Received: 3 December 2022 | Revised: 30 January 2023 | Accepted: 17 February 2023

DOI: 10.1111/jeu.12972

ORIGINAL ARTICLE

The Mixoplankton Database (MDB): Diversity of photo-phago-trophic plankton in form, function, and distribution across the global ocean

Aditee Mitra[1] | **David A. Caron**[2] | **Emile Faure**[3] | **Kevin J. Flynn**[4] | **Suzana Gonçalves Leles**[5] | **Per J. Hansen**[6] | **George B. McManus**[7] | **Fabrice Not**[8] | **Helga do Rosario Gomes**[9] | **Luciana F. Santoferrara**[10] | **Diane K. Stoecker**[11] | **Urban Tillmann**[12]

[1]School of Earth and Environmental Sciences, Cardiff University, Cardiff, UK

[2]Department of Biological Sciences, University of Southern California, Los Angeles, California, USA

[3]CNRS, Ifremer, UMR6197 BEEP, Université Brest, Plouzané, France

[4]Plymouth Marine Laboratory, Plymouth, UK

[5]Department of Marine and Environmental Biology, University of Southern California, Los Angeles, California, USA

[6]Department of Biology, University of Copenhagen, Helsingør, Denmark

[7]Department of Marine Sciences, University of Connecticut, Groton, Connecticut, USA

[8]CNRS, AD2M-UMR7144 Station Biologique de Roscoff, Sorbonne Université, Roscoff, France

[9]Biology and Paleo Environment Division, Lamont Doherty Earth Observatory, Columbia University, Palisades, New York, USA

[10]Department of Biology, Hofstra University, Hempstead, New York, USA

[11]Horn Point Laboratory, University of Maryland Center for Environmental Science, Cambridge, Maryland, USA

[12]Alfred-Wegener-Institut, Helmholtz-Zentrum für Polar- und Meeresforschung, Bremerhaven, Germany

Correspondence
Aditee Mitra, School of Earth and Environmental Sciences, Cardiff University, Main Building, Park Place, Cardiff CF10 3AT, UK.
Email: mitraa2@cardiff.ac.uk

Funding information
Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant/Award Number: Science Without Borders 232845/2014-0; European Regional Development Fund, Grant/Award Number: Sêr Cymru II Living with the Perfect Beast 82372; European Union's Horizon 2020 Research and Innovation Program under the Marie Skłodowska-Curie Grant Agreement, Grant/Award Number: MixITiN 766327; UKRI NERC, Grant/Award Number: NE/W004461/1

Abstract

Protist plankton are major members of open-water marine food webs. Traditionally divided between phototrophic phytoplankton and phagotrophic zooplankton, recent research shows many actually combine phototrophy and phagotrophy in the one cell; these protists are the "mixoplankton." Under the mixoplankton paradigm, "phytoplankton" are incapable of phagotrophy (diatoms being exemplars), while "zooplankton" are incapable of phototrophy. This revision restructures marine food webs, from regional to global levels. Here, we present the first comprehensive database of marine mixoplankton, bringing together extant knowledge of the identity, allometry, physiology, and trophic interactivity of these organisms. This mixoplankton database (MDB) will aid researchers that confront difficulties in characterizing life traits of protist plankton, and it will benefit modelers needing to better appreciate ecology of these organisms with their complex functional and allometric predator–prey interactions. The MDB also identifies knowledge gaps, including the need to better understand, for different mixoplankton functional types, sources of nutrition (use of nitrate, prey types, and nutritional states), and to obtain vital rates (e.g. growth, photosynthesis, ingestion, factors affecting photo' vs. phago' -trophy). It is now possible to revisit and re-classify protistan "phytoplankton" and "zooplankton" in extant databases of plankton life forms so as to clarify their roles in marine ecosystems.

KEYWORDS
Allometry, Biogeography, Metagenomics, Microzooplankton, Mixoplankton, Mixotroph, Phytoplankton, Protist, Symbiosis, Traits, Zooplankton

INTRODUCTION

A new paradigm for marine plankton ecology

Understanding mechanisms that drive life in the single largest ecosystem of our planet, the ocean, remains a pivotal research theme in natural sciences. About half of Earth's carbon fixation and oxygen production have been attributed to the activities of microscopic marine plankton (Field et al., 1998), with a disproportionate importance in the coastal zones that are affected, and exploited, most by human activity (Ducklow et al., 2022). Traditional interpretations of marine plankton ecology mirror the plant–animal dichotomy of terrestrial ecology, with the microbial planktonic communities considered as occupying separate food web niches, such as phototrophic primary producers (phytoplankton), heterotrophic primary consumers (zooplankton) and remineralizers (bacterioplankton). In such a food web, single-celled phytoplankton produce food that is consumed by single-celled zooplankton and the smaller metazoan zooplankton, which are in turn consumed by larger zooplankton, and on up through to higher trophic levels (Mitra et al. 2014). The dichotomic division between phytoplankton and zooplankton has formed the bedrock of marine ecology and biological oceanography for over a century. Over the last decade, however, there has been an increasing awareness that protist plankton engaging in various forms of mixotrophy (the coupling of autotrophy and heterotrophy) involving photoautotrophy plus phago-heterotrophy comprise important, and in some cases dominant, sub-groups of marine plankton communities (Flynn et al., 2013).

Mixotrophy in protist plankton invariably involves phototrophy, but the heterotrophic component may be supported by osmotrophy (the use of dissolved organics) and/or by phagotrophy (a generic term used to describe the ingestion of particulate organic matter). Mixotrophy in the plankton is far from being new as a research topic in marine ecology. However, the use of the term "mixotrophy" is not so common, perhaps because much of the earlier work on phototrophic plankton (microalgae) considered the heterotrophic component to provide nutrients such as N and P (such as dissolved free amino and nucleic acids; Antia et al., 1981; Flynn & Butler, 1986; Zubkov et al., 2003) rather than supplying C (Coe et al., 2016; Lewin & Hellebust, 1970). The latter is consistent with the traditional definition of "mixotrophy," for the supply of energy and C (Lawrence, 2011), while current day uses of the term take a wider view to include contributions of nutrients in addition to C (Raven et al., 2009; Selosse et al., 2017).

Given how wide ranging are the results of studies of osmotrophy in microalgae, from prokaryotic phytoplankton (Yelton et al., 2016) to eukaryotic flagellates (Burkholder et al., 2008) and diatoms (Meyer et al., 2022), we may assume with almost certainty that all these organisms are capable of photo-osmo-mixotrophy. What is not clear, however, is whether this osmotrophy to acquire organics (excluding the acquisition of vitamins—Droop, 1968, 2007; Tang et al., 2010) presents a significant net gain to these organisms, or if it represents primarily a mechanism to recover metabolites that inevitably leak from these microbes (Flynn & Berry, 1999). It has long been held that prokaryotes are more likely the main exploiters of dissolved organics in the ocean (Ferrer-González et al., 2021; Keil & Kirchman, 1993; Wheeler & Kirchman, 1986).

As photo-osmo-mixotrophy is likely ubiquitous, a generalized physiological trait of "mixotrophy" cannot provide a clear discriminatory functional characteristic. In contrast, mixotrophy that involves phagotrophy is certainly not ubiquitous and the means by which this physiology is exploited also differs greatly across the protist plankton (Mitra et al., 2016). Not only does photo-phago-mixotrophy provide clear discriminatory power between organisms, but it also has clear consequences for the functioning of the food web. Mixoplankton directly affect trophic dynamics by being able to consume other organisms, be those competitors or even their own predators. The growth of mixoplankton restructure food webs by consuming other organisms, but it also produces different waste streams (e.g. remnants from partial digestion of prey) that will stimulate microbial loop activities (Azam et al., 1983; Jiao & Azam, 2011; Mitra & Flynn, 2010). The activity of non-phagotrophic mixotrophs (e.g. cyanobacteria, diatoms) is quite different; their growth does not directly lead to the death of other organisms, while their consumption of dissolved organics brings them into competition with bacteria and other osmotrophs.

Photo-phago-trophy has traditionally been considered to be of relatively minor importance in ecology, although reports of organisms capable of such activity date from the early 20th century (e.g. Pringsheim, 1958; Schiller, 1933). As research progressed, it was also noted that the forms of phototrophy and phagotrophy vary between organisms (Stoecker et al., 2009). Now, even species traditionally assumed as exemplar "phytoplankton" (e.g. *Emiliania huxleyi*, Avrahami & Frada, 2020; *Phaeocystis globosa*, Koppelle et al., 2022) are recorded as capable of phagotrophy, while over a third of species traditionally labeled as

"microzooplankton" have been found to be capable of engaging in acquired phototrophy (e.g. *Laboea strobila*, Stoecker et al., 2009; various species of Foraminifera and Radiolaria, Anderson, 1983; Bé et al., 1977; Gast & Caron, 1996; Michaels, 1988). Prey ingestion is also more complicated than is often appreciated; it may entail mechanisms other than, or in addition to, phagocytosis (Jeong et al., 2005; Tillmann, 1998), which is used as a generic term to describe feeding by protists. Feeding may involve, as alternatives to phagocytosis, semi-extracellular phagocytosis (Kamennaya et al., 2018), the use of a peduncle as a feeding straw to extract the contents of a prey cell (Larsen, 1988; Nagai et al., 2008), toxins to lyse prey (Granéli et al., 2012; Tillmann, 2003), and/or mucus traps (Blossom et al., 2017; Larsson et al., 2022). The ecological importance of oceanic plankton deploying such physiologies developed from the 1980s (Sanders & Porter, 1988; Stoecker et al., 1988a, 1988b) and is now widely recognized (Stoecker et al., 2017).

The protist plankton, which traditionally were labeled as either "phytoplankton" or "microzooplankton," thus includes photosynthetic organisms that also eat, and phagotrophic organisms that also photosynthesize; both contribute to primary and secondary production simultaneously in the same cell. To help emphasize the shift in understanding of the categorization of plankton functional types, and equally important also to separate the likely ubiquitous photo-osmo-mixotrophs from organisms that can also engage phagotrophy, Flynn et al. (2019) proposed the use of the term "mixoplankton." Mixoplankton are defined as planktonic protists that engage in photo-autotrophy *plus* phago-heterotrophy; they are also able to engage in osmotrophy. Thus, phytoplankton are non-phagotrophic (e.g. diatoms, which are mixotrophs via photo-osmo-trophy only) and protistan zooplankton are non-phototrophic (e.g. tintinnids).

Mixoplankton types

Mixoplankton comprise a diverse sub-group of protist plankton that can be functionally divided firstly between those with a constitutive (innate) ability to photosynthesize ("constitutive mixoplankton"—CM), and those which need to acquire phototrophic capabilities ("non-constitutive mixoplankton"—NCM; Mitra et al., 2016; Flynn et al., 2019; Figure 1). The NCM can be sub-divided according to how they acquire their phototrophic potential by:

1. stealing plastids from variable prey types (generalists: GNCM; e.g. *Laboea strobila*—McManus & Fuhrman, 1986; Stoecker et al., 1988a; *Strombidinium conicum*—Stoecker et al., 1988b),
2. stealing photosynthetic machinery (including nuclear material) from only specific prey (plastidic specialists: pSNCM; e.g. *Mesodinium rubrum*—Gustafson et al., 2000; Johnson et al., 2016; *Dinophysis acuminata*—Jacobson & Andersen, 1994; Park et al., 2006), or,
3. harboring endosymbionts (endosymbiotic specialists: eSNCM; e.g. green *Noctiluca scintillans*—Subrahmanyan, 1954; Wang et al., 2016; various species belonging to Acantharia, Foraminifera, Radiolaria—Caron et al., 1995; Decelle et al., 2012; Spero & Parker, 1985).

A dichotomous key to these mixoplankton types, according to their mode and sources of phototrophy, is shown in Figure 1A. Figure 1B illustrates the trophic categories of organisms found in the plankton, emphasizing mixoplankton lineages. Saprophytes, including fungi, labyrinthulids, etc., have neither photo- nor phagotrophic capabilities, obtaining nutrition by osmotrophy aided by extracellular digestion of organic matter. Protistan zooplankton exploit phagotrophy (and, also potentially osmotrophy) but are incapable of phototrophy, though it should be noted that some extant strict phagotrophic groups show genetic evidence of having had chloroplasts in the ancient past (Raven et al., 2009). Phytoplankton, as now more rigorously defined (Flynn et al., 2019), lack the ability for phagotrophy. While the lack of phagotrophy usually cannot be proven, accumulating evidence suggests that, of the protist plankton, only diatoms and probably some very small protists (e.g. *Ostreococcus*) are completely incapable of particle ingestion.

The CM are most readily mistaken as phytoplankton, as they can often be grown, (at least for some time) without a need to consume prey, as phototrophs using inorganic nutrients. However, there is great variability among CM species in their ability to grow as phototrophs or heterotrophs, and to shift between those forms of nutrition. Some species of the chrysophyte genus *Ochromonas*, for example, grow well without light when sufficient numbers of bacteria are provided as prey, while others appear to have a requirement for some degree of phototrophy (Lie et al., 2018). In contrast, growth of the harmful bloom-forming haptophyte, *Prymnesium parvum*, is strongly dependent on light, although its ability to attack and kill prey is highly developed (Tillmann, 2003). Long-term culture of CM species as phytoplankton, with no provision of suitable prey as food, may result in the loss of an ability to eat (Blossom & Hansen, 2021).

NCM species may be confused with protistan zooplankton, the presence of their phototrophic pigmentation being misidentified as that from prey held within digestive vacuoles. However, unlike such zooplankton, NCM typically cannot be grown solely heterotrophically in total darkness; in contrast, some phytoplankton can be grown in darkness via osmo-heterotrophy (Villanova & Spetea, 2021). While in CM the phototrophic organelles (plastids) are tightly integrated with cellular metabolic and reproductive cycles, the NCM species need

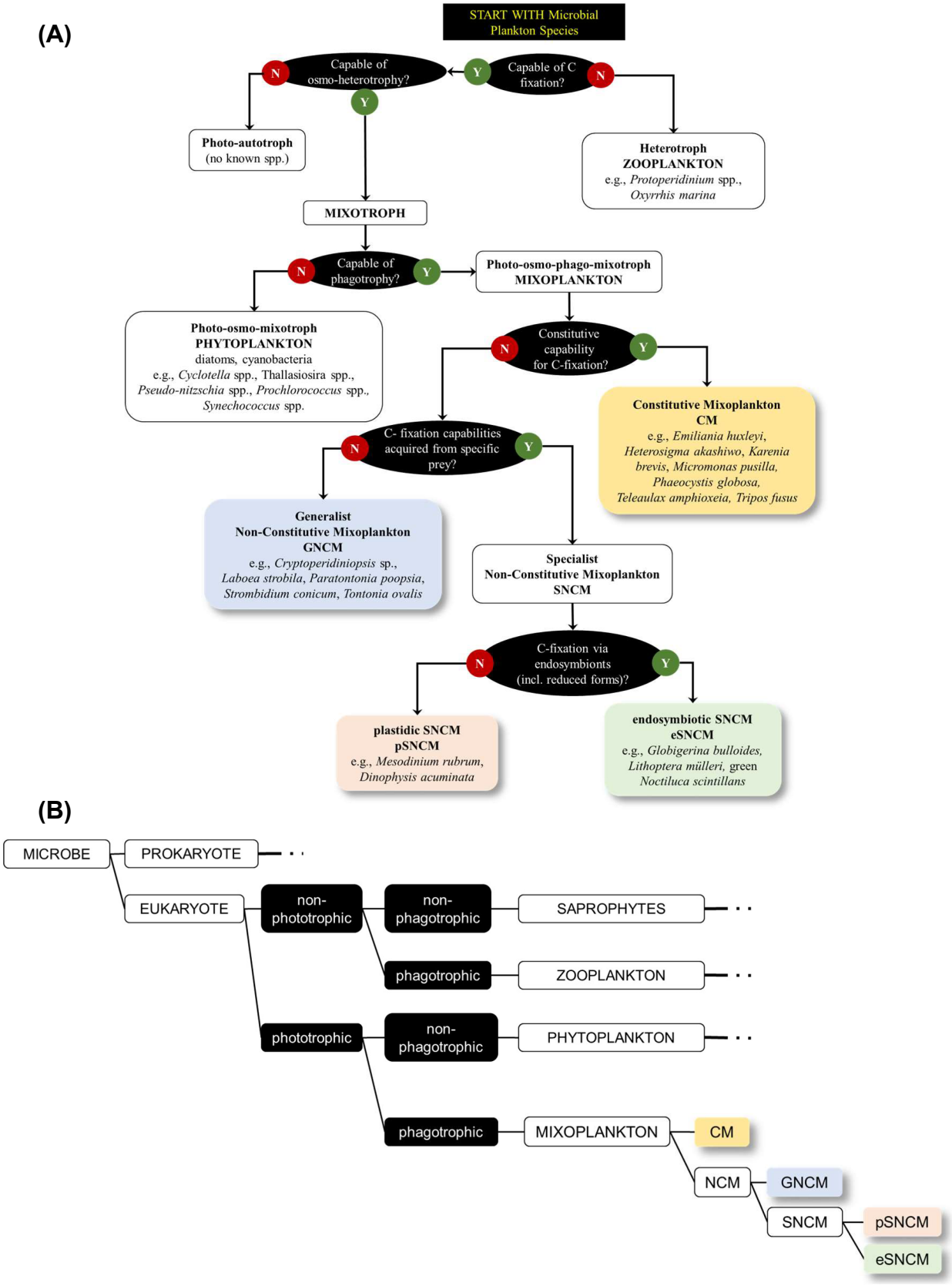

FIGURE 1 Functional group classification for marine microbial plankton. Panel (A): classification key; modified from Mitra and Flynn (2021). Panel (B): Marine microbial plankton traits tree leading to mixoplankton; dash-dotted lines indicate other tree branches not detailed here.

to re-acquire phototrophy periodically via symbionts or plastids retained from ingested prey. GNCM species, need very frequent (1–3 days) re-acquisitions as they are incapable of maintaining their acquired photosystems in a viable state for long (Stoecker et al., 2009), being unable to divide ingested chloroplasts. In contrast, some of the pSNCM species have significant control of the ingested chloroplasts, as seen in species of *Mesodinium* and *Dinophysis*, with their abilities to retain and/or horizontally transfer essential genes involved in photosynthesis, from prey to mixoplankton host genome (Stoecker et al., 2017; Wisecaver & Hackett, 2010). SNCM species are thus less dependent on the frequency of acquisition of phototrophy compared to GNCM, such that the temporal and perhaps even the spatial co-occurrence of the SNCM species and their source of acquired phototrophy may not be necessary; such a potential mismatch can have important implications for ecology of these mixoplankton (Anschütz et al., 2022).

The need for the Mixoplankton Database (MDB)

The recognition that oceanic primary producers cannot correctly be analogized as "miniature plants" and their primary consumers as "miniature animals", but includes the mixoplankton, has led to a paradigm shift in the understanding of marine ecology (Flynn et al., 2019; Glibert & Mitra, 2022; Mitra et al., 2016). While there are various databases which contain members from the microbial plankton community, none of these consider facets of protist plankton such as their ecophysiology and/or trophic interactions (including predator–prey dynamics with protists as prey or predators). For example: AlgaeBase focusses on microalgae and thus does not consider protists with acquired phototrophy; IOC-UNESCO HAB database focuses on harmful algal bloom (HAB) species but not on ecosystem disruptive bloom (EDAB; Sunda et al., 2006) species (such as green *Noctiluca*); the WoRMs database attributes, at best, functional type characteristics to species but like the others mentioned above provides none of the trophic linkages required to support mixoplankton science.

This work explicitly concerns "mixoplankton," rather than mixotrophic plankton in general (see above; Figure 1A), and presents the first comprehensive database for mixoplankton (MDB; Mitra et al., 2023). The MDB is required for marine research because of the increasing understanding of the importance of mixoplankton. This is especially to clarify which protist plankton species are where and what they are doing. In moving from a paradigm of phytoplankton+zooplankton, to phytoplankton+mixoplankton+zooplankton (Glibert & Mitra, 2022) we have not just added a new functional group, but we have to redefine the original groups and realign their life-form designations that form the core underpinning of marine science. Datasets used to support modeling efforts will also need to be reappraised in this context, and the models themselves will require significant attention to reflect the biogeochemical and ecological consequences of including "mixoplankton" (Ghyoot et al., 2017; Leles et al., 2018, 2021).

To support such developments, the MDB brings together not just a list of species which have been evidenced as mixoplankton, but also collates information on the sizes and types of the mixoplankton species as well as the types of and sizes of their prey, including (as applicable) similar data about organisms from whence phototrophy is acquired. This database, also for the first time, brings together taxonomic and genetic data on mixoplankton species. The MDB, therefore, provides a platform for future marine plankton research and applications that depend on an understanding of protist plankton and the microbial food web.

METHODS

Building the MDB

The MDB is available as Mitra et al. (2023). The MDB comprises a Microsoft Excel file with the data on one sheet, with a separate sheet explaining the data categories. The data sheet can be manipulated (options selected or deselected) using dropdown menus across multiple descriptors simultaneously.

Identification of mixoplankton species

The genus and species designation of an organism remains the most accurate and widely used identifier, and most reports referencing mixoplankton (and plankton protists in general) identify them that way. Accordingly, for the MDB, data were compiled according to species name as the primary feature. Assembly commenced by building from our previously published datasets, as used for biogeography papers on NCM (Leles et al., 2017) and CM (Faure et al., 2019; Leles et al., 2019). The original definitions of different mixoplankton types by Mitra et al. (2016), modified as per Flynn et al. (2019) to introduce the term "mixoplankton" in order to avoid the inherent ambiguity of using "mixotroph," were used to differentiate between mixoplankton and strict phototrophs or strict phagotrophs (as per Figure 1A).

Only species with clear evidence of mixoplanktonic activity (i.e. with documented phototrophy and phagotrophy) were included in the database. To ensure that we included all currently known mixoplankton species within the MDB, we exploited the expert knowledge of the authors with extensive literature searches in the electronic databases—ISI Web of Science and Elsevier over 3 years starting from June 2019. Our literature reviews were conducted in English, German, and French.

Evidence of mixoplanktonic activity was traced to the original source, reviewing articles published over a 135-year time span (the earliest being, Haeckel, 1887). Additional mixoplankton identifications have been added as new reports emerged during our analysis, with the most recent additions made in October 2022.

A recent dataset, where trophic modes based on Mitra et al. (2016) were assigned to protist plankton species (Schneider et al., 2020), was also interrogated. Schneider et al. (2020) assumed all species within each genus as listed in our original datasets (i.e. Faure et al., 2019; Leles et al., 2017, 2019) to be mixoplankton irrespective of whether, or not, there was evidence of photo-phagotrophy for individual species. On inspection, some of these assumptions do not appear to be based on published evidence and we found some errors in identification of mixoplankton species within this dataset. Because some of the errors in mixoplankton identification by Schneider et al. (2020) appear to have been brought across into the World Register of Marine Species (WoRMS) database, we have not referred to the WoRMS database to verify mixoplankton status of any species; as noted above, we relied solely on primary sources for verification.

The taxonomic grouping of all mixoplankton species within our database has been made in accordance with Adl et al. (2019). The WoRMS database (https://www.marinespecies.org/index.php) was used to provide AphiaID for most species. In instances where a species was not listed in the WoRMS database, we used AlgaeBase (http://www.algaebase.org/) to confirm the validity of the species name. Various species have undergone name changes over the years; we provide synonyms, basonyms, and allied information for widely studied species only. For example, *Alexandrium pacificum* has, in previous publications, been confused with other species from the *Alexandrium tamarense/catenella/fundyense* species complex. Expert knowledge of our team coupled with AlgaeBase was used to resolve synonyms and basonyms of some species.

The recent proliferation of large-scale environmental DNA sequencing projects in marine ecology offers the opportunity to detect the presence of specific organisms in hundreds of samples of the global ocean (Santoferrara et al., 2020; Vernette et al., 2021). To facilitate the detection of mixoplankton species in such datasets, the MDB compiles all marker genes of mixoplankton species available in the Protist Ribosomal Reference database (PR^2, Guillou et al., 2013). The list of mixoplankton taxa included in the MDB was used to retrieve the corresponding reference marker sequences in the PR^2 database by use of the PR^2 R package (code available at https://github.com/MixoHUB/MixoMaps). For each taxon, the MDB gives access to all associated PR^2 and GenBank accession numbers, along with the type of marker (18S rRNA gene from nucleus or 16S rRNA gene from plastids) and references the publications that generated these sequences.

Assignment of mixoplankton functional traits

Each mixoplankton species was classified into one of the four functional types—CM (constitutive mixoplankton), GNCM (generalist non-constitutive mixoplankton), pSNCM (plastidic specialist non-constitutive mixoplankton), or eSNCM (endosymbiotic specialist non-constitutive mixoplankton)—according to the classification key (Mitra et al., 2016; Mitra & Flynn, 2021; Figure 1A). This classification was based on published evidence of the form of mixoplanktonic physiology displayed by that species. Thus, species with innate capability to photosynthesize were categorized as CM only if there is documented evidence of phagotrophy through feeding observations and/or the presence of digestive vacuoles containing ingested material. Acquired phototrophy in species classified as NCM was based on evidence of the presence of functioning photosynthetic apparatus within the cell (i.e. plastids in GNCM and pSNCM, endosymbionts in eSNCM). In most instances, these judgments have been made by microscopy indicating prolonged retention of the chloroplasts or intact cells (i.e. retention times well beyond normal times required for prey digestion), although in a few cases, measurements of photosynthesis have been reported.

The MDB also documents for each species whether there are distinct life forms other than being unicellular planktonic. Within this life-form trait, we consider whether the species is capable of forming colonies, or has "benthic" (e.g. *Mesodinium chamaeleon*, Moestrup et al., 2012; *Phaeocystis globosa*, Peperzak & Gäbler-Schwarz, 2012) or "parasitic" (e.g. *Blastodinium galatheanum*, Skovgaard & Salomonsen, 2009; *Protoodinium chattoni*, Cachon & Cachon, 1977) forms. Those with a "benthic" life form include species with either a part-benthic stage or those that could be primarily benthic but become planktonic (and thence mixoplanktonic) through suspension in the water column. The "parasitic" life form includes species which can become parasitic or can become mixoplanktonic through suspension in the water body. Within the category of life-form traits, we also identify which mixoplankton are documented as HAB species; this was done primarily by interrogating the database on HAB species curated by IOC-UNESCO (https://marinespecies.org/hab/). Most of these species produce secondary metabolites recognized as toxins (Hallegraeff et al., 2021; Reguera et al., 2012). Some other mixoplankton that are not recorded as "HAB" species in the IOC-UNESCO are harmful to ecology (EDAB species), notably the eSNCM green *Noctiluca scintillans*; these are also tagged as "HAB" within the database.

Where possible, the numeric cell size (or its range) for each species was documented; these data were obtained from published literature or estimated by the co-authors from published photographs in the Radiolaria database (https://radiolaria.org/index.php) and Galerie de l'Observatoire Océanologique de Villefranche-sur-Mer

(http://gallery.obs-vlfr.fr/gallery2/main.php). In some instances, cell size was measured by co-authors of this work using plankton cultures to which they have access (e.g. data for *Noctiluca scintillans*). Further, to aid ecological studies (and in the absence of a numeric cell size value), species were categorized according to the traditional standard plankton size categories, namely as—pico: 0.2–2 μm; nano: 2–20 μm; micro: 20–200 μm; meso: 200 μm–20 mm; macro: 20 mm–2 cm. For those species capable of forming colonies, the colony size is described both by explicit dimensions (where available) and size categories.

The capability of mixoplankton to engage in diverse resource acquisition strategies is a critical trait. A major inorganic nutrient driving primary production in marine systems is nitrate (NO_3^-); here we have recorded the ability of mixoplankton species to use nitrate as a nitrogen source. Also in this resource acquisition category, the database includes each mixoplankton species and their mode of feeding. Colloquially termed "phagotrophy," actual feeding methods involve some combination of raptorial feeding, capture using filters or traps, engulfment of prey, prey lysis (with engulfment or osmotrophy of the remnants), and ingestion using a feeding tube; see Introduction.

Identifying sources of prey and acquired phototrophy

Data for the size and taxonomic group of prey ingested by each mixoplankton species are provided within the database. The prey size data include, where available, the size range of prey tested for each mixoplankton species. Additional information about prey sizes is provided for the different ontogenetic stages of the eSNCM Foraminifera within the MDB. As documented for each mixoplankton species, the prey is also classified according to the traditional size categories used in marine plankton science (including also femto, 0.02–0.2 μm, for viruses).

GNCM and pSNCM acquire phototrophic capability through sequestration of plastids from photosynthetic prokaryotes or eukaryotes, while acquired phototrophy in eSNCM is through endosymbiosis. We provide data on the prey sources for acquired phototrophy for those three functional types, including taxonomic grouping and size of the source organisms.

Global occurrence

Data for the global distribution of the mixoplankton species within the database were acquired through interrogation of the Ocean Biogeographic Information System database (OBIS; http://www.iobis.org/) with the most recent data extracted on October 26, 2022. Species names were matched against those in the WoRMS database, which is utilized by OBIS for taxonomic quality control. Geographical coordinates corresponding to the locations where each mixoplankton species was recorded were obtained. Records with possible spatial errors, such as data points located inland, were excluded from the analysis. Geo-referenced occurrence data were retrieved from OBIS using the R package "robis" (Provoost & Bosch, 2021). Global distribution maps were then generated based on mixoplankton functional type (MFT) and size class across the different Longhurst biogeographic provinces (Longhurst, 2007). Grids corresponding to Longhurst's provinces used in the maps were obtained from http://www.marineregions.org/. At least one record was necessary to assume that mixoplankton occurred in any province. The code used to convert OBIS occurrence data to counts and allocated to Longhurst provinces, and also to construct the biogeography maps, is publicly accessible on GitHub (https://github.com/MixoHUB/MixoMaps); this code can be applied to map the distribution of any species present in the OBIS database by Longhurst province.

Marker gene records were retrieved for exemplar mixoplankton taxa through metaPR^2 (metabarcoding Protist Ribosomal Reference database) and the corresponding R package (Vaulot et al., 2022). At the time of the last retrieval made (October 28, 2022) metaPR^2 included 4000 samples from all oceans and depths (surface, euphotic, mesopelagic, bathypelagic, under-ice and bottom), spanning six size fractions (from pico- to meso-). For each considered taxon, the corresponding amplicon sequence variants (ASVs) and number of reads (with a minimum of 100 total reads as the default threshold in metaPR^2) across samples were retrieved. For selected taxa, OBIS and metaPR^2 data were compared; these taxa were selected based on (1) their common use as exemplars in the literature for each MFT, (2) the commonality of their distribution, and (3) their utility to show key similarities or discrepancies between OBIS and metaPR2.

Data analysis

Relationship between MFT and geographic distribution

In order to identify exemplar species within each MFT, we conducted a frequency analysis to determine the most frequently (commonly) recorded species in the OBIS database within each MFT, as well as for each size class within each MFT. We also determined the percentage of occurrence of each mixoplankton species across the Longhurst provinces (% LP) using the following equation

through which we identified those provinces which had at least 10 independent observations:

$$\%LP = \frac{\text{CountIF(LP records} \geq 10)}{\text{total\#LP}} \cdot 100$$

OBIS records versus metaPR^2 records

The MDB offers a unique opportunity to compare the global distribution of different mixoplankton species between the OBIS and the metaPR^2 databases. Here we present a study case where we compare the distribution of selected species across the global ocean. Our goal was to evaluate whether the distributions of species differ when looking at OBIS and metaPR^2 databases to describe the limitations and the strengths associated with these databases. We performed a Non-metric Multi-Dimensional Scaling (NMDS) analysis (Table S1). Our matrix contained the number of records for each species within each biogeographic province from both databases. The NMDS technique ordinates the species based on their dissimilarities so that species with similar distributions will be positioned closer to each other relative to other species. The distance matrix used in the NMDS analysis was calculated using the Jaccard distance after transforming both datasets to presence/absence data; this is because counts between metaPR^2 and OBIS are not comparable. Ordination was performed using the "metaMDS" function in the "vegan" package in R. We also generated individual global maps for selected species based on the geo-referenced data to illustrate the similarities and dissimilarities between the OBIS and the metaPR^2 sampling effort and coverage.

Quality control

The authors, collectively, bring a wide range of skills—taxonomy, genetics, field, laboratory, ecology, modeling—to control the quality of the MDB. As noted above, all data incorporated within the database were curated from original sources. The MDB will be reviewed periodically (at least every 2 years) and updated as science advances; this approach is similar to that employed for updating the classification, nomenclature, and diversity of eukaryotes (Adl et al., 2019).

RESULTS

The following presents an analysis of the current MDB. It should be noted that we have only recorded protists with clear evidence of phototrophic + phagotrophic potential. Over time, we expect the MDB list of species to grow; we consider the challenges in confirming a mixoplanktonic status in "Discussion" section.

Diversity in mixoplankton species and MFTs

The MDB comprises a total of 435 species. Of these, 150 species had been previously identified as mixoplankton in the datasets of Leles et al. (2017, 2019) and Faure et al. (2019). Over 50% of the species listed within the MDB belong to the eSNCM functional group (Figure 2). The CM functional group, which includes species commonly identified as "phytoplankton," is the second most abundant with 36% of the species in the database belonging to this group. The GNCM and pSNCM functional groups each contain 30 species within the database (Figure 2A). Of the total mixoplankton, ~9% of the CM and ~3% of the pSNCM species have been recorded as HAB species within the IOC-UNESCO database (Figure 2A). Analysis of the size class distribution (Figure 2B) within each MFT (Figure 1) showed the greatest range for eSNCM, which span across the nano to macro sizes, though the majority belong to the micro size class. The size range distribution of the reported CM species falls within the nano and micro sizes.

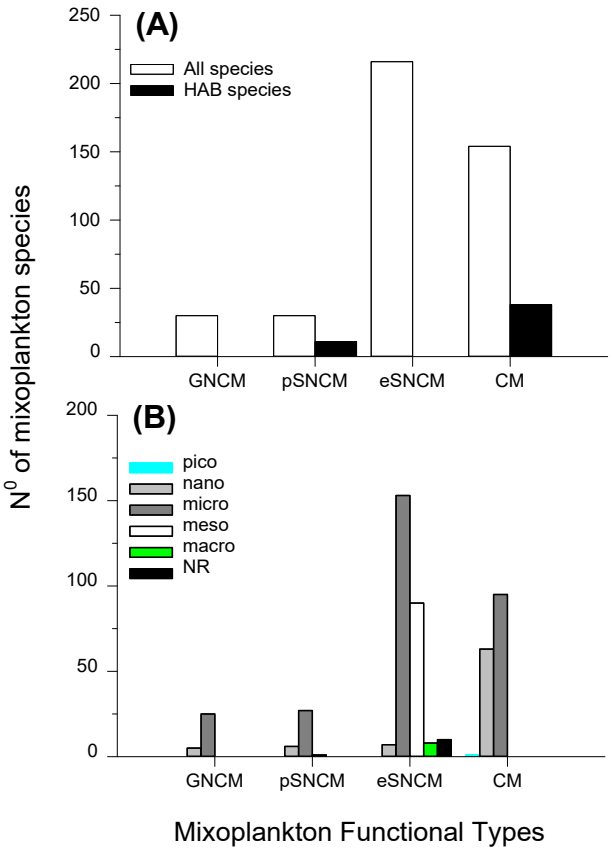

FIGURE 2 Diversity of mixoplankton species. Panel (A): species categorized according to MFT, also showing species recorded in the IOC-UNESCO database as Harmful Algal Bloom species (HAB, https://marinespecies.org/hab/). Panel (B): size class distribution of species within each MFT. NR, size not recorded. See Figure 1 for MFT definitions.

Examination of the taxonomic diversity of the MFTs indicates that the vast bulk of the GNCM was comprised of ciliates (Figure 3), with a minor contribution from dinoflagellates. Of the pSNCM species, ~75% were dinoflagellates, with the balance being ciliates or Foraminifera. Most eSNCM species were Radiolaria, followed by Dinoflagellata and Foraminifera. The CM contained the greatest taxonomic diversity among the mixoplankton types, but dinoflagellates dominated. Mapping the taxonomic diversity of MFTs upon the eukaryotic tree of life indicated that multiple evolutionary events led to the diversification of mixoplankton across protists (Figure 4). Chlorophyta, Cryptophyceae, Haptophyta, Ochrophyta, and Cercozoa include only CM species, with CM as the most widespread MFT in the tree. Rhizaria includes representatives of all MFTs, except GNCM. Alveolata is the most heterogeneous lineage, with Ciliophora including three MFTs (with the prevalence of GNCM species and no CM species) and Dinoflagellata including all four MFTs (with the prevalence of CM species, a strong presence of eSNCM species, and minimum contribution of GNCM species).

Diversity in resource acquisition

The allometric relationships for predator–prey combinations (Figure 5) demonstrate the breadth of the potential food web interactions for mixoplankton. There are many instances of within-size group interactions (i.e. similar sizes of prey and their predators), and also a significant minority of above-size predation. These data reflect the different modes of prey capture exhibited by mixoplankton; the colloquial vision of these organisms feeding through phagocytosis sensu stricto, which requires a significant superiority in mixoplankton size over their prey, gives a false impression. As supplementary figures, we present the data we have collated on feeding mechanisms with respect to the size of the mixoplankton (Figure S1), and to the size of its prey (Figure S2). These evidence great varieties of feeding modes employed across the MFTs. The exception are the GNCM species which, perhaps in keeping within their abilities to exploit varied diet for acquired phototrophy, have been documented to exploit "filter-feeding."

To enable acquired phototrophy, SNCM species require specific prey cells that serve as symbionts (in eSNCM), or exploited for chloroplasts and other organelles (in pSNCM). The allometric relationships between the source of acquired phototrophy and the SNCM (Figure 6A,B) show some instances where the acquisition is made from organisms within the same size group as the SNCM. However, and especially for eSNCM species, most acquisitions are from smaller if not very much smaller organisms (Figure 6B). The acquisitions for pSNCM, in the form of chloroplasts and smaller subcellular components, more readily enable interactions with prey of similar size. The taxonomic sources of those acquisitions into pSNCM cover a narrow range (Figure 6C); 2/3^{rds} of pSNCM species source their phototrophy from cryptophytes, with haptophytes and diatoms comprising the most important other sources. The source of phototrophy for ca. 10% of the pSNCM is unrecorded. The sources of phototrophy (as symbionts) used by different eSNCM cover a wide taxonomic range (Figure 6D), with approaching half of all eSNCM species exploiting dinoflagellates. Cyanobacteria are also important sources, as are haptophytes, but ca. 20% remain unrecorded.

Diversity in occurrence across the global ocean

The global distribution of mixoplankton retrieved from OBIS provides greater resolution according to their functional type and size class (Figure 7). CM species ranging from <10 to 300 µm are ubiquitous across the global ocean. OBIS lacks data for the smallest GNCM (<20 µm) and SNCM (10–20 µm) species; most GNCM species and pSNCM species appear to be constrained to the 20–200 µm size group. NCM species within 20–200 µm are ubiquitous, but OBIS records for GNCM show a more limited global distribution, followed by pSNCM and eSNCM. It is noteworthy that an absence in a given province should not be interpreted as a "true" absence since it might reflect a lack of data held by OBIS. Moreover, large eSNCM have complex life cycles involving the production of minute (<10 µm) juveniles whose distributions are very poorly understood (Anderson, 1980; Hembleben et al., 1988). Therefore, these smaller sizes must be present, albeit

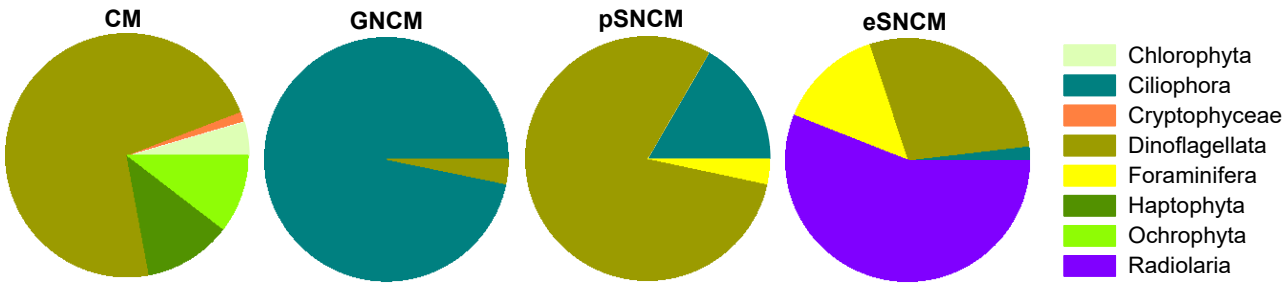

FIGURE 3 Taxonomic diversity within each MFT. See Figure 1 for MFT definitions.

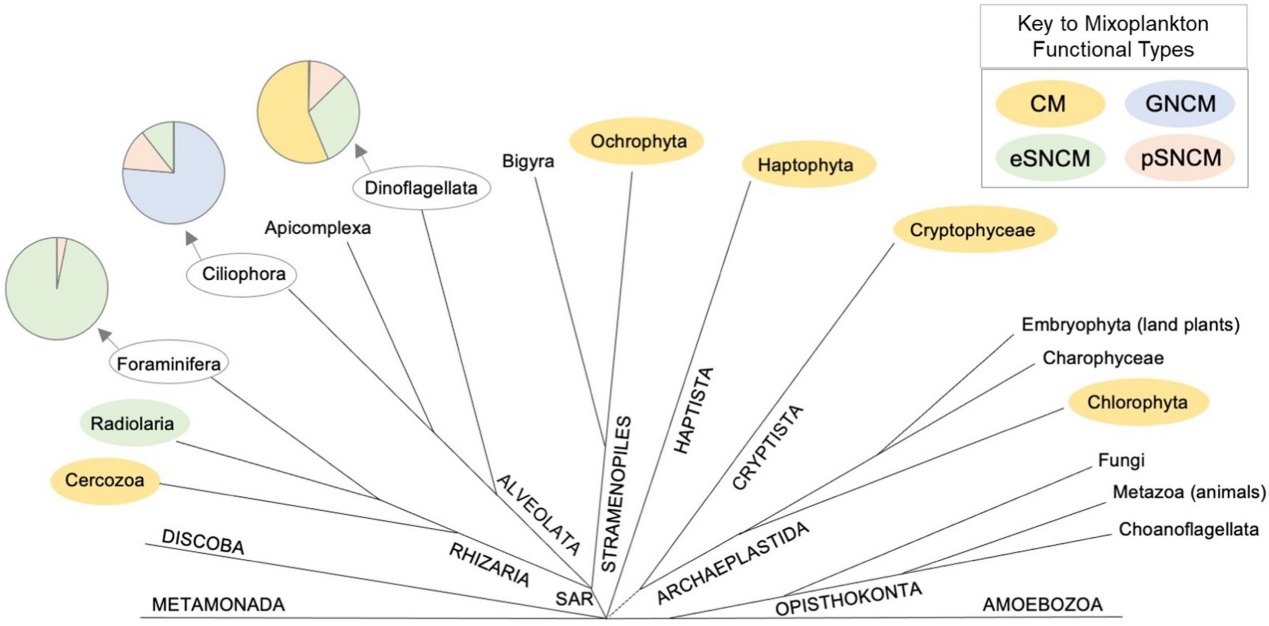

FIGURE 4 Mixoplankton placement within the eukaryotic tree of life. Lineages with representatives of only one functional group are indicated by colored ovals, while lineages with multiple functional types are indicated by inset pie charts. Schematic phylogenic tree adapted from Keeling and Burki (2019); dotted line represents groups with uncertain monophyly. See Figure 1 for MFT definitions.

undocumented, in the same provinces as the larger specimens. The overall most important size category for mixoplankton according to the OBIS records is the 20–200 µm group with representatives across all provinces. While the 20–200 µm size range includes most sampled eSNCM species, this MFT also extends to globally distributed examples of >300 µm.

Table 1 documents the most frequently recorded species in each MFT, while Table 2 further characterizes these MFTs within size categories. For species with extensive ontogenetic development (e.g. many Foraminifera), only the maximum sizes are provided in Table 1. Such species commence as much smaller forms (often as nanoplankton) and progress through to meroplankton; this information is present in the database. Most other protists do not change as much in size during reproduction because binary fission is the dominant form of reproduction. Species of the genus *Tripos* (previously included in *Ceratium*) are the most important frequently recorded mixoplankton (Table 1). These contributors to the larger CM are robust dinoflagellates that more readily survive plankton sampling such as the Continuous Plankton Recorder. It is noteworthy that, despite being so widely encountered, the prey types consumed by *Tripos* spp., other than *T. furca*, are unrecorded (Table 1); their phagotrophic potential is signaled by the presence of digestive vacuoles. As individual species, GNCM appear restricted in their distribution (Table 1), possibly due to cell fragility and/or under-sampling of the corresponding protist groups; even the most common species, the ciliate *Laboea strobila*, is present in only 20% of provinces. However, the GNCM species of *Strombidium* collectively appear quite common. *Mesodinium rubrum* (which can span the nano and micro size classes) and *Dinophysis* spp are the most frequently encountered pSNCM, present in 25%–30% of provinces. *Dinophysis* acquires its phototrophic potential from *Mesodinium* (Park et al., 2006), the latter in turn acquiring plastids from the CM cryptophyte species of the *Teleaulax* and allied clades (Gustafson et al., 2000). Individual species of eSNCM are of wide global distribution, a testament to their ecological success in global oceanic gyres and boundary currents (Table 2).

Data for the 18S rRNA gene marker were available in the PR^2 database for 229 mixoplankton species, comprising 105 CM, 89 eSNCM, 13 GNCM, and 22 pSNCM species (Figure S3). Of these, 32 of the 105 CM species also had available records of plastidic 16S rRNA genes. The species with the most gene records was the eSNCM *Pulleniatina obliquiloculata* (with 274 sequences registered in PR^2), followed by the eSNCM species *Globigerinoides ruber* and *G. elongatus* (248 and 183 entries, respectively). In summary, records of 50% and 41% of the eSNCM species in the MDB were located within both the OBIS and metaPR^2 databases, respectively. The OBIS database recorded the presence of 55% of the CM species, 57% of the GNCM species, and 67% of pSNCM species; the metaPR^2 holds records for 67% of the CM species, 43% of the GNCM species, and 73% of the pSNCM species.

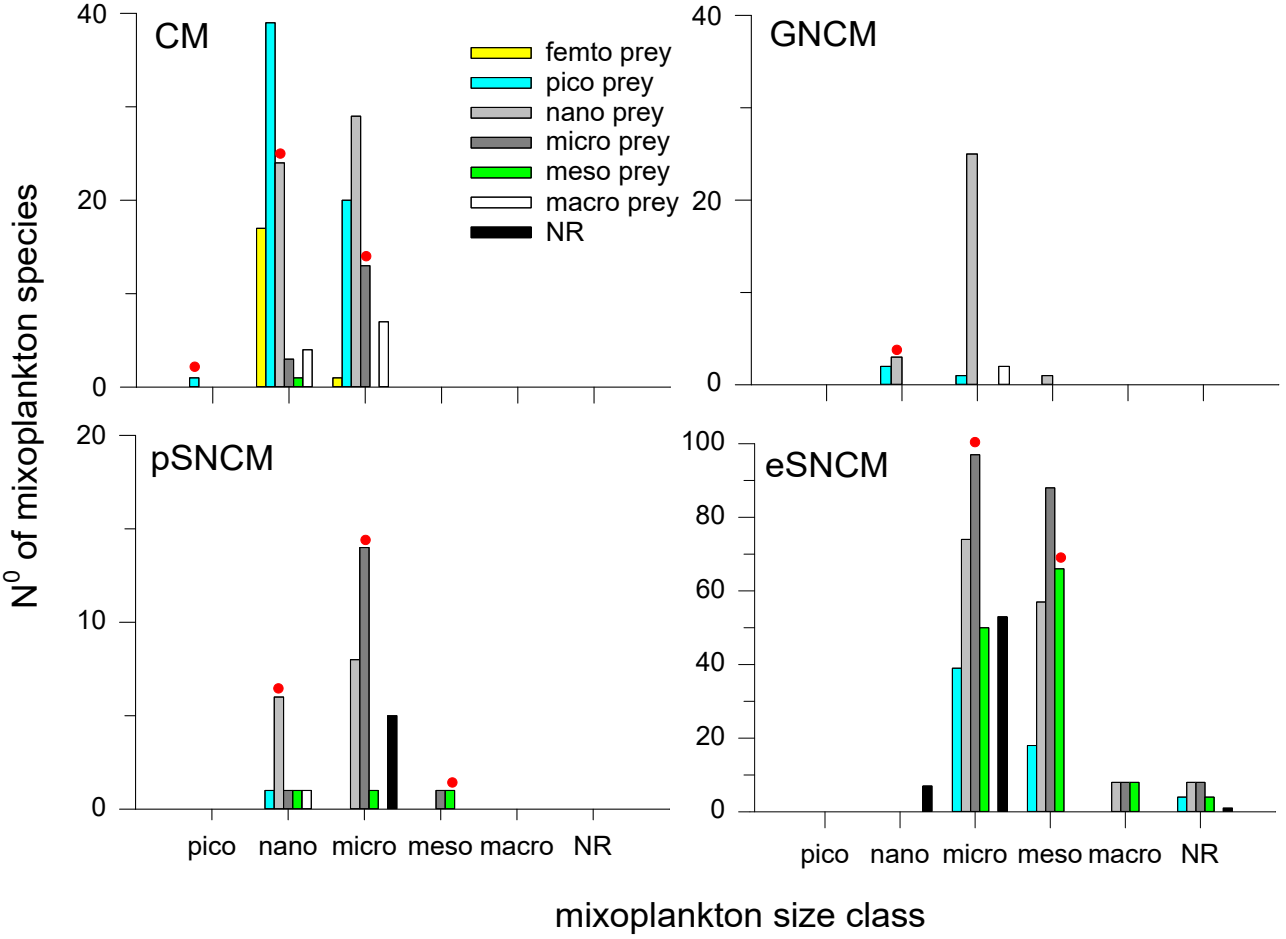

FIGURE 5 Number of mixoplankton species of different size for each functional type, consuming prey of different sizes. X-axes indicate the mixoplankton size class, with bar colors indicating the respective prey size classes. Red dot indicates predation within the same size range. NR, not recorded. See Figure 1 for MFT definitions. Size classes: femto, 0.02–0.2 µm; pico, 0.2–2 µm; nano, 2–20 µm; micro, 20–200 µm; meso, 200–20 mm; macro, 20 mm–2 cm.

Mixoplankton occurrence according to OBIS versus metaPR²

The global distribution of mixoplankton species by Longhurst province differs considerably between OBIS and metaPR² (Figure 8); NMDS shows a clear separation between samples derived from OBIS and metaPR². A closer analysis suggests that this separation occurs independently of size class or mixoplankton type (Figure S4). The sampling effort can partly explain these differences between databases because metaPR² contains a majority of open ocean samples, while OBIS includes also coastal regions.

Global maps comparing the OBIS and metaPR² datasets provide further insight into why the distribution patterns differ; this is shown for selected species in Figures 9 and 10. Among CM species (Figure 9), the cosmopolitan coccolithophorid *Emiliania huxleyi* is found in OBIS but absent from metaPR², while members of the HAB (IOC-UNESCO) database such as *Phaeocystis globosa* and *Chrysochromulina leadbeateri* are better covered by metaPR². The large CM dinoflagellate *Tripos furca* has a similar distribution between databases, probably due to its easier morphological identification and higher probability of being sampled intact due to its larger robust cells (see also Tables 1 and 2).

Fewer data are available for GNCM and pSNCM species compared to CM species but we were able to compare a few key representatives, such as *Mesodinium rubrum*, *Dinophysis acuminata*, *D. acuta*, and *Laboea strobila* (Figure 10A). The clear distinction based on the oceanic regions in which the species were observed (mainly in open seas by metaPR2 and in coastal regions by OBIS) suggests that these two databases can be complementary in the investigation of mixoplankton distribution. Similarly, these databases can provide further information on the biogeography of eSNCM species (Figure 10B) when used side-by-side since OBIS is superior in providing distribution records for Foraminifera (e.g. *Globigerina glutinata* and *Orbulina universa*), while metaPR² is superior in depicting the distribution of Radiolaria (e.g. *Acanthometra pellucida* and *Collozoum inerme*). While Foraminifera have well-defined morphospecies, the presence of gene inserts complicates analysis in gene surveys. In contrast, Acantharia

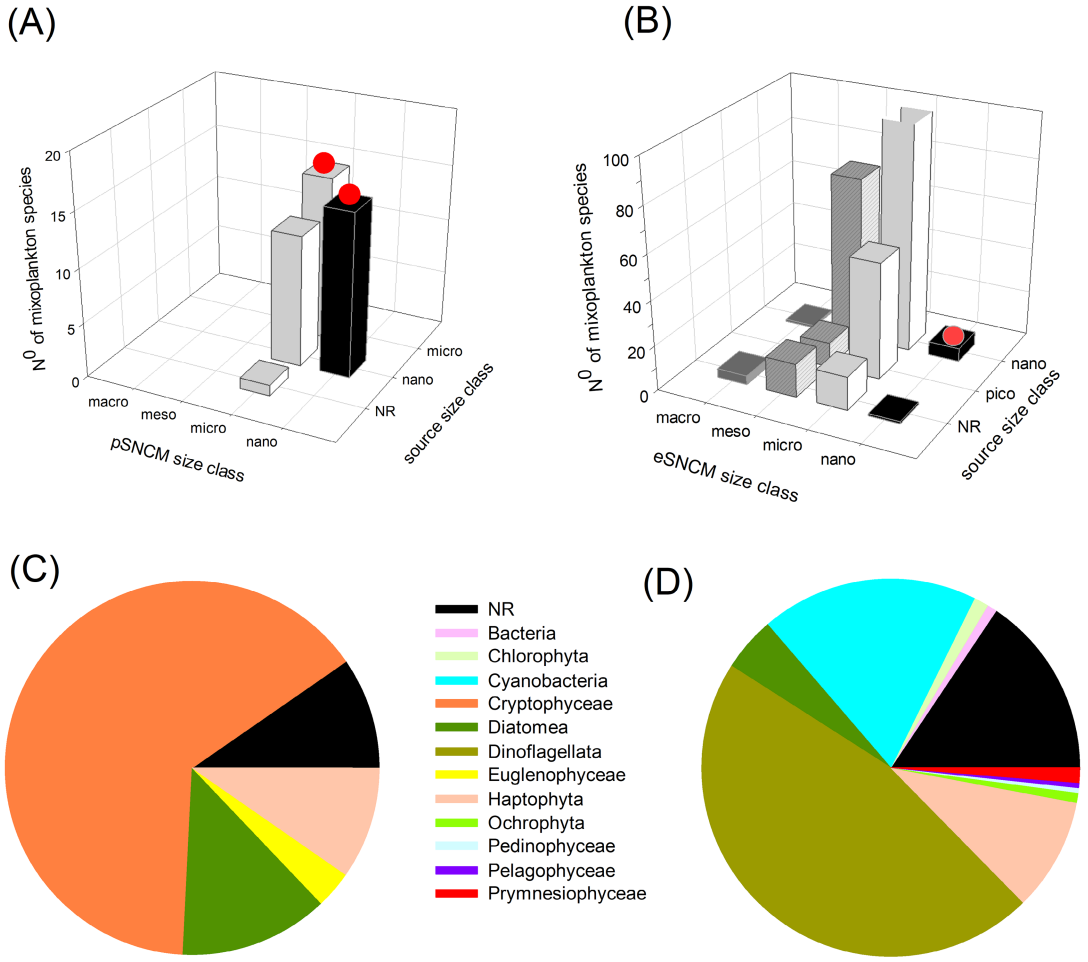

FIGURE 6 Sources of acquired phototrophy for plastidic- and endosymbiotic-specialist non-constitutive mixoplankton (pSNCM and eSNCM, respectively). Panels (A) and (B) show the allometric relationships between the mixoplankton and the source organisms for the acquired phototrophy. Panels (C) and (D) portray the taxonomic groups contributing photosynthetic material to pSNCM and endosymbionts to eSNCM, respectively. NR, not recorded. "Bacteria," purple sulfur bacteria. Size classes: pico, 0.2–2 μm; nano, 2–20 μm; micro, 20–200 μm; meso, 200 μm–20 mm; macro, 20 mm–2 cm. Red dot indicates where phototrophy is acquired from prey within the same size range as the mixoplankton species.

and shell-less Radiolaria are often poorly identified in morphospecies analyses (Acantharia dissolve in most fixatives, while there is no shell in Collozoum to use for morphospecies identification).

DISCUSSION

Updating plankton life-form databases

The MDB provides the first comprehensive, species-specific, collection of data for marine photo-phagotrophic protist plankton. Analysis of this database provides an evaluation of what we know, and equally important what we do not know, about a group of organisms that have hitherto been largely ignored by mainstream marine science (from laboratory, field, and modeling work, through to management). Only the HAB sector has previously recognized the physiological diversity of mixoplankton (Burkholder et al., 2008; Flynn et al., 2018), though even there the vast bulk of the research effort and allied ecosystem management strategies have treated these organisms as phytoplankton, emphasizing the roles of light and inorganic nutrients in their ecology.

Identifying those "phytoplankton" and "zooplankton" that are actually "mixoplankton" is only part of the task going forward. Additionally, it is important that we revise databases of "phytoplankton" and "zooplankton", to either delete those species entries that are actually "mixoplankton", or at the very least to explicitly flag them as "mixoplankton". The former is clearly the more robust route; an individual species, with very few exceptions (most notably "red" vs. "green" *Noctiluca scintillans*; Gomes et al., 2018), cannot properly be a member of two high-level trophic-linked functional categories. Such a development needs then to also be mirrored by updates to catalogs of living forms. Thus, while the diatom database of Leblanc et al. (2012) is unaffected by the mixoplankton paradigm (no known diatoms being phagotrophic), there

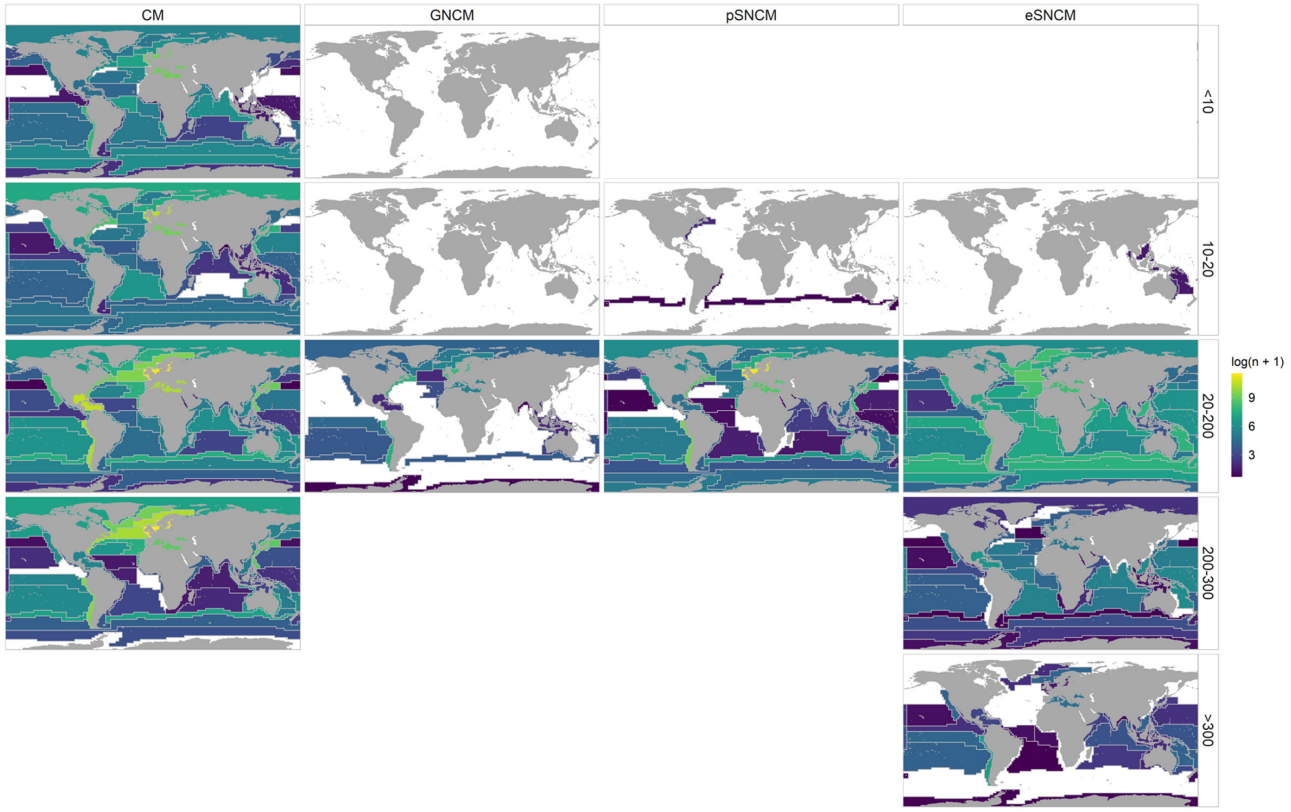

FIGURE 7 Global distribution of mixoplankton across Longhurst's biogeographical provinces. Distribution maps are shown for different MFT across different size classes (*Y*-axes in μm). The color-casts indicate the number of records (as log ($n+1$)) for each combination of MFT and size class; white provinces indicate no data. The absence of maps indicates that there are no known members of MFT of that size class. See Figure 1 for MFT definitions.

are many species within the phytoplankton database of Righetti et al. (2020) that are actually mixoplankton, and which at the least would benefit from being tagged as being mixoplanktonic.

To not recognize mixoplanktonic species as mixoplankton, to continue to label these organisms as something that they are not, has implications not only for science but also for stakeholders such as policy makers and ecosystem managers. For example, neither the OBIS nor metaPR^2 datasets discriminate between the zooplanktonic red *Noctiluca scintillans* and the ecosystem disruptive bloom-forming eSNCM green *N. scintillans* (Figure 11). Green *N. scintillans* is spreading in the Indian Ocean and adjoining provinces with deleterious impact on ecosystem services (Goes & Gomes, 2016). There is, therefore, a clear need to routinely and explicitly differentiate between the red (zooplankton) and green (mixoplankton) forms of this species.

Environmental genomics and detection of mixoplankton

The proliferation of global-scale metabarcoding studies documenting distributions of organisms according to DNA-based analyses, usually 18S rRNA gene sequences (e.g. TARA Oceans, Malaspina, as well as many local and regional-scale surveys; Caracciolo et al., 2022; de Vargas et al., 2015; Gutiérrez-Rodríguez et al., 2022; Hu et al., 2016; Massana et al., 2015), is greatly expanding our knowledge of plankton distributions. This could potentially allow us to quantify the global extent of mixoplankton importance in the ocean's food web. However, when characterizing plankton as clusters of identical or similar sequences (amplicon sequence variants—ASV or operational taxonomic units—OTUs), there is often no direct link available to a known morphological species. Identification to the genus level is often more feasible, but we know that many protist genera contain both mixoplankton and strict heterotrophs (Cf. Schneider et al., 2020 vs. the MDB). For example, the oligotrich ciliate genus *Strombidium* includes several species that have been brought into culture for laboratory studies, of which some are GNCM based on their retention of chloroplasts from ingested prey, some are known to be purely heterotrophic zooplankton, while many remain cryptic (e.g. McManus et al., 2010). Thus, identification to genus would not be enough to validate the presence of a mixoplankton from such a group in a meta-barcode dataset with genus level resolution. In cases where all known members of a genus are mixoplanktonic (e.g. the oligotrich genera *Laboea* and *Tontonia*), this difficulty is

TABLE 1 Ten most frequently recorded species for each MFT in OBIS database; species in bold are recorded as HAB forming mixoplankton in the database.

MFT	Species	Taxonomic group	OBIS records	# LP[a]	% LP[b]	Mixoplankton indicative size	prey indicative size
CM	*Tripos fusus*	Dinoflagellata	118,677	38	70.37	micro	not recorded[c]
CM	*Tripos furca*	Dinoflagellata	85,255	36	66.67	micro	nano-micro
CM	*Tripos muelleri*	Dinoflagellata	77,123	34	62.96	micro	not recorded
CM	***Karenia brevis***	Dinoflagellata	37,289	7	12.96	micro	pico
CM	*Prorocentrum micans*	Dinoflagellata	35,713	29	53.70	micro	nano
CM	*Tripos longipes*	Dinoflagellata	31,017	19	35.19	micro	not recorded[c]
CM	*Heterocapsa rotundata*	Dinoflagellata	23,742	12	22.22	nano	nano
CM	***Prorocentrum cordatum***	Dinoflagellata	22,446	24	44.44	nano	nano
CM	*Emiliania huxleyi*	Haptophyta	20,308	36	66.67	nano	femto-pico
CM	*Scrippsiella acuminata*	Dinoflagellata	12,416	21	38.89	micro	nano
eSNCM	*Globigerina bulloides*	Foraminifera	44,885	50	92.59	micro-meso	micro-meso
eSNCM	*Globigerinoides ruber*	Foraminifera	38,824	42	77.78	micro-meso	micro-meso
eSNCM	*Globigerinita glutinata*	Foraminifera	37,113	45	83.33	micro-meso	micro-meso
eSNCM	*Orbulina universa*	Foraminifera	25,162	44	81.48	micro-meso	micro-meso
eSNCM	*Globigerinoides sacculifer*[d]	Foraminifera	22,098	37	68.52	micro-meso	micro-meso
eSNCM	*Globigerinella siphonifera*[e]	Foraminifera	22,192	40	74.07	micro-meso	micro-meso
eSNCM	***Noctiluca scintillans***[f]	Dinoflagellata	21,089	24	44.44	meso	nano-micro
eSNCM	*Neogloboquadrina dutertrei*	Foraminifera	20,562	44	81.48	micro-meso	micro-meso
eSNCM	*Globigerina falconensis*	Foraminifera	17,024	38	70.37	micro-meso	micro-meso
eSNCM	*Globorotalia menardii*	Foraminifera	15,632	38	70.37	micro-meso	micro-meso
pSNCM	*Mesodinium rubrum*	Ciliophora	46,629	19	35.19	nano-micro	nano
pSNCM	***Dinophysis acuminata***	Dinoflagellata	33,000	23	42.59	micro	micro
pSNCM	***Dinophysis norvegica***	Dinoflagellata	13,674	6	11.11	micro	micro
pSNCM	***Dinophysis caudata***	Dinoflagellata	10,433	18	33.33	micro	micro
pSNCM	***Dinophysis acuta***	Dinoflagellata	7868	14	25.93	micro	micro
pSNCM	***Dinophysis sacculus***	Dinoflagellata	2726	4	7.41	micro	micro
pSNCM	*Amylax triacantha*	Dinoflagellata	2190	7	12.96	micro	micro
pSNCM	***Dinophysis fortii***	Dinoflagellata	1937	13	24.07	micro	micro
pSNCM	*Elphidium*	Foraminifera	1810	18	33.33	micro-meso	micro-meso
pSNCM	***Dinophysis tripos***	Dinoflagellata	1331	10	18.52	micro	micro
GNCM	*Laboea strobila*	Ciliophora	3665	11	20.37	micro	nano
GNCM	*Strombidium conicum*	Ciliophora	2176	9	16.67	micro	nano
GNCM	*Strombidium vestitum*	Ciliophora	599	5	9.26	nano	nano
GNCM	*Strombidium acutum*	Ciliophora	355	3	5.56	micro	nano
GNCM	*Paratontonia gracillima*	Ciliophora	208	3	5.56	micro	pico-nano
GNCM	*Tontonia ovalis*	Ciliophora	194	1	1.85	micro	nano
GNCM	*Pseudotontonia simplicidens*	Ciliophora	89	3	5.56	micro	nano
GNCM	*Strombidium capitatum*	Ciliophora	83	2	3.70	micro	nano
GNCM	*Strombidium reticulatum*	Ciliophora	44	1	1.85	micro	nano
GNCM	*Strombidium dalum*	Ciliophora	39	2	3.70	nano	pico

Note: "OBIS records" indicate total number of observations per species in the OBIS database. Size classes: femto, <0.2 μm; pico, 0.2–2 μm; nano, 2–20 μm; micro, 20–200 μm; meso, 200 μm–20 mm.

[a]Number of Longhurst provinces (LP) where ≥10 observations have been reported for the species.

[b]% Longhurst provinces (LP) of occurrence of the mixoplankton species; total LP = 54.

[c]Prey unknown; mixoplankton activity evidenced through the presence of food vacuoles.

[d]Synonym, *Trilobatus sacculifer*.

[e]Synonym, *Globigerinella aequilateralis*.

[f]OBIS records no distinction between green and red *Noctiluca scintillans* forms.

TABLE 2 Fifteen most frequently observed species in each MFT within each size range in OBIS database; species in bold are recorded as HAB forming mixoplankton in the database.

MFT	mixoplankton indicative size	Species	Taxonomic group	OBIS records	# LP[a]	% LP[b]	prey indicative size
CM	pico	*Micromonas pusilla*	Chlorophyta	1995	3	5.56	pico
CM	nano	*Heterocapsa rotundata*	Dinoflagellata	23,742	12	22.22	nano
CM	nano	***Prorocentrum cordatum***	Dinoflagellata	22,446	24	44.44	nano
CM	nano	*Emiliania huxleyi*	Haptophyta	20,308	36	66.67	femto-pico
CM	nano	***Phaeocystis globosa***	Haptophyta	6501	6	11.11	pico
CM	nano	*Dinobryon faculiferum*	Ochrophyta	5944	5	9.26	pico
CM	nano	*Calcidiscus leptoporus*	Haptophyta	5222	34	62.96	femto-pico
CM	nano	*Dinobryon balticum*	Ochrophyta	4979	7	12.96	pico
CM	nano	***Karlodinium veneficum***	Dinoflagellata	1556	7	12.96	pico/nano
CM	nano	*Cymbomonas tetramitiformis*	Chlorophyta	556	3	5.56	pico
CM	nano	***Amphidinium carterae***	Dinoflagellata	342	6	11.11	nano
CM	nano	***Prymnesium polylepis***	Haptophyta	298	2	3.70	femto-pico
CM	nano	***Prymnesium parvum***	Haptophyta	98	2	3.70	femto-meso
CM	nano	*Chrysochromulina pringsheimii*	Haptophyta	96	2	3.70	femto-nano
CM	nano	*Haptolina ericina*	Haptophyta	81	1	1.85	femto-pico
CM	nano	*Haptolina hirta*	Haptophyta	65	3	5.56	femto-pico
CM	micro	*Tripos fusus*	Dinoflagellata	118,677	38	70.37	not recorded[c]
CM	micro	*Tripos furca*	Dinoflagellata	85,255	36	66.67	nano-micro
CM	micro	*Tripos muelleri*	Dinoflagellata	77,123	34	62.96	not recorded[c]
CM	micro	***Karenia brevis***	Dinoflagellata	37,289	7	12.96	pico
CM	micro	*Prorocentrum micans*	Dinoflagellata	35,713	29	53.70	nano
CM	micro	*Tripos longipes*	Dinoflagellata	31,017	19	35.19	not recorded[c]
CM	micro	*Scrippsiella acuminata*	Dinoflagellata	12,416	21	38.89	nano
CM	micro	***Karenia mikimotoi***	Dinoflagellata	8910	12	22.22	pico-nano
CM	micro	***Lingulodinium polyedra***	Dinoflagellata	8675	17	31.48	nano-micro
CM	micro	***Gonyaulax spinifera***	Dinoflagellata	8081	29	53.70	pico
CM	micro	***Akashiwo sanguinea***	Dinoflagellata	7613	17	31.48	nano-micro
CM	micro	***Protoceratium reticulatum***	Dinoflagellata	5127	22	40.74	not recorded[c]
CM	micro	***Alexandrium catenella***	Dinoflagellata	4269	8	14.81	pico-nano
CM	micro	*Gonyaulax polygramma*	Dinoflagellata	4257	22	40.74	pico-nano
CM	micro	*Tripos lunula*	Dinoflagellata	3642	9	16.67	not recorded[c]
eSNCM	nano	*Durinskia agilis*	Dinoflagellata	343	1	1.85	not recorded
eSNCM	micro	*Globigerina bulloides*	Foraminifera	44,885	50	92.59	micro-meso
eSNCM	micro	*Globigerinoides ruber*	Foraminifera	38,824	42	77.78	micro-meso
eSNCM	micro	*Globigerinita glutinata*	Foraminifera	37,113	45	83.33	micro-meso
eSNCM	micro	*Orbulina universa*	Foraminifera	25,162	44	81.48	micro-meso
eSNCM	micro	*Globigerinoides sacculifer*[d]	Foraminifera	22,098	37	68.52	micro-meso
eSNCM	micro	*Globigerinella siphonifera*[e]	Foraminifera	22,192	40	74.07	micro-meso
eSNCM	micro	*Neogloboquadrina dutertrei*	Foraminifera	20,562	44	81.48	micro-meso
eSNCM	micro	*Globigerina falconensis*	Foraminifera	17,024	38	70.37	micro-meso
eSNCM	micro	*Globorotalia menardii*	Foraminifera	15,632	38	70.37	micro-meso
eSNCM	micro	*Pulleniatina obliquiloculata*	Foraminifera	12,241	37	68.52	micro-meso
eSNCM	micro	*Globigerinoides conglobatus*	Foraminifera	11,560	37	68.52	micro-meso
eSNCM	micro	*Globorotalia hirsute*	Foraminifera	6450	33	61.11	micro-meso
eSNCM	micro	*Globorotalia tumida*	Foraminifera	5545	31	57.41	micro-meso
eSNCM	micro	*Turborotalita humilis*	Foraminifera	3043	28	51.85	micro-meso
eSNCM	micro	*Globoquadrina conglomerata*	Foraminifera	2934	25	46.30	micro-meso

(Continues)

TABLE 2 (Continued)

MFT	mixoplankton indicative size	Species	Taxonomic group	OBIS records	# LP[a]	% LP[b]	prey indicative size
eSNCM	meso	*Globigerina bulloides*	Foraminifera	44,885	50	92.59	micro-meso
eSNCM	meso	*Globigerinoides ruber*	Foraminifera	38,824	42	77.78	micro-meso
eSNCM	meso	*Globigerinita glutinata*	Foraminifera	37,113	45	83.33	micro-meso
eSNCM	meso	*Orbulina universa*	Foraminifera	25,162	44	81.48	micro-meso
eSNCM	meso	*Trilobatus sacculifer*	Foraminifera	22,098	37	68.52	micro-meso
eSNCM	meso	*Globigerinella siphonifera*	Foraminifera	22,192	40	74.07	micro-meso
eSNCM	meso	***Noctiluca scintillans***[f]	Dinoflagellata	21,089	24	44.44	nano-micro
eSNCM	meso	*Neogloboquadrina dutertrei*	Foraminifera	20,562	44	81.48	micro-meso
eSNCM	meso	*Globigerina falconensis*	Foraminifera	17,024	38	70.37	micro-meso
eSNCM	meso	*Globorotalia menardii*	Foraminifera	15,632	38	70.37	micro-meso
eSNCM	meso	*Pulleniatina obliquiloculata*	Foraminifera	12,241	37	68.52	micro-meso
eSNCM	meso	*Globigerinoides conglobatus*	Foraminifera	11,560	37	68.52	micro-meso
eSNCM	meso	*Globorotalia hirsute*	Foraminifera	6450	33	61.11	micro-meso
eSNCM	meso	*Globorotalia tumida*	Foraminifera	5545	31	57.41	micro-meso
eSNCM	meso	*Turborotalita humilis*	Foraminifera	3043	28	51.85	micro-meso
pSNCM	nano	*Mesodinium rubrum*	Ciliophora	46,629	19	35.19	nano
pSNCM	micro	***Dinophysis acuminata***	Dinoflagellata	33,000	23	42.59	micro
pSNCM	micro	***Dinophysis norvegica***	Dinoflagellata	13,674	6	11.11	micro
pSNCM	micro	***Dinophysis caudata***	Dinoflagellata	10,433	18	33.33	micro
pSNCM	micro	***Dinophysis acuta***	Dinoflagellata	7868	14	25.93	micro
pSNCM	micro	***Dinophysis sacculus***	Dinoflagellata	2726	4	7.41	micro
pSNCM	micro	*Amylax triacantha*	Dinoflagellata	2190	7	12.96	micro
pSNCM	micro	***Dinophysis fortii***	Dinoflagellata	1937	13	24.07	micro
pSNCM	micro	*Elphidium*	Foraminifera	1810	18	33.33	micro-meso
pSNCM	micro	***Dinophysis tripos***	Dinoflagellata	1331	10	18.52	micro
pSNCM	micro	*Phalacroma rapa*	Dinoflagellata	689	8	14.81	not recorded
pSNCM	micro	*Kryptoperidinium foliaceum*	Dinoflagellata	630	2	3.70	not recorded
pSNCM	micro	*Phalacroma cuneus*	Dinoflagellata	524	3	5.56	not recorded
pSNCM	micro	***Phalacroma mitra***	Dinoflagellata	229	6	11.11	not recorded
pSNCM	micro	*Phalacroma favus*	Dinoflagellata	74	4	7.41	not recorded
GNCM	nano	*Strombidium vestitum*	Ciliophora	599	5	9.26	nano
GNCM	nano	*Strombidium dalum*	Ciliophora	39	2	3.70	pico
GNCM	micro	*Laboea strobila*	Ciliophora	3665	11	20.37	nano
GNCM	micro	*Strombidium conicum*	Ciliophora	2176	9	16.67	nano
GNCM	micro	*Strombidium acutum*	Ciliophora	355	3	5.56	nano
GNCM	micro	*Paratontonia gracillima*	Ciliophora	208	3	5.56	pico-nano
GNCM	micro	*Tontonia ovalis*	Ciliophora	194	1	1.85	nano
GNCM	micro	*Pseudotontonia simplicidens*	Ciliophora	89	3	5.56	nano
GNCM	micro	*Strombidium capitatum*	Ciliophora	83	2	3.70	nano
GNCM	micro	*Strombidium reticulatum*	Ciliophora	44	1	1.85	nano
GNCM	micro	*Pseudotontonia cornuta*	Ciliophora	24	1	1.85	nano
GNCM	micro	*Strombidium chlorophilum*	Ciliophora	11	1	1.85	nano

Note: In some instances the number of species <15. "OBIS records" indicate the total number of observations per species in the OBIS database. Size class: femto, <0.2 μm; pico, 0.2–2 μm; nano, 2–20 μm; micro, 20–200 μm; meso, 200 μm–20 mm.

[a]Number of Longhurst provinces (LP) where ≥10 observations for the species have been reported in OBIS.

[b]% Longhurst provinces (LP) of occurrence of the mixoplankton species; total LP = 54.

[c]Prey unknown; mixoplankton activity evidenced through the presence of food vacuoles.

[d]Synonym, *Trilobatus sacculifer*.

[e]Synonym, *Globigerinella aequilateralis*.

[f]OBIS records no distinction between green and red *Noctiluca scintillans* forms.

Journal of Eukaryotic Microbiology ISOP Published by the International Society of Protistologists

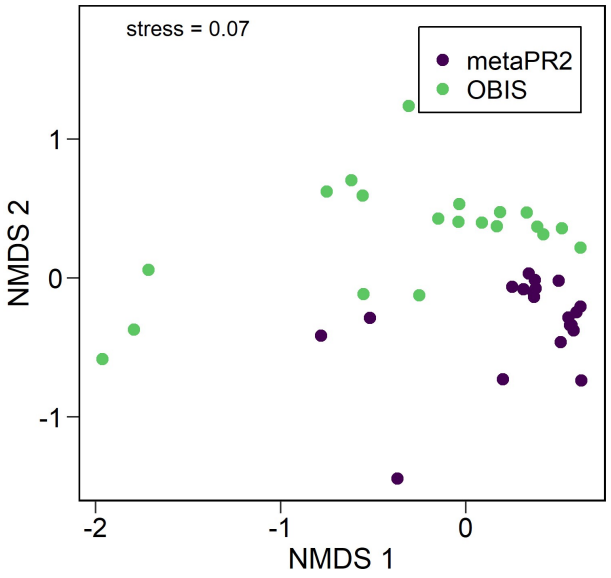

FIGURE 8 Comparison of species distributions between the OBIS and the metaPR^2 databases. NMDS ordination of species was based on the number of occurrences across the Longhurst biogeographic provinces. Symbol color indicates species data derived from metaPR^2 (purple) and OBIS (green) databases. Differences between OBIS and metaPR^2 are observed across size classes and MFT (see also Figure S4).

not as great. Currently there remain various limitations inherent to meta-barcoding; these include quantification biases linked with 18S rRNA gene copy number variations across taxa, amplification biases, and the inability to measure functional expression of genes and/or specific traits unique to mixoplankton (Sandin et al., 2022; Santoferrara, 2019). The emergence of meta-genomics and meta-transcriptomics offers the promise of extending beyond the ribosomal RNA genes to possibly predict mixoplanktonic activity in uncultivated species based on genomic information (Lambert et al., 2022), and to quantify the expression of genes directly related to mixoplankton activity, although this possibility has not yet been fully explored.

The plasticity of photo-phago-mixotrophy as a trophic mode, the diversity of mixoplankton types based on fundamentally different metabolisms (e.g. constitutive vs. non-constitutive or even kleptoplastidic vs. symbiotic), and unresolved evolutionary histories, illustrated by taxa exhibiting multiple types of mixotrophy (photo-osmo vs. photo-osmo-phago) even at the genus level, are all challenging hurdles to overcome for defining effective genomic and transcriptomic markers of diverse mixotrophic activity (e.g. between photo-osmo-mixotrophic phytoplankton vs. photo-osmo-phago-mixotrophic mixoplankton). The presence of prey genetic material within individual mixoplankton provides additional challenges. Lambert et al. (2022) highlighted the potential of large-scale transcriptome comparisons to identify such markers, using a public database with only limited information on organisms' trophic modes and lacking measures of mixotrophic activities in phytoplankton and/or mixoplankton.

Ultimately, the value of meta-omics approaches depends on applications of traditional taxonomy and experimental approaches to confirm phototrophy and phagotrophy, and hence a mixoplanktonic status. To be of use in accessing ecological contributions, rate measurements are essential. Development of molecular techniques to determine vital rates, and especially for application to marine plankton communities, remains far in the future (Strzepek et al., 2022), and will need calibrating against experimental methods. Going forward, it is clear that concomitant measurements will be required of mixoplanktonic activity (relative and absolute rates of growth, photosynthesis, feeding, and respiration) and meta-transcriptomics in order to obtain a genomic "silver bullet" for in situ mixoplankton identification and quantification. Until that time, evidence of mixoplanktonic activity, as we catalogue in the MDB, at least flags the importance of the photo-phago-trophic potential for ecology. Further, as evidence from culture work indicates that a failure to exploit phagotrophy results in the gradual loss of the trait in at least some species (Blossom & Hansen, 2021), we should perhaps assume that a documented ability for a given species to be able to photosynthesize and also to eat most likely indicates that the organism in question indeed exploits those potentials in nature.

Mismatch in most frequently observed mixoplankton species versus exemplar laboratory species

The most well documented mixoplankton species across the global oceans are, inevitably, the more robust and larger forms that are captured and identified in routine surveys, such as the Continuous Plankton Recorder (Leles et al., 2017, 2019). Notably, these include the dinoflagellate *Tripos* sp. (formerly *Ceratium* sp.) and various Foraminifera species (Table 1). In contrast, mixoplankton that are often exploited in laboratory studies are ranked relatively low within the OBIS database according to the frequency of observations. These include the HAB species *Alexandrium catenella* (ranked #43 in the MDB and observed in eight Longhurst Provinces), *Alexandrium minutum* (ranked #50, observed in eight Longhurst Provinces), *Karlodinium veneficum* (ranked #60, observed in seven Longhurst Provinces), and *Prymnesium parvum* (ranked #111, observed in two Longhurst Provinces). The ciliate *Laboea strobila* is the most commonly and widespread recorded GNCM (#41 in the MDB, observed in 11 Longhurst Provinces; Table 1). The non-HAB CM genus, *Ochromonas*, which has been the subject of various experimental studies (e.g. Lie et al., 2018; Sanders & Porter, 1988), appears to be of minor importance when one considers the OBIS and

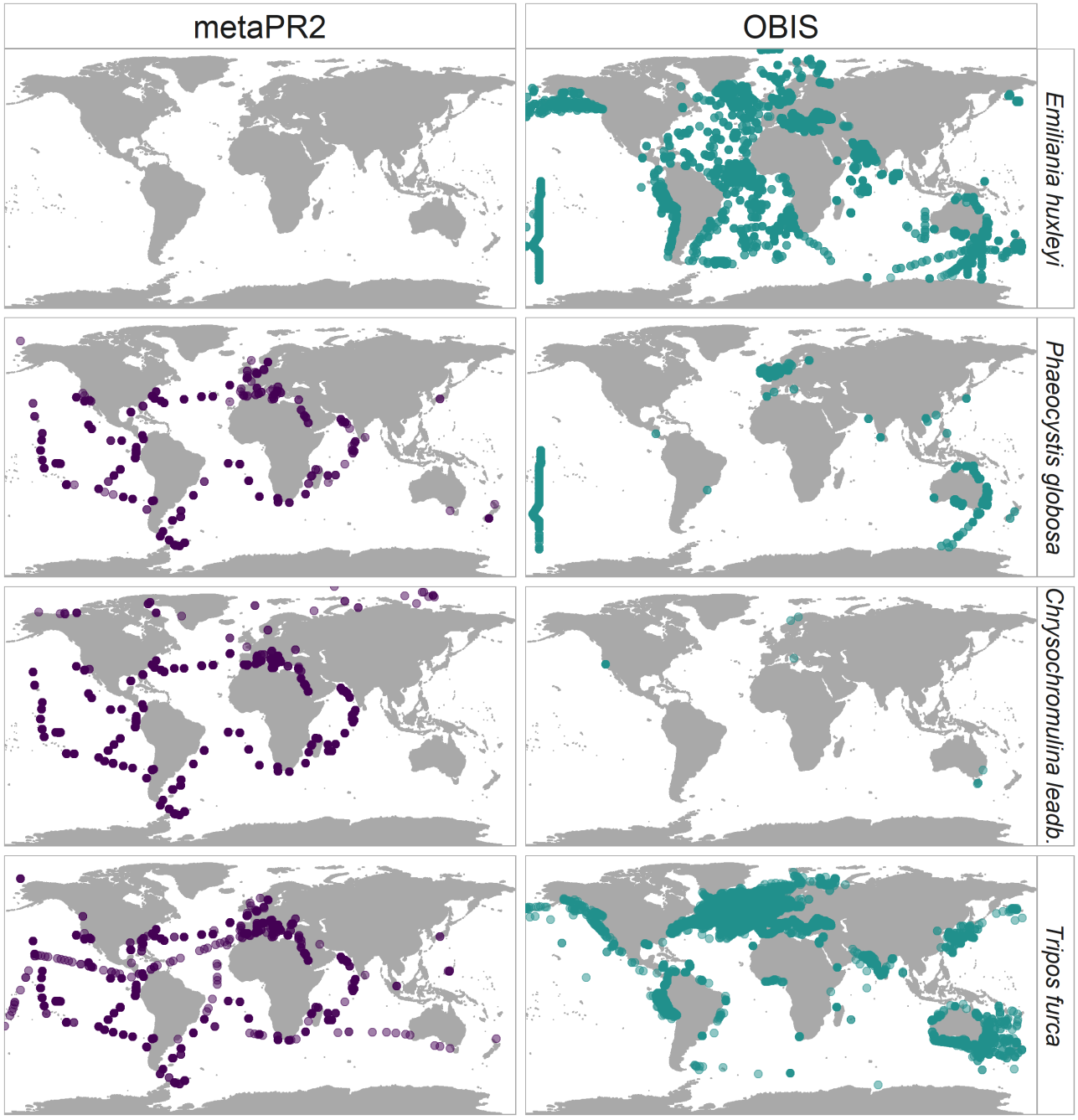

FIGURE 9 Geographic records of example CM species from the metaPR^2 and OBIS databases.

metaPR^2 databases, However, such an absence could be attributed to a range of different reasons, including difficulties in identifying these small organisms to species level. Similar problems likely affect the identification of other CM species which are <5 μm in size, and also delicate species that are easily damaged during sampling. Until routine sampling approaches for surveying and monitoring plankton take better account of the very many delicate and smaller species (including mixoplankton), science will continue to have a very skewed vision of marine plankton biodiversity and of the contribution of mixoplankton to ecology.

Identifying and confirming the mixoplanktonic status of protist plankton requires the observation of phagocytosis and ideally measurement of ingestion rates. Using only the presence of digestive vacuoles is a secondary, imperfect, approach for confirming phagotrophy (e.g. Jacobson & Anderson, 1996). Ingestion rates of significance to the organism may be very low, perhaps only a few events each day (Avrahami & Frada, 2020; Koppelle et al., 2022). In contrast, confirmation of phototrophy can be undertaken easily, most readily using PAM fluorometry. Bringing organisms into culture provides the best way to study their photo-phago-physiology. With

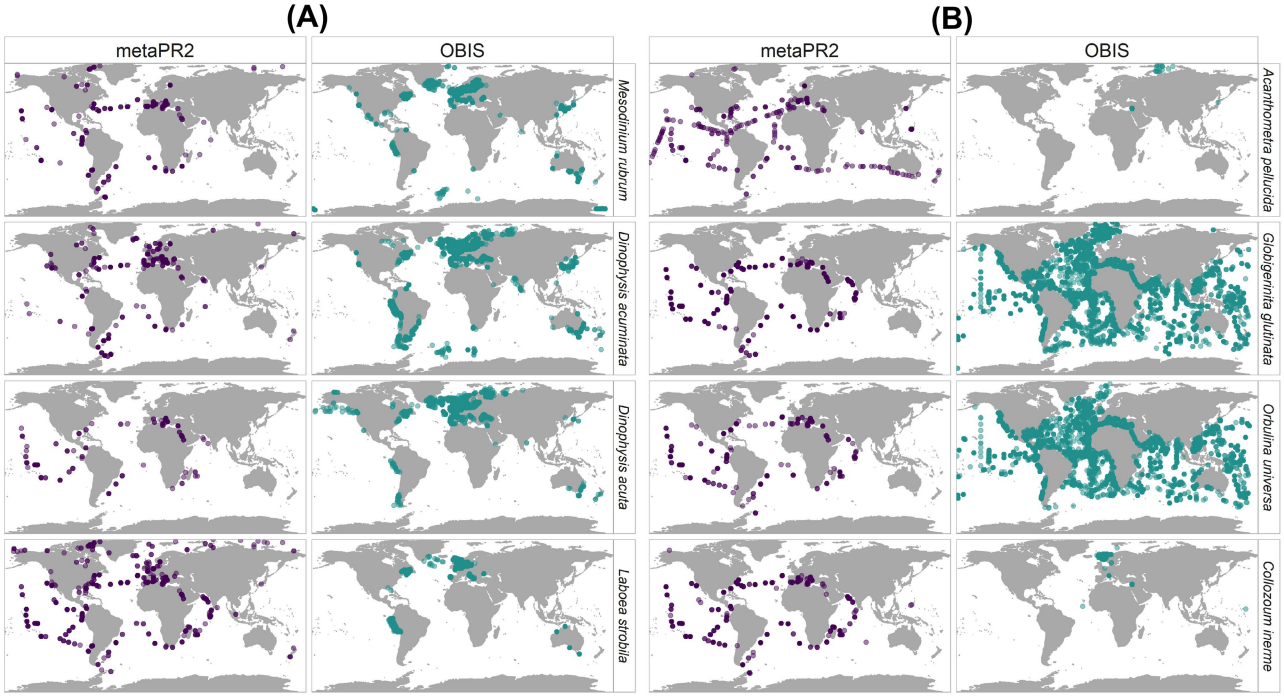

FIGURE 10 Geographic records of selected NCM species from the metaPR^2 and OBIS databases. Panel (A) shows data for selected GNCM (*Laboea strobila*) and pSNCM (*Mesodinium rubrum*, *Dinophysis acuminata*, *D. acuta*) species. Panel (B) shows data for selected eSNCM species.

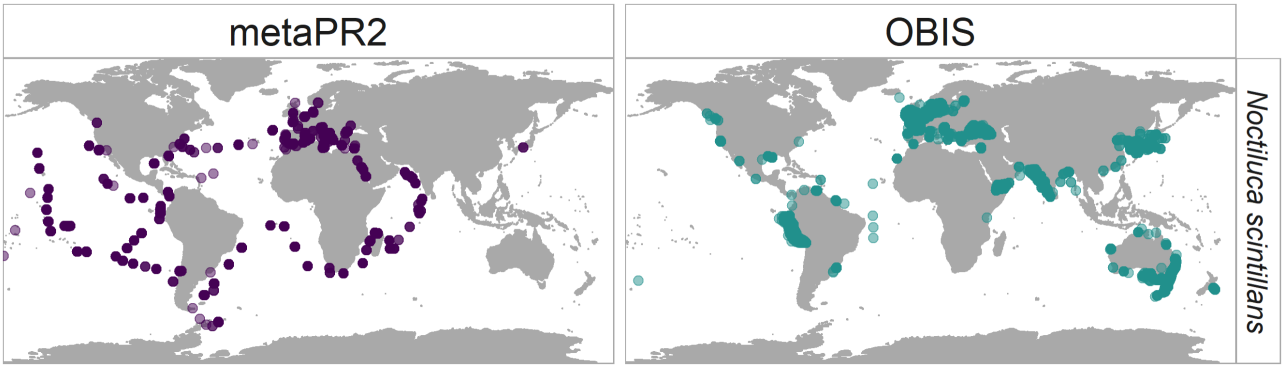

FIGURE 11 Geographic records of the occurrence of *Noctiluca scintillans* from the metaPR^2 and OBIS databases. These databases do not discriminate between the zooplanktonic red *N. scintillans* versus the mixoplanktonic green *N. scintillans*.

old cultures, we need more and better coverage in reference sequence data for an evaluation of which, if any of these species (i.e. those maintained from time before sequencing was standard procedure) are of "ecological significance." Ideally, we need new, fresh, isolates with which to work. However, in general mixoplankton are not easy to grow in culture, contributing to our lack of understanding of their ecophysiology. Indeed, most species are not robust to the interventions required to bring them into culture (Hansen et al., 2021), and there is evidence that phagotrophy may be lost if cultures are maintained as phytoplankton, with no addition of prey (Blossom & Hansen, 2021). Supplying prey, aside from adding to the logistic challenges in culturing and then supplying what is perhaps not the ideal natural prey species, can also be problematic. It is all too common for the prey species in nutrient-rich culture media to take over the culture, while those mixoplankton that produce traps (Larsson et al., 2022) can rapidly foul their cultures with discarded rotting traps containing decaying prey.

(Dis)continuums in mixoplanktonic activity

Mixoplankton comprise a highly diverse group of organisms with respect to their taxonomy (Figure 4), their broad physiological functionalities linked to the sources of their phototrophic potential (Figure 1) and

use of nitrate, feeding mechanisms (Figures S1 and S2 that impact the allometric relationships with their prey (Figures 5 and 6), and also the balance of their expressed levels of phototrophy versus phagotrophy (Adolf et al., 2006; Anschütz & Flynn, 2020; Jones, 1997; Millette et al., 2017; Mitra & Flynn, 2010; Stoecker, 1998). Taking just the aspect of feeding, across the database we see a wide range of mechanisms enabling prey acquisition by immobilization, killing, lysing or otherwise capturing prey using trichocysts (Li et al., 1999), haptonema (Kawachi et al., 1991), mucocysts or other "sticky" surfaces (Gowing, 1989; Jeong et al., 2010; Sugiyama et al., 2008), mucus traps (Blossom et al., 2017; Larsson et al., 2022), toxins (Granéli et al., 2012; Tillmann, 2003), and raptorial capture (Riisgård & Larsen, 2009). Mechanisms for ingestion include engulfment (Jeong et al., 2005; Tillmann, 1998), semi-extracellular phagocytosis (Kamennaya et al., 2018), use of a peduncle (akin to a feeding straw inserted into the prey to suck out material; Larsen, 1988; Nagai et al., 2008), and also osmotrophy to acquire materials leaked by lysed prey. The availability of data on such matters, factors that affect the generality or specificity of mixoplankton–prey interactions, is limited. Research is required to better provide information on who-is-eating-whom to enhance the MDB with the information that is key for the reconstruction of food web dynamics. Such information will also help us better gauge how the success of these organisms will play out with climate change.

The diversity of mixoplankton physiologies, which greatly exceeds that of the phytoplankton, makes the inclusion of these organisms in models particularly challenging (Anschütz et al., 2022; Leles et al., 2021). An approach to simplify the situation is to identify general biological "rules," such as an allometric relationship for phototrophy in small protists versus phagotrophy in large protists. From analysis of the MDB, we find no robust evidence for such a trait relationship; diversities in size, predator–prey allometry, mode of feeding within each MFT and also across the mixoplankton in totality, are too great to allow such a simple rule to be of value. Interpretations of such proposed rules for mixoplankton are also much complicated by the different physiological roles that phagotrophy and phototrophy may have for each species in a given environment. For example, it is apparent that many CM may feed primarily to acquire nutrients (N, P, Fe) rather than C, with the relative importance of phototrophy versus phagotrophy being most likely a function of resource availability (including light) in their respective environments (Jones, 1997, 2000; Stoecker, 1998) rather than according to a size spectrum. Except for strongly phototrophic forms such as the pSNCM *Mesodinium*, which in many ways could be considered analogous to a CM or phytoplankton (Crawford, 1989; Johnson, 2011), a significant role for mixotrophy, sensu stricto as a means to derive C and energy (Lawrence, 2011), is clearest in NCM.

It is apparent from the variability in the types and detail of data available for each species in the MDB, that science is missing much quantitative data on both phagotrophy and also the interactions between photo- and phago-trophies under different conditions of light, temperature, and resource availability (inorganic nutrients, prey quality, and quantity). The complexity of interactions, and the challenges for meaningful modeling of them, are apparent for those few instances where they have been studied (e.g. Lin et al., 2018; Lundgren et al., 2016). The very many gaps in the MDB is indicative of the research effort needed before science can more comprehensively appreciate the ecophysiology of these organisms and thence their ecological significance. This is all the more pressing given the involvement of climate change in re-shaping plankton communities (López Urrutia & Morán, 2015; Schmidt et al., 2020) and the representation of mixoplankton as HAB species (Burkholder et al., 2008; Mitra & Flynn, 2021).

Future directions

Although mixoplankton represent ancient lineages and have been known to science for over a century, only over the last decade or so has their importance started to be registered within mainstream marine science. The recently convened SCOR group on mixotrophs (SCOR Working Group 165; https://scor-int.org/group/mixotrophy-in-the-oceans-novel-experimental-designs-and-tools-for-a-new-trophic-paradigm-mixonet/) bears witness to this recognition. Given the time that it took other paradigm-shifting developments in marine science to establish a foothold in mainstream research and thence into teaching (notably the microbial loop and viral shunt, and the allied microbial carbon pump concept—Azam et al., 1983; Suttle, 2007; Jiao & Azam, 2011), it will take perhaps another decade for "mixoplankton" to become part of day-to-day plankton language. The creation of the MDB is a pivotal part of this journey, mirrored as it will need to be by the consequential re-appraisal of the content of phytoplankton and zooplankton databases and life-form registers.

Future developments will see a gradual expansion of the MDB through the addition of other species, and critically also the procurement of data to fill the very many gaps present in the current iteration of the database. It is important that the database is expanded, and the authors welcome additional information in this regard. Some of the most important aspects include data for potential growth rate and temperature optima. Information on photosynthetic and feeding parameters is also needed to contextualize the role of mixoplankton in biogeochemistry and ecology. Much of the focus of future work will find its way into models, and eventually into digital twins of planktonic ecosystems (Flynn et al., 2022) for use by scientists and ecosystem managers. Models inevitably require simplifications; an

extension of the types of information in Figures 2 and 4 would help greatly in this regard.

An important outcome of building the MDB has been an enhanced appreciation of the variation in physiological form-and-function within genera. We cannot assume that all species within a single genus are capable of mixoplanktonic activity just because one of those species has been evidenced to be a mixoplankton. This requires a rather painstaking effort to determine "who can do what, and to whom." The skills required to conduct such investigations are not common, and at least for the foreseeable future, they cannot be replaced by exploiting "omics" (Strzepek et al., 2022). The gaps in the MDB thus also flag the need for a new generation of plankton microbial ecophysiologists and new technological developments.

AUTHOR CONTRIBUTIONS

The original concept of this paper arose from discussions between AM, DKS, FN, and GBM following a special session on mixoplankton at the International Society of Protistology (ISOP) conference in Rome (2019). For construction of the MDB, taxonomic groups were reviewed as follows—Cercozoa, Chlorophyta, Cryptophyceae, Dinoflagellata, Haptophyta, Ochrophyta: UT, PJH, and KJF; Ciliophora: DKS, GBM, and LS; Foraminifera: DAC and AM; Radiolaria: AM, FN, and KJF; HdRG provided data for green *Noctiluca scintillans*. Literature reviews in German were undertaken by UT and in French by FN. IOC-UNESCO HABs list interrogated by AM. WoRMS database interrogation for AphiaID and cross-referencing to AlgaeBase by AM and SGL. Radiolaria database interrogation by AM and KJF; Galerie de l'Observatoire Océanologique de Villefranche-sur-Mer was interrogated by FN. R-scripts were developed by SGL. Data from OBIS were extracted and compiled by SGL. Genetic data were extracted and compiled by EF and LS. Data analysis was conducted by AM, SGL, EF, and LS. Visualization by AM, SGL, and LS. Writing (original draft, reviewing, and editing) of the text was undertaken by AM, KJF, GBM, EF, SGL, and LS; additional review and editing of the text were undertaken by FN, DKS, DAC, PJH, and UT.

FUNDING INFORMATION

This work was supported by ISOP via a special session *Mixotrophic planktonic protists: living with the "Perfect Beast*," at the 2019 annual meeting; the European Union's Horizons 2020 Research and Innovation Program under the Marie Skłodowska-Curie Grant Agreement No. 766327 MixITiN, the Sêr Cymru II WEFO ERDF Programme *Living with the Perfect Beast* 82372, UKRI NERC Funded Global Partnerships Grant NocSym (NE/W004461/1) and the Brazilian government program Science Without Borders (232845/2014-0) through CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico). This is a contribution to SCOR WG #165 *MixONET* which is supported by grant OCE-214035 from the National Science Foundation to the Scientific Committee on Oceanic Research (SCOR) and contributions from SCOR National Committees. This is Cardiff EARTH CRediT contribution 5.

ORCID

Aditee Mitra https://orcid.org/0000-0001-5572-9331
David A. Caron https://orcid.org/0000-0003-2164-3606
Emile Faure https://orcid.org/0000-0002-9049-2269
Kevin J. Flynn https://orcid.org/0000-0001-6913-5884
Suzana Gonçalves Leles https://orcid.org/0000-0002-7819-5851
Per J. Hansen https://orcid.org/0000-0003-0228-9621
George B. McManus https://orcid.org/0000-0002-2768-1286
Fabrice Not https://orcid.org/0000-0002-9342-195X
Helga do Rosario Gomes https://orcid.org/0000-0002-6287-354X
Luciana F. Santoferrara https://orcid.org/0000-0001-5587-2111
Diane K. Stoecker https://orcid.org/0000-0002-8963-4303
Urban Tillmann https://orcid.org/0000-0002-8207-4382

REFERENCES

Adl, S.M., Bass, D., Lane, C.E., Lukeš, J., Schoch, C.L., Smirnov, A. et al. (2019) Revisions to the classification, nomenclature, and diversity of eukaryotes. *Journal of Eukaryotic Microbiology*, 66, 4–119.

Adolf, J.E., Stoecker, D.K. & Harding, L.W., Jr. (2006) The balance of autotrophy and heterotrophy during mixotrophic growth of *Karlodinium micrum* (Dinophyceae). *Journal of Plankton Research*, 28, 737–751.

Anderson, O.R. (1980) Radiolaria. In: Levandowsky, M. & Hutner, S.H. (Eds.) *Biochemistry and physiology of protozoa*. New York: Academic Press, pp. 1–42.

Anderson, O.R. (1983) *Radiolaria*. New York: Springer-Verlag, p. 355.

Anschütz, A.-A. & Flynn, K.J. (2020) Niche separation between different functional types of mixoplankton: results from NPZ-style N-based model simulations. *Marine Biology*, 167, 3. Available from: https://doi.org/10.1007/s00227-019-3612-3

Anschütz, A.-A., Flynn, K.J. & Mitra, A. (2022) Acquired phototrophy and its implications for bloom dynamics of the *Teleaulax-Mesodinium-Dinophysis*-complex. *Frontiers in Marine Science*, 8, 799358. Available from: https://doi.org/10.3389/fmars.2021.799358

Antia, N.J., Harrison, P.J. & Oliveira, L. (1981) The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology. *Phycologia*, 30, 1–89.

Avrahami, Y. & Frada, M.J. (2020) Detection of phagotrophy in the marine phytoplankton group of the coccolithophores (Calcihaptophycidae, Haptophyta) during nutrient-replete and phosphate-limited growth. *Journal of Phycology*, 56, 1103–1108.

Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, L.A. & Thingstad, F. (1983) The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series*, 10, 257–263.

Bé, A.W., Hemleben, C., Anderson, O.R., Spindler, M., Hacunda, J. & Tuntivate-Choy, S. (1977) Laboratory and field observations of living planktonic foraminifera. *Micropaleontology*, 1, 155–179.

Blossom, H.E., Bædkel, T.D., Tillmann, U. & Hansen, P.J. (2017) A search for mixotrophy and mucus trap production in *Alexandrium* spp. and the dynamics of mucus trap formation in *Alexandrium pseudogonyaulax*. *Harmful Algae*, 64, 51–62.

Blossom, H.E. & Hansen, P.J. (2021) The loss of mixotrophy in *Alexandrium pseudogonyaulax*: implications for trade-offs

between toxicity, mucus trap production, and phagotrophy. *Limnology and Oceanography*, 66, 528–542.

Burkholder, J.M., Glibert, P.M. & Skelton, H.M. (2008) Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. *Harmful Algae*, 8, 77–93.

Cachon, J. & Cachon, M. (1977) Observations on the mitosis and on the chromosome evolution during the lifecycle of *Oodinium*, a parasitic dinoflagellate. *Chromosoma*, 60, 237–251.

Caracciolo, M., Rigaut-Jalabert, F., Romac, S., Mahé, F., Forsans, S., Gac, J.P. et al. (2022) Seasonal dynamics of marine protist communities in tidally mixed coastal waters. *Molecular Ecology*, 31, 3761–3783.

Caron, D.A., Michaels, A.F., Swanberg, N.R. & Howse, F.A. (1995) Primary productivity by symbiont-bearing planktonic sarcodines (Acantharia, Radiolaria, foraminifera) in surface waters near Bermuda. *Journal of Plankton Research*, 17, 103–129.

Coe, A., Ghizzoni, J., LeGault, K., Biller, S., Roggensack, S.E. & Chisholm, S.W. (2016) Survival of *Prochlorococcus* in extended darkness. *Limnology and Oceanography*, 61, 1375–1388.

Crawford, D.W. (1989) *Mesodinium rubrum*: the phytoplankter that wasn't. *Marine Ecology Progress Series*, 58, 161–174.

de Vargas, C., Audic, S., Henry, N., Decelle, J., Mahé, F., Logares, R. et al. (2015) Eukaryotic plankton diversity in the sunlit ocean. *Science*, 348, 1261605.

Decelle, J., Probert, I., Bittner, L., Desdevises, Y., Colin, S., de Vargas, C. et al. (2012) An original mode of symbiosis in open ocean plankton. *Proceedings of the National Academy of Sciences*, 109, 18000–18005.

Droop, M.R. (1968) Vitamin B_{12} and marine ecology. IV. The kinetics of uptake, growth and inhibition in *Monochrysis lutheri*. *Journal of the Marine Biological Association of the United Kingdom*, 48, 689–733.

Droop, M.R. (2007) Vitamins, phytoplankton and bacteria: symbiosis or scavenging? *Journal of Plankton Research*, 29, 107–113.

Ducklow, H., Cimino, M., Dunton, K.H., Fraser, W.R., Hopcroft, R.R., Ji, R. et al. (2022) Marine pelagic ecosystem responses to climate variability and change. *Bioscience*, 72, 827–850.

Faure, E., Not, F., Benoiston, A.S., Labadie, K., Bittner, L. & Ayata, S.D. (2019) Mixotrophic protists display contrasted biogeographies in the global ocean. *The ISME Journal*, 13, 1072–1083.

Ferrer-González, F.X., Widner, B., Holderman, N.R., Glushka, J., Edison, A.S., Kujawinski, E.B. et al. (2021) Resource partitioning of phytoplankton metabolites that support bacterial heterotrophy. *The ISME Journal*, 15, 762–773.

Field, C.B., Behrenfeld, M.J., Randerson, J.T. & Falkowski, P. (1998) Primary production of the biosphere: integrating terrestrial and oceanic components. *Science*, 281, 237–240.

Flynn, K.J. & Berry, L.S. (1999) The loss of organic nitrogen during marine primary production may be overestimated significantly when estimated using ^{15}N substrates. *Proceedings of the Royal Society B: Biological Sciences*, 266, 641–647.

Flynn, K.J. & Butler, I. (1986) Nitrogen sources for the growth of marine microalgae; role of dissolved free amino acids. *Marine Ecology Progress Series*, 34, 281–304.

Flynn, K.J., Mitra, A., Anestis, K., Anschütz, A.A., Calbet, A., Ferreira, G.D. et al. (2019) Mixotrophic protists and a new paradigm for marine ecology: where does plankton research go now? *Journal of Plankton Research*, 41, 375–391.

Flynn, K.J., Mitra, A., Glibert, P.M. & Burkholder, J.M. (2018) Mixotrophy in harmful algal blooms: by whom, on whom, when, why, and what next. In: Glibert, P., Berdalet, E., Burford, M., Pitcher, G. & Zhou, M. (Eds.) *Global ecology and oceanography of harmful algal blooms, ecological studies*. Cham: Springer, pp. 113–132.

Flynn, K.J., Stoecker, D.K., Mitra, A., Raven, J.A., Glibert, P.M., Hansen, P.J. et al. (2013) Misuse of the phytoplankton–zooplankton dichotomy: the need to assign organisms as mixotrophs within plankton functional types. *Journal of Plankton Research*, 35, 3–11.

Flynn, K.J., Torres, R., Irigoien, X. & Blackford, J.C. (2022) Plankton digital twins—a new research tool. *Journal of Plankton Research*, 44, 805–813.

Gast, R.J. & Caron, D.A. (1996) Molecular phylogeny of symbiotic dinoflagellates from planktonic foraminifera and radiolaria. *Molecular Biology and Evolution*, 13, 1192–1197.

Ghyoot, C., Lancelot, C., Flynn, K.J., Mitra, A. & Gypens, N. (2017) Introducing mixotrophy into a biogeochemical model describing an eutrophied coastal ecosystem: the southern North Sea. *Progress in Oceanography*, 157, 1–11.

Glibert, P.M. & Mitra, A. (2022) From webs, loops, shunts, and pumps to microbial multitasking: evolving concepts of marine microbial ecology, the mixoplankton paradigm, and implications for a future ocean. *Limnology and Oceanography*, 67, 585–597.

Goes, J.I. & Gomes, H.D.R. (2016) An ecosystem in transition: the emergence of mixotrophy in the Arabian Sea. In: Glibert, P.M. & Kana, T.M. (Eds.) *Aquatic microbial ecology and biogeochemistry: a dual perspective*. Switzerland: Springer, pp. 155–170.

Gomes, H.D.R., McKee, K., Mile, A., Thandapu, S., Al-Hashmi, K., Jiang, X. et al. (2018) Influence of light availability and prey type on the growth and photo-physiological rates of the mixotroph *Noctiluca scintillans*. *Frontiers in Marine Science*, 5, 374.

Gowing, M.M. (1989) Abundance and feeding ecology of Antarctic phaeodarian radiolarians. *Marine Biology*, 103, 107–118.

Granéli, E., Evardsen, B., Roelke, D.L. & Hagström, J.A. (2012) The ecophysiology and bloom dynamics of *Prymnesium* spp. *Harmful Algae*, 14, 260–270.

Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L. et al. (2013) The Protist ribosomal reference database (PR^2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. *Nucleic Acids Research*, 41, 597–604.

Gustafson, D.E., Stoecker, D.K., Johnson, M.D., Van Heukelem, W.F. & Sneider, K. (2000) Cryptophyte algae are robbed of their organelles by the marine ciliate *Mesodinium rubrum*. *Nature*, 405, 1049–1052.

Gutiérrez-Rodríguez, A., dos Santos, A.L., Safi, K., Probert, I., Not, F., Fernández, D. et al. (2022) Planktonic protist diversity across contrasting subtropical and Subantarctic waters of the Southwest Pacific. *Progress in Oceanography*, 206, 102809.

Haeckel, E. (1887) Report on the Radiolaria collected by HMS challenger during the years 1873-1876. Report of the voyage of HMS challenger. *Zoology*, 18, 1–188.

Hallegraeff, G., Enevoldsen, H. & Zingone, A. (2021) Global harmful algal bloom status reporting. *Harmful Algae*, 102, 101992.

Hansen, P.J., Flynn, K.J., Mitra, A., Calbet, A., Saiz, E., Pitta, P. et al. (2021) A manual for isolation and culture of mixoplankton to support experimental studies. *Zenodo*, 37. Available from: https://doi.org/10.5281/zenodo.5520863

Hembleben, C., Spindler, M. & Anderson, O.R. (1988) *Modern planktonic foraminifera*. New York: Springer-Verlag, p. 363.

Hu, S.K., Campbell, V., Connell, P., Gellene, A.G., Liu, Z., Terrado, R. et al. (2016) Protistan diversity and activity inferred from RNA and DNA at a coastal ocean site in the eastern North Pacific. *FEMS Microbiology Ecology*, 92, 4.

Jacobson, D.M. & Andersen, R.A. (1994) The discovery of mixotrophy in photosynthetic species of *Dinophysis* (Dinophyceae): light and electron microscopical observations of food vacuoles in *Dinophysis acuminata*, *D. norvegica* and two heterotrophic dinophysoid dinoflagellates. *Phycologia*, 33, 97–110.

Jacobson, D.M. & Anderson, D.M. (1996) Widespread phagocytosis of ciliates and other protists by marine mixotrophic and heterotrophic thecate dinoflagellates. *Journal of Phycology*, 32, 279–285.

Jeong, H.J., Park, J.Y., Nho, J.H., Park, M.O., Ha, J.H., Seong, K.A. et al. (2005) Feeding by red-tide dinoflagellates on the cyanobacterium *Synechococcus*. *Aquatic Microbial Ecology*, 41, 131–143.

Jeong, H.J., Seong, K.A., Kang, N.S., Du Yoo, Y., Nam, S.W., Park, J.Y. et al. (2010) Feeding by raphidophytes on the cyanobacterium *Synechococcus* sp. *Aquatic Microbial Ecology*, 58, 181–195.

Jiao, N. & Azam, F. (2011) Microbial carbon pump and its significance for carbon sequestration in the ocean. *Microbial Carbon Pump in the Ocean*, 10, 43–45.

Johnson, M.D. (2011) Acquired phototrophy in ciliates: a review of cellular interactions and structural adaptations 1. *Journal of Eukaryotic Microbiology*, 58, 185–195.

Johnson, M.D., Beaudoin, D.J., Laza-Martinez, A., Dyhrman, S.T., Fensin, E., Lin, S. et al. (2016) The genetic diversity of *Mesodinium* and associated cryptophytes. *Frontiers in Microbiology*, 7, 2017.

Jones, H. (1997) A classification of mixotrophic protists based on their behaviour. *Freshwater Biology*, 37, 35–43.

Jones, R.I. (2000) Mixotrophy in planktonic protists: an overview. *Freshwater Biology*, 45, 219–226.

Kamennaya, N.A., Kennaway, G., Fucha, B.M. & Zubkov, M.V. (2018) "Pomacytosis" - semi-extracellular phagocytosis of cyanobacteria by the smallest marine algae. *PLoS Biology*, 16, e2003502.

Kawachi, M., Inouye, I., Maeda, O. & Chirara, M. (1991) The haptonema as a food-capturing device: observations on *Chrysochromulina hirta* (Prymnesiophyceae). *Phycologia*, 30, 563–573.

Keeling, P.J. & Burki, F. (2019) Progress towards the tree of eukaryotes. *Current Biology*, 29, R808–R817.

Keil, R.G. & Kirchman, D.L. (1993) Dissolved combined amino acids: chemical form and utilization by marine bacteria. *Limnology and Oceanography*, 38, 1256–1270.

Koppelle, S., López-Escardó, D., Brussaard, C.P., Huisman, J., Philippart, C.J., Massana, R. et al. (2022) Mixotrophy in the bloom-forming genus *Phaeocystis* and other haptophytes. *Harmful Algae*, 117, 102292.

Lambert, B.S., Groussman, R.D., Schatz, M.J., Coesel, S.N., Durham, B.P., Alverson, A.J. et al. (2022) The dynamic trophic architecture of open-ocean protist communities revealed through machine-guided metatranscriptomics. *Proceedings of the National Academy of Sciences*, 119, e2100916119.

Larsen, J. (1988) An ultrastructural study of *Amphidinium poecilochroum* (Dinophyceae), a phagotrophic dinoflagellate feeding on small species of cryptophytes. *Phycologia*, 27, 366–377.

Larsson, M.E., Bramucci, A.R., Collins, S., Hallegraeff, G., Kahlke, T., Raina, J.B. et al. (2022) Mucospheres produced by a mixotrophic protist impact ocean carbon cycling. *Nature Communications*, 13, 1–15.

Lawrence, E. (2011) *Henderson's dictionary of biology*. Harlow, England: Pearson Education.

Leblanc, K., Arístegui, J., Armand, L., Assmy, P., Beker, B., Bode, A. et al. (2012) A global diatom database–abundance, biovolume and biomass in the world ocean. *Earth System Science Data*, 4, 149–165.

Leles, S.G., Bruggeman, J., Polimene, L., Blackford, J., Flynn, K.J. & Mitra, A. (2021) Differences in physiology explain succession of mixoplankton functional types and affect carbon fluxes in temperate seas. *Progress in Oceanography*, 190, 102481.

Leles, S.G., Mitra, A., Flynn, K.J., Stoecker, D.K., Hansen, P.J., Calbet, A. et al. (2017) Oceanic protists with different forms of acquired phototrophy display contrasting biogeographies and abundance. *Proceedings of the Royal Society B*, 284, 20170664.

Leles, S.G., Mitra, A., Flynn, K.J., Tillmann, U., Stoecker, D., Jeong, H.J. et al. (2019) Sampling bias misrepresents the biogeographic significance of constitutive mixotrophs across global oceans. *Global Ecology and Biogeography*, 28, 418–428.

Leles, S.G., Polimene, L., Bruggeman, J., Blackford, J., Ciavatta, S., Mitra, A. et al. (2018) Modelling mixotrophic functional diversity and implications for ecosystem function. *Journal of Plankton Research*, 40, 627–642.

Lewin, J. & Hellebust, J.A. (1970) Heterotrophic nutrition of the marine pennate diatom, *Cylindrotheca fusiformis*. *Canadian Journal of Microbiology*, 16, 1123–1129.

Li, A., Stoecker, D.K. & Adolf, J.E. (1999) Feeding, pigmentation, photosynthesis and growth of the mixotrophic dinoflagellate *Gyrodinium galatheanum*. *Aquatic Microbial Ecology*, 19, 163–176.

Lie, A.A.Y., Liu, Z., Terrado, R., Tatters, A.O., Heidelberg, K.B. & Caron, D.A. (2018) A tale of two mixotrophic chrysophytes: insights into the metabolisms of two *Ochromonas* species (Chrysophyceae) through a comparison of gene expression. *PLoS One*, 13, e0192439.

Lin, C.-H., Flynn, K.J., Mitra, A. & Glibert, P.M. (2018) Simulating effects of variable stoichiometry and temperature on mixotrophy in the harmful dinoflagellate *Karlodinium veneficum*. *Frontiers in Marine Science*, 5, 320.

Longhurst, A. (2007) *Ecological Geography of the sea*. MA: Academic Press.

López Urrutia, Á. & Morán, X.A.G. (2015) Temperature affects the size-structure of phytoplankton communities in the ocean. *Limnology and Oceanography*, 60, 733–738.

Lundgren, V.M., Glibert, P.M., Granéli, E., Vidyarathna, N.K., Fiori, E., Ou, L. et al. (2016) Metabolic and physiological changes in *Prymnesium parvum* when grown under, and grazing on prey of, variable nitrogen:phosphorus stoichiometry. *Harmful Algae*, 55, 1–12.

Massana, R., Gobet, A., Audic, S., Bass, D., Bittner, L., Boutte, C. et al. (2015) Marine protist diversity in European coastal waters and sediments as revealed by high-throughput sequencing. *Environmental Microbiology*, 17, 4035–4049.

McManus, G., Xu, D., Costas, B. & Katz, L. (2010) Genetic identities of cryptic species in the *Strombidium stylifer/apolatum/oculatum* cluster, including a description of *Strombidium rassoulzadegani* n. sp. *Journal of Eukaryotic Microbiology*, 57, 369–378.

McManus, G.B. & Fuhrman, J.A. (1986) Photosynthetic pigments in the ciliate *Laboea strobila* from Long Island sound, USA. *Journal of Plankton Research*, 8, 317–327.

Meyer, N., Rydzyk, A. & Pohnert, G. (2022) Pronounced uptake and metabolism of organic substrates by diatoms revealed by pulse-labeling metabolomics. *Frontiers in Marine Science*, 9, 821167.

Michaels, A.F. (1988) Vertical distribution and abundance of Acantharia and their symbionts. *Marine Biology*, 97, 559–569.

Millette, N.C., Pierson, J.J., Aceves, A. & Stoecker, D.K. (2017) Mixotrophy in *Heterocapsa rotundata*: a mechanism for dominating the winter phytoplankton. *Limnology and Oceanography*, 62, 836–845.

Mitra, A., Caron, D.A., Faure, E., Flynn, K.J., Gonçalves Leles, S., Hansen, P.J. et al. (2023) The Mixoplankton database (MDB). *Zenodo*. Available from: https://doi.org/10.5281/zenodo.7560583

Mitra, A., Castellani, C., Gentleman, W.C., Jónasdóttir, S.H., Flynn, K.J., Bode, A. et al. (2014) Bridging the gap between marine biogeochemical and fisheries sciences; configuring the zooplankton link. *Progress in Oceanography*, 129, 176–199.

Mitra, A. & Flynn, K.J. (2010) Modelling mixotrophy in harmful algal blooms: more or less the sum of the parts? *Journal of Marine Systems*, 83, 158–169.

Mitra, A. & Flynn, K.J. (2021) HABs and the Mixoplankton paradigm. *Harmful Algae News*, 67, 4–6. Available from: https://doi.org/10.5281/zenodo.5109703

Mitra, A., Flynn, K.J., Tillmann, U., Raven, J.A., Caron, D., Stoecker, D.K. et al. (2016) Defining planktonic protist functional groups on mechanisms for energy and nutrient acquisition; incorporation of diverse mixotrophic strategies. *Protist*, 167, 106–120.

Moestrup, Ø., Garcia-Cuetos, L., Hansen, P.J. & Fenchel, T. (2012) Studies on the genus *Mesodinium* I: ultrastructure and description of *Mesodinium chamaeleon* n. sp., a benthic marine species with green or red chloroplasts. *Journal of Eukaryotic Microbiology*, 59, 20–39.

Nagai, S., Nitshitani, G., Tomaru, Y., Sakiyama, S. & Kamiyama, T. (2008) Predation by the toxic dinoflagellate *Dinophysis fortii* on the ciliate *Myrionecta rubra* and observation of sequestration of ciliate chloroplasts. *Journal of Phycology*, 44, 909–922.

Park, M.G., Kim, S., Kim, H.S., Myung, G., Kang, Y.G. & Yih, W. (2006) First successful culture of the marine dinoflagellate *Dinophysis acuminata*. *Aquatic Microbial Ecology*, 45, 101–106.

Peperzak, L. & Gäbler-Schwarz, S. (2012) Current knowledge of the life cycles of *Phaeocystis globosa* and *Phaeocystis Antarctica* (Prymnesiophyceae) 1. *Journal of Phycology*, 48, 514–517.

Pringsheim, E.G. (1958) Über Mixotrophie bei Flagellaten. *Planta*, 52, 405–430.

Provoost, P. & Bosch, S. (2021) Robis: ocean biodiversity information system (OBIS) client. R package version 2.8.2. https://CRAN.R-project.org/package=robis

Raven, J.A., Beardall, J., Flynn, K.J. & Maberly, S.C. (2009) Phagotrophy in the origins of photosynthesis in eukaryotes and as a complementary mode of nutrition in phototrophs: relation to Darwin's insectivorous plants. *Journal of Experimental Botany*, 60, 3975–3987.

Reguera, B., Velo-Suárez, L., Raine, R. & Park, M.G. (2012) Harmful *Dinophysis* species: a review. *Harmful Algae*, 14, 87–106. Available from: https://doi.org/10.1016/j.hal.2011.10.016

Righetti, D., Vogt, M., Zimmermann, N.E., Guiry, M.D. & Gruber, N. (2020) PhytoBase: a global synthesis of open-ocean phytoplankton occurrences. *Earth System Science Data*, 12, 907–933.

Riisgård, H.U. & Larsen, P.S. (2009) Ciliary-propelling mechanism, effect of temperature and viscosity on swimming speed, and adaptive significance of 'jumping' in the ciliate *Mesodinium rubrum*. *Marine Biology Research*, 5, 585–595.

Sanders, R.W. & Porter, K.G. (1988) Phagotrophic phytoflagellates. In: *Advances in microbial ecology*. Boston, MA: Springer, pp. 167–192.

Sandin, M.M., Romac, S. & Not, F. (2022) Intra-genomic rRNA gene variability of Nassellaria and Spumellaria (Rhizaria, Radiolaria) assessed by sanger, MinION and Illumina sequencing. *Environmental Microbiology*, 24, 2979–2993.

Santoferrara, L. (2019) Current practice in plankton metabarcoding: optimization and error management. *Journal of Plankton Research*, 41, 571–582.

Santoferrara, L., Burki, F., Filker, S., Logares, R., Dunthorn, M. & McManus, G. (2020) Perspectives from ten years of protist studies by high-throughput metabarcoding. *Journal of Eukaryotic Microbiology*, 67, 612–622.

Schiller, L. (1933) Dinoflagellatae (Peridineae) in monographischer Behandlung. I. Teil, Leiferung 3. In: *Dr L. Rabenhort's Kryptogamen-Flora von Deutscheland, Oesterreich und der Schweiz*. Leipzig: Akademische Verlagsgesellschaft.

Schmidt, K., Birchill, A.J., Atkinson, A., Brewin, R.J., Clark, J.R., Hickman, A.E. et al. (2020) Increasing picocyanobacteria success in shelf waters contributes to long-term food web degradation. *Global Change Biology*, 26, 5574–5587.

Schneider, L.K., Anestis, K., Mansour, J., Anschütz, A.A., Gypens, N., Hansen, P.J. et al. (2020) A dataset on trophic modes of aquatic protists. *Biodiversity Data Journal*, 8, e56648.

Selosse, M.-A., Charpin, M. & Not, F. (2017) Mixotrophy everywhere on land and in water: the grand écart hypothesis. *Ecology Letters*, 20, 246–263.

Skovgaard, A. & Salomonsen, X.M. (2009) *Blastodinium galatheanum* sp. nov. (Dinophyceae) a parasite of the planktonic copepod *Acartia negligens* (Crustacea, Calanoida) in the Central Atlantic Ocean. *European Journal of Phycology*, 44, 425–438.

Spero, H.J. & Parker, S.L. (1985) Photosynthesis in the symbiotic planktonic foraminifer *Orbulina universa*, and its potential contribution to oceanic primary productivity. *Journal of Foraminiferan Research*, 15, 273–281.

Stoecker, D.K. (1998) Conceptual models of mixotrophy in planktonic protists and some ecological and evolutionary implications. *European Journal of Protistology*, 34, 281–290.

Stoecker, D.K., Hansen, P.J., Caron, D.A. & Mitra, A. (2017) Mixotrophy in the marine plankton. *Annual Review of Marine Science*, 9, 311–335.

Stoecker, D.K., Johnson, M.D., de Vargas, C. & Not, F. (2009) Acquired phototrophy in aquatic protists. *Aquatic Microbial Ecology*, 57, 279–310.

Stoecker, D.K., Silver, M.W., Michaels, A.E. & Davis, L.H. (1988a) Obligate mixotrophy in *Laboea strobila*, a ciliate which retains chloroplasts. *Marine Biology*, 99, 415–423.

Stoecker, D.K., Silver, M.W., Michaels, A.E. & Davis, L.H. (1988b/1989) Enslavement of algal chloroplasts by four *Strombidium* spp. (Ciliophora, Oligotrichida). *Marine Microbial Food Webs*, 3, 79–100.

Strzepek, R.F., Nunn, B.L., Bach, L.T., Berges, J.A., Young, E.B. & Boyd, P.W. (2022) The ongoing need for rates: can physiology and omics come together to co-design the measurements needed to understand complex oceanbiogeochemistry? *Journal of Plankton Research*, 44, 485–495.

Subrahmanyan, R. (1954) A new member of the Euglenineæ, *Protoeuglena Noctilucæ* gen. Et sp. nov., occurring in *Noctiluca miliaris* suriray, causing green discoloration of the sea off Calicut. In: *Proceedings of the Indian Academy of Sciences-section B*, Vol. 39. India: Springer, pp. 118–127.

Sugiyama, K., Hori, R.S., Kusunoki, Y. & Matsuoka, A. (2008) Pseudopodial features and feeding behavior of living nassellarians *Eucyrtidium hexagonatum* Haeckel, *Pterocorys zancleus* (Müler) and *Dictyocodon prometheus* Haeckel. *Paleontological Research*, 12, 209–222.

Sunda, W.G., Graneli, E. & Gobler, C.J. (2006) Positive feedback and the development and persistence of ecosystem disruptive algal blooms. *Journal of Phycology*, 42, 963–974.

Suttle, C.A. (2007) Marine viruses—major players in the global ecosystem. *Nature Reviews Microbiology*, 5, 801–812.

Tang, Y.Z., Koch, F. & Gobler, C.J. (2010) Most harmful algal bloom species are vitamin B_1 and B_{12} auxotrophs. *Proceedings of the National Academy of Sciences*, 107, 20756–20761.

Tillmann, U. (1998) Phagotrophy by a plastidic haptophyte, *Prymnesium patelliferum*. *Aquatic Microbial Ecology*, 14, 155–160.

Tillmann, U. (2003) Kill and eat your predator: a winning strategy of the planktonic flagellate *Prymnesium parvum*. *Aquatic Microbial Ecology*, 32, 73–84.

Vaulot, D., Sim, C.W.H., Ong, D., Teo, B., Biwer, C., Jamy, M. et al. (2022) metaPR2: a database of eukaryotic 18S rRNA metabarcodes with an emphasis on protists. *Molecular Ecology Resources*, 22, 3188–3201. Available from: https://doi.org/10.1111/1755-0998.13674

Vernette, C., Henry, N., Lecubin, J., de Vargas, C., Hingamp, P. & Lescot, M. (2021) The ocean barcode atlas: a web service to explore the biodiversity and biogeography of marine organisms. *Molecular Ecology Resources*, 21, 1347–1358.

Villanova, V. & Spetea, C. (2021) Mixotrophy in diatoms: molecular mechanism and industrial potential. *Physiologia Plantarum*, 173, 603–611.

Wang, L., Lin, X., Goes, J.I. & Lin, S. (2016) Phylogenetic analyses of three genes of *Pedinomonas noctilucae*, the green endosymbiont of the marine dinoflagellate *Noctiluca scintillans*, reveal its affiliation to the order Marsupiomonadales (Chlorophyta, Pedinophyceae) under the reinstated name *Protoeuglena noctilucae*. *Protist*, 167, 205–216.

Wheeler, P.A. & Kirchman, D.L. (1986) Utilization of inorganic and organic nitrogen by bacteria in marine systems 1. *Limnology and Oceanography*, 31, 998–1009.

Wisecaver, J.H. & Hackett, J.D. (2010) Transcriptome analysis reveals nuclear-encoded proteins for the maintenance of temporary plastids in the dinoflagellate *Dinophysis acuminata*. *BMC Genomics*, 11, 1–10.

Yelton, A.P., Acinas, S.G., Sunagawa, S., Bork, P., Pedrós-Alió, C. & Chisholm, S.W. (2016) Global genetic capacity for mixotrophy in marine picocyanobacteria. *ISME Journal*, 10, 2946–2957.

Journal of Eukaryotic Microbiology ISOP Published by the International Society of Protistologists

Zubkov, M.V., Fuchs, B.M., Tarran, G.A., Burkill, P.H. & Amann, R. (2003) High rates of uptake of organic nitrogen compounds by *Prochlorococcus* cyanobacteria as a key to their dominance in oligotrophic oceanic waters. *Applied Environmental Microbiology*, 69, 1299–1304.

SUPPORTING INFORMATION

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

How to cite this article: Mitra, A., Caron, D.A., Faure, E., Flynn, K.J., Leles, S.G., Hansen, P.J. et al. (2023) The mixoplankton database: Diversity of photo-phago-trophic plankton in form, function, and distribution across the global ocean. *Journal of Eukaryotic Microbiology*, 00, e12972. Available from: https://doi.org/10.1111/jeu.12972