelagic Habitat Thematic Assessment, in: OSPAR Commission (Ed.), The 2023 Quality Status Report for the Northeast Atlantic. OSPAR, London.
McQuatters-Gollop, A., Guérin, L., Arroyo, N.L., Aubert, A., Artigas, L.F., Bedford, J., Corcoran, E., Dierschke, V., Elliott, S.A.M., Geelhoed, S.C.V., Gilles, A., González-Irusta, J.M., Haelters, J., Johansen, M., Le Loc'h, F., Lynam, C.P., Niquil, N., Meakins, B., Mitchell, I., Padegimas, B., Pesch, R., Preciado, I., Rombouts, I., Safi, G., Schmitt, P., Schückel, U., Serrano, A., Stebbing, P., De la Torriente, A., Vina-Herbon, C., 2022. Assessing the state of marine biodiversity in the Northeast Atlantic. Ecol. Ind. 141, 109148 https://doi.org/10.1016/j.ecolind.2022.109148.
Mehner, T., Lischke, B., Scharnweber, K., Attermeyer, K., Brothers, S., Gaedke, U., Hilt, S., Brucet, S., 2018. Empirical correspondence between trophic transfer efficiency in freshwater food webs and the slope of their size spectra. Ecology 99, 1463–1472.
Menden-Deuer, S., Lessard, E.J., 2000. Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol. Oceanogr. 45, 569–579. https://doi.org/10.4319/lo.2000.45.3.0569.
Morris, R.M., Rappé, M.S., Connon, S.A., Vergin, K.L., Siebold, W.A., Carlson, C.A., Giovannoni, S.J., 2002. SAR11 clade dominates ocean surface bacterioplankton communities. Nature 420, 806–810.
Naselli-Flores, L., Padisák, J., 2022. Ecosystem services provided by marine and freshwater phytoplankton. Hydrobiologia 1–16.
Not, F., Latasa, M., Marie, D., Cariou, T., Vaulot, D., Simon, N., 2004. A single species, Micromonas pusilla (Prasinophyceae), dominates the eukaryotic picoplankton in the Western English Channel. Appl. Environ. Microbiol. 70, 4064–4072.
O'Brien, T.D., Lorenzoni, L., Isensee, K., Valdés, L., 2017. What are Marine Ecological Time Series telling us about the ocean. A status report. IOC Tech. Ser 129, 1–297.
OSPAR, 2017. PH1/FW5: Changes in phytoplankton and zooplankton communities., in: OSPAR (Ed.), OSPAR Intermediate Assessment 2017. OSPAR, London, UK, p. 2.
Ostle, C., Paxman, K., Graves, C.A., Arnold, M., Artigas, L.F., Atkinson, A., Aubert, A., Baptie, M., Bear, B., Bedford, J., Best, M., Bresnan, E., Brittain, R., Broughton, D., Budria, A., Cook, K., Devlin, M., Graham, G., Halliday, N., Hélaouët, P., Johansen, M., Johns, D.G., Lear, D., Machairopoulou, M., McKinney, A., Mellor, A., Milligan, A., Pitois, S., Rombouts, I., Scherer, C., Tett, P., Widdicombe, C., McQuatters-Gollop, A., 2021. The Plankton Lifeform Extraction Tool: a digital tool to increase the discoverability and usability of plankton time-series data. Earth Syst. Sci. Data 13, 5617–5642. https://doi.org/10.5194/essd-13-5617-2021.
Pedrotti, M.L., Mousseau, L., Marro, S., Passafiume, O., Gossaert, M., Labat, J.-P., 2017. Variability of ultraplankton composition and distribution in an oligotrophic coastal ecosystem of the NW Mediterranean Sea derived from a two-year survey at the single cell level. PLoS One 12, e0190121.
Quere, C.L., Harrison, S.P., Colin Prentice, I., Buitenhuis, E.T., Aumont, O., Bopp, L., Claustre, H., Cotrim Da Cunha, L., Geider, R., Giraud, X., 2005. Ecosystem dynamics based on plankton functional types for global ocean biogeochemistry models. Glob. Chang. Biol. 11, 2016–2040.
Quinones, R.A., Platt, T., Rodríguez, J., 2003. Patterns of biomass-size spectra from oligotrophic waters of the Northwest Atlantic. Prog. Oceanogr. 57, 405–427.
Rassoulzadegan, F., Laval-Peuto, M., Sheldon, R., 1988. Partitioning of the food ration of marine ciliates between pico-and nanoplankton. Hydrobiologia 159, 75–88.
Reuman, D.C., Anderson, T.L., Walter, J.A., Zhao, L., Sheppard, L.W., 2021. wsyn: Wavelet Approaches to Studies of Synchrony in Ecology and Other Fields. R Package Version 1, 4. http://CRAN.R-project.org/package=wsyn.
Robinson, C., Poulton, A.J., Holligan, P.M., Baker, A.R., Forster, G., Gist, N., Jickells, T. D., Malin, G., Upstill-Goddard, R., Williams, R.G., 2006. The Atlantic Meridional Transect (AMT) programme: a contextual view 1995–2005. Deep-Sea Res. II Top. Stud. Oceanogr. 53, 1485–1515.
Sanders, R.W., Berninger, U.-G., Lim, E.L., Kemp, P.F., Caron, D.A., 2000. Heterotrophic and mixotrophic nanoplankton predation on picoplankton in the Sargasso Sea and on Georges Bank. Mar. Ecol. Prog. Ser. 192, 103–118.
Sang, M., Wang, M., Liu, J., Zhang, C., Li, A., 2012. Effects of temperature, salinity, light intensity, and pH on the eicosapentaenoic acid production of Pinguiococcus pyrenoidosus. J. Ocean Univ. China 11, 181–186.
Scharlemann, J.P.W., Brock, R.C., Balfour, N., Brown, C., Burgess, N.D., Guth, M.K., Ingram, D.J., Lane, R., Martin, J.G.C., Wicander, S., Kapos, V., 2020. Towards understanding interactions between Sustainable Development Goals: the role of environment–human linkages. Sustain. Sci. 15, 1573–1584. https://doi.org/10.1007/s11625-020-00799-6.
Schmidt, K., Birchill, A.J., Atkinson, A., Brewin, R.J., Clark, J.R., Hickman, A.E., Johns, D.G., Lohan, M.C., Milne, A., Pardo, S., 2020. Increasing picocyanobacteria success in shelf waters contributes to long-term food web degradation. Glob. Chang. Biol. 26, 5574–5587.
Schoenle, A., Živaljić, S., Prausse, D., Voß, J., Jakobsen, K., Arndt, H., 2019. New phagotrophic euglenids from deep sea and surface waters of the Atlantic Ocean (Keelungia nitschei, Petalomonas acorensis, Ploeotia costaversata). Eur. J. Protistol. 69, 102–116.
Schreiber, T., Schmitz, A., 2000. Surrogate time series. Physica D 142, 346–382. https://doi.org/10.1016/S0167-2789(00)00043-9.
Sheppard, L.W., Defriez, E.J., Reid, P.C., Reuman, D.C., 2019. Synchrony is more than its top-down and climatic parts: interacting Moran effects on phytoplankton in British seas. PLoS Comput. Biol. 15, e1006744.
Sheppard, L.W., Reid, P.C., Reuman, D.C., 2017. Rapid surrogate testing of wavelet coherences.
Sieburth, J.M., Smetacek, V., Lenz, J., 1978. Pelagic ecosystem structure: Heterotrophic compartments of the plankton and their relationship to plankton size fractions 1. Limnol. Oceanogr. 23, 1256–1263. https://doi.org/10.4319/lo.1978.23.6.1256.
Sohm, J.A., Ahlgren, N.A., Thomson, Z.J., Williams, C., Moffett, J.W., Saito, M.A., Webb, E.A., Rocap, G., 2016. Co-occurring Synechococcus ecotypes occupy four major oceanic regimes defined by temperature, macronutrients and iron. ISME J. 10, 333–345.
Stern, R.F., Picard, K.T., Hamilton, K.M., Walne, A., Tarran, G.A., Mills, D., McQuatters-Gollop, A., Edwards, M., 2015. Novel lineage patterns from an automated water sampler to probe marine microbial biodiversity with ships of opportunity. Prog. Oceanogr. 137, 409–420.
Stern, R., Picard, K., Clarke, J., Walker, C.E., Martins, C., Marshall, C., Amorim, A., Woodward, E.M.S., Widdicombe, C., Tarran, G., Edwards, M., 2023. Composition and patterns of taxa assemblages in the western channel assessed by 18s sequencing, microscopy and flow cytometry. J. Mar. Sci. Eng. 11, 480.
Tarran, G.A., May, R.S., 2019. Abundance of phytoplankton, heterotrophic nanoflagellates and bacteria through the water column at time series station L4 in the Western English Channel, 2007-2017. British Oceanographic Data Centre - Natural Environment Research Council, UK. UK. doi:10.5285/908f84ec-d20c-7c88-e053-6c86abc08fda.
Tarran, G.A., Bruun, J.T., 2015. Nanoplankton and picoplankton in the Western English Channel: abundance and seasonality from 2007–2013. Prog. Oceanogr. 137, 446–455.
Tarran, G.A., May, R.S., 2020. Plankton abundance measured by flow cytometry from long term time series at Station L4 in the Western English Channel for 2019. British Oceanographic Data Centre - Natural Environment Research Council, UK.

Tarran, G.A., May, R.S., 2021. Abundance of phytoplankton, heterotrophic nanoflagellates and bacteria through the water column at time series station L4 in the Western English Channel for 2020. British Oceanographic Data Centre - Natural Environment Research Council, UK.

Tett, P., Carreira, C., Mills, D., Van Leeuwen, S., Foden, J., Bresnan, E., Gowen, R., 2008. Use of a Phytoplankton Community Index to assess the health of coastal waters. ICES J. Mar. Sci. 65, 1475–1482.

Tett, P., Edwards, A., Grantham, B., Jones, K., Turner, M., 1988. Microplankton dynamics in an enclosed coastal water column in summer. Algae and the Aquatic Environment (Contributions in honour of JWG Lund, FRS). Biopress, Bristol, UK, 339-368.

Tett, P., Gowen, R., Mills, D., Fernandes, T., Gilpin, L., Huxham, M., Kennington, K., Read, P., Service, M., Wilkinson, M., 2007. Defining and detecting undesirable disturbance in the context of marine eutrophication. Mar. Pollut. Bull. 55, 282-297.

Tett, P., McQuatters-Gollop, A., Bresnan, E., Best, M., Sheppard, L., Stern, R., Tarran, G., 2022. Picoplankton & Nanoplankton LifeForms: theoretical basis and assessment of candidate lifeforms.

Thyssen, M., Grégori, G., Créach, V., Lahbib, S., Dugenne, M., Aardema, H.M., Artigas, L.-F., Huang, B., Barani, A., Beaugeard, L., 2022. Interoperable vocabulary for marine microbial flow cytometry. Front. Mar. Sci. 9, 975877.

Ting, C.S., Rocap, G., King, J., Chisholm, S.W., 2002. Cyanobacterial photosynthesis in the oceans: the origins and significance of divergent light-harvesting strategies. Trends Microbiol. 10, 134–142.

Turner, J.T., 2015. Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump. Prog. Oceanogr. 130, 205–248.

Uitz, J., Claustre, H., Gentili, B., Stramski, D., 2010. Phytoplankton class-specific primary production in the world's oceans: Seasonal and interannual variability from satellite observations. Global Biogeochem. Cycles 24.

Utermöhl, H., 1958. Zur vervollkommnung der quantitativen phytoplankton-methodik: Mit 1 Tabelle und 15 abbildungen im Text und auf 1 Tafel. Internationale Vereinigung Für Theoretische Und Angewandte Limnologie: Mitteilungen 9, 1–38.

Vaulot, D., Eikrem, W., Viprey, M., Moreau, H., 2008. The diversity of small eukaryotic phytoplankton (≤3 μm) in marine ecosystems. FEMS Microbiol. Rev. 32, 795–820. https://doi.org/10.1111/j.1574-6976.2008.00121.x.

Vázquez-Domínguez, E., Duarte, C.M., Agustí, S., Jürgens, K., Vaqué, D., Gasol, J.M., 2008. Microbial plankton abundance and heterotrophic activity across the Central Atlantic Ocean. Prog. Oceanogr. 79, 83–94.

Visintini, N., Martiny, A.C., Flombaum, P., 2021. Prochlorococcus, Synechococcus, and picoeukaryotic phytoplankton abundances in the global ocean. Limnol. Oceanogr. Lett. 6, 207–215.

Ward, B.B., Rees, A.P., Somerfield, P.J., Joint, I., 2011. Linking phytoplankton community composition to seasonal changes in f-ratio. ISME J. 5, 1759–1770.

Widdicombe, C.E., Harbour, D., 2021. Phytoplankton taxonomic abundance and biomass time-series at Plymouth Station L4 in the Western English Channel, 1992-2020. NERC EDS British Oceanographic Data Centre NOC. doi:10.5285/c9386b5c-b459-782f-e053-6c86abc0d129. doi:10.5285/c9386b5c-b459-782f-e053-6c86abc0d129.

Widdicombe, C.E., Eloire, D., Harbour, D., Harris, R.P., Somerfield, P.J., 2010. Long-term phytoplankton community dynamics in the Western English Channel. J. Plankton Res. 32, 643–655.

Winder, M., Cloern, J.E., 2010. The annual cycles of phytoplankton biomass. Philos. Trans. R. Soc., B 365, 3215–3226.

Wyatt, T., 2014. Margalef's mandala and phytoplankton bloom strategies. Deep-Sea Res. II Top. Stud. Oceanogr. 101, 32–49.

Yung, C.C., Rey Redondo, E., Sanchez, F., Yau, S., Piganeau, G., 2022. Diversity and Evolution of Mamiellophyceae: Early-Diverging Phytoplanktonic Green Algae Containing Many Cosmopolitan Species. J. Mar. Sci. Eng. 10, 240.

Zarauz, L., Irigoien, X., Fernandes, J.A., 2008. Modelling the influence of abiotic and biotic factors on plankton distribution in the Bay of Biscay, during three consecutive years (2004–06). J. Plankton Res. 30, 857–872.

Zubkov, M.V., Burkill, P.H., 2006. Syringe pumped high speed flow cytometry of oceanic phytoplankton. Cytometry A 69, 1010–1019.

Zubkov, M.V., Sleigh, M.A., Burkill, P.H., 1998. Measurement of bacterivory by protists in open ocean waters. FEMS Microbiol. Ecol. 27, 85–102.

Zubkov, M.V., Tarran, G.A., 2008. High bacterivory by the smallest phytoplankton in the North Atlantic Ocean. Nature 455, 224–226.