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Oceanic protists with different forms of acquired phototrophy display contrasting biogeographies and abundance

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This first comprehensive analysis of the global biogeography of marine protistan plankton with acquired phototrophy shows these mixotrophic organisms to be ubiquitous and abundant; however, their biogeography differs markedly between different functional groups. These mixotrophs, lacking a constitutive capacity for photosynthesis (i.e. non-constitutive mixotrophs, NCMs), acquire their phototrophic potential through either integration of prey-plastids or through endosymbiotic associations with photosynthetic microbes. Analysis of field data reveals that 40–60% of plankton traditionally labelled as (non-phototrophic) microzooplankton are actually NCMs, employing acquired phototrophy in addition to phagotrophy. Specialist NCMs acquire chloroplasts or endosymbionts from specific prey, while generalist NCMs obtain chloroplasts from a variety of prey. These contrasting functional types of NCMs exhibit distinct seasonal and spatial global distribution patterns. Mixotrophs reliant on 'stolen' chloroplasts, controlled by prey diversity and abundance, dominate in high-biomass areas. Mixotrophs harbouring intact symbionts are present in all waters and dominate particularly in oligotrophic open ocean systems. The contrasting temporal and spatial patterns of distribution of different mixotroph functional types across the oceanic provinces, as revealed in this study, challenges traditional interpretations of marine food web structures. Mixotrophs with acquired phototrophy (NCMs) warrant greater recognition in marine research.

1. Background

Primary production in the oceans is critical for life on the Earth, with almost half of global photosynthesis undertaken by marine plankton [1]. This production is traditionally viewed as mediated by 'plant-like' phytoplankton, which are in turn
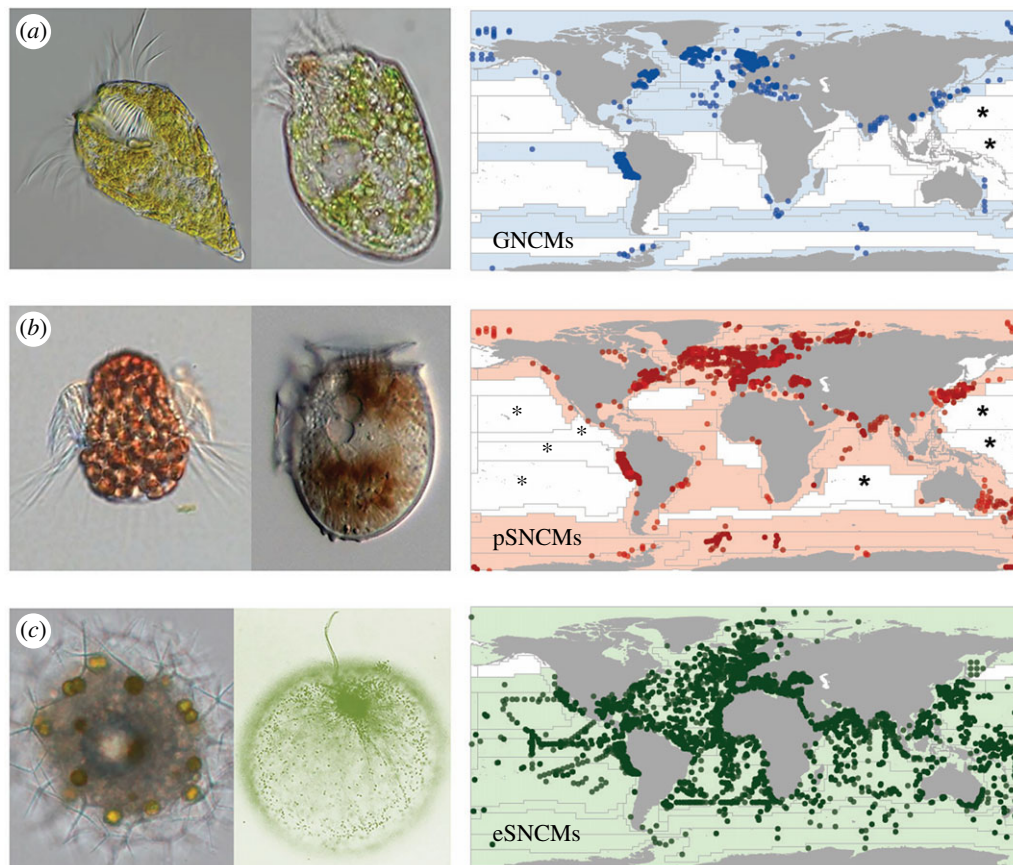
Figure 1. Global distribution of protists with acquired phototrophy (non-constitutive mixotrophs, NCMs). Functional groups identify protists which acquire plastids from a variety of prey (generalist NCMs, GNCMs; blue (a)), from specific prey (plastidic specialist NCMs, pSNCMs; red (b)), or enslave entire specific autotrophic prey as symbionts (endosymbiotic SNCMs, eSNCMs; green (c)). Images next to each map provide protist genus examples within each functional group. From left to right (size as length): (a) GNCMs *Laboea* (100 μm) and *Strombidium* (50 μm); (b) pSNCMs, *Mesodinium* (60 μm) and *Dinophysis* (40 μm); (c) eSNCMs, *Sphaerozoum* (200 μm) and *Noctiluca* (500 μm). On maps, symbols correspond to the exact location where mixotrophic species/taxa were found (from more than 110 000 records); the grid indicates biogeographic provinces. Colour-cast provinces indicate the presence of NCMs and white provinces correspond to the absence. Provinces marked with asterisks indicate that studies conducted in these areas did not record the presence of mixotrophic species; unmarked white provinces indicate a lack of field studies providing information on acquired phototrophy among microzooplankton.

grazed by 'animal-like' zooplankton. However, it is now recognized that a large proportion of marine plankton do not follow this traditional 'plant–animal' dichotomy but are actually mixotrophic protists—single-celled organisms that can perform both photosynthesis and phagocytosis simultaneously [2–5]. In reflection of this shift in our understanding of the marine trophic paradigm, a new functional group classification of marine planktonic protists has been proposed [6] in which mixotrophic protists are broadly divided into constitutive mixotrophs (i.e. those that have a constitutive ability to photosynthesize; CMs), and non-constitutive mixotrophs (i.e. those that do not possess an innate ability to photosynthesize; NCMs).

The CM group conforms to the popular perception of a planktonic mixotroph as a 'plant that eats' [7–9]. This group includes many harmful algal bloom (HAB) species, which have traditionally been considered strictly phototrophic [9]. NCMs, on the other hand, acquire the ability to photosynthesize either by 'stealing' and using plastids from a variety of prey (generalists; GNCMs) or acquiring plastids from specific prey (plastidic specialists; pSNCMs); in some instances, they harbour intact photosynthetic prey as symbionts (endosymbiotic specialists; eSNCMs) [10–12]. However, the presence and importance of NCMs is often overlooked, except for some notable cases such as the ciliate *Mesodinium rubrum* and the HAB dinoflagellates 'green *Noctiluca*' and *Dinophysis* [13–16].

Here, we report the first analysis of the global biogeography of non-constitutive mixotrophs—marine predatory plankton that exploit/use/recycle the photosynthetic machinery of their prey. Our analysis shows that these organisms are not only ubiquitous and abundant, but their biogeography differs markedly between the different groups. Our study establishes NCMs as important members of marine planktonic food webs across different biogeographic provinces at large spatial and temporal scales.

2. Methods

We conducted a global analysis of field data for the different groups of non-constitutive mixotrophs (NCMs); see the electronic supplementary material for detailed methods. Species were classified *a priori* to the GNCM, pSNCM and eSNCM groups according to their physiology. We adapted the Longhurst biogeographic classification system for oceanic provinces (electronic supplementary material, figure S1 and table S1) [17]; these provinces were grouped into seven principal biomes according to primary production and physical forcing. Coordinates corresponding to the locations where mixotrophic species had been recorded were aligned with these biogeographic provinces.

Qualitative data were obtained mainly from the Ocean Biogeographic Information System (OBIS) database; over 110 000 distribution records of greater than 60 species were obtained
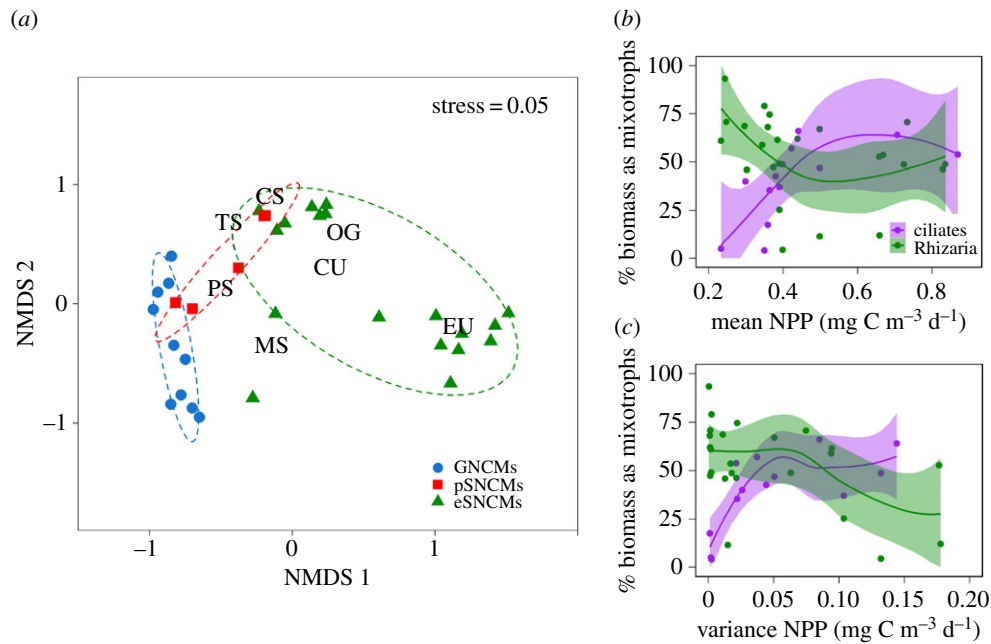
Figure 2. Differences in the biogeography of non-constitutive mixotrophs (NCMs). (*a*) Results from the NMDS analysis showing the ordination of species and biomes in a two-dimensional space. Species were classified according to the non-constitutive mixotroph functional groups (GNCMs, pSNCMs and eSNCMs). Each symbol represents an NCM species; different symbols and dashed ellipses (at 80% CI) represent different functional groups. The different biomes are: MS, Mediterranean Sea; PS, Polar Seas; TS, Temperate Seas; CS, Coastal Seas; OG, Oligotrophic Gyres; CU, Coastal Upwelling; EU, Equatorial Upwelling (see also the electronic supplementary material, figure S3). (*b,c*) Relative contribution of mixotrophs (% biomass) as a function of (*b*) nutrient load and (*c*) system variability. Contribution of mixotrophic ciliate biomass (GNCMs + the pSNCM *Mesodinium*; purple) is plotted relative to total ciliate biomass while contribution of mixotrophic Rhizarian biomass (green) is presented relative to total Rhizarian biomass. Annual average and variance of net primary productivity (NPP) were used as a proxy for nutrient load and system variability, respectively (data obtained from [17]); each symbol represents a biogeographic province (see also the electronic supplementary material). Loess regressions were fitted to data using R package ggplot2; 95% CIs shown.

(electronic supplementary material, table S2). Quantitative data were obtained through a bibliographic survey of the published literature (1970 to present) using the ISI Web of Science database on 13 March 2017. We targeted works from which the quantitative contribution of mixotrophs to the microzooplankton assemblage could be estimated. Over 180 articles were examined (electronic supplementary material, table S3), of which approximately 45 articles provided quantitative data for mixotrophic oligotrich ciliates (GNCMs) and the mixotrophic *Mesodinium* spp. (pSNCM), hereafter referred to as *Mesodinium* (electronic supplementary material, table S4). The relative contribution of mixotrophic Rhizaria (eSNCMs) to the planktonic assemblage within the topmost 100 m was estimated from recent research that used a non-destructive *in situ* imaging system [18].

Non-metric multidimensional scaling (NMDS) was used to explore dissimilarities between non-constitutive mixotrophic species according to their spatial distributions. For this analysis, we used the qualitative data (electronic supplementary material, table S2) to build a presence–absence matrix of species occurrences across the different provinces and biomes (electronic supplementary material, table S1). The quantitative distribution of the NCMs across the seven biomes was also analysed, as was the seasonal progression of biomass for mixotrophic ciliates (both GNCMs and pSNCMs) and Rhizaria (eSNCMs). Two-way ANOVAs were conducted to compare mixotrophic biomass (relative and absolute values) across time and space. All analyses were carried out using R software [19].

3. Results

Our analysis revealed that acquired phototrophy is ubiquitous in the global oceans; however, the biogeography of the three functional groups differed markedly (figure 1). While the eSNCMs were observed to be widely distributed, GNCMs

and pSNCMs were more restricted spatially (figure 1*a,b* versus figure 1*c*). Indeed, each of the three major plankton taxa within the eSNCM functional grouping (dinoflagellates, Radiolaria and Foraminifera) has a wider distribution than the GNCMs and the pSNCMs (electronic supplementary material, figure S2).

The NMDS analysis based on the dissimilarities among species distributions (derived from their presence or absence within the biogeographic provinces) revealed species clustered together according to the NCM functional groups (figure 2*a*). Notably, pSNCMs were positioned between the GNCMs and eSNCMs; certain pSNCM species (e.g. *Amylax triacantha* and *Dinophysis mitra*) were closer to the GNCMs, while others (e.g. *Mesodinium rubrum* and *Dinophysis acuminata*) were closer to the eSNCMs. Spatially, while GNCMs were mainly associated with the Temperate Seas, Polar Seas and Mediterranean Sea biomes, eSNCMs were primarily associated with the Oligotrophic Gyres, Coastal Upwelling and Equatorial Upwelling biomes (figure 2*a*). The eSNCM distribution was observed to follow two distinctly diverse patterns; one group was closely related to the Equatorial Upwelling biome, while a second cosmopolitan group occurred in the intersection between most biomes (figure 2*a*). The Coastal Sea biome was not associated with any single functional group; all the NCM functional groups occurred at least in one biogeographic province within this biome. Our results suggest that the contribution of mixotrophic ciliates increases towards more productive and less variable systems, while the opposite pattern is expected for mixotrophic Rhizaria (figure 2*b,c*; see also the electronic supplementary material).

The quantitative data analysis revealed that the biomass contribution of acquired phototrophy across biomes varied
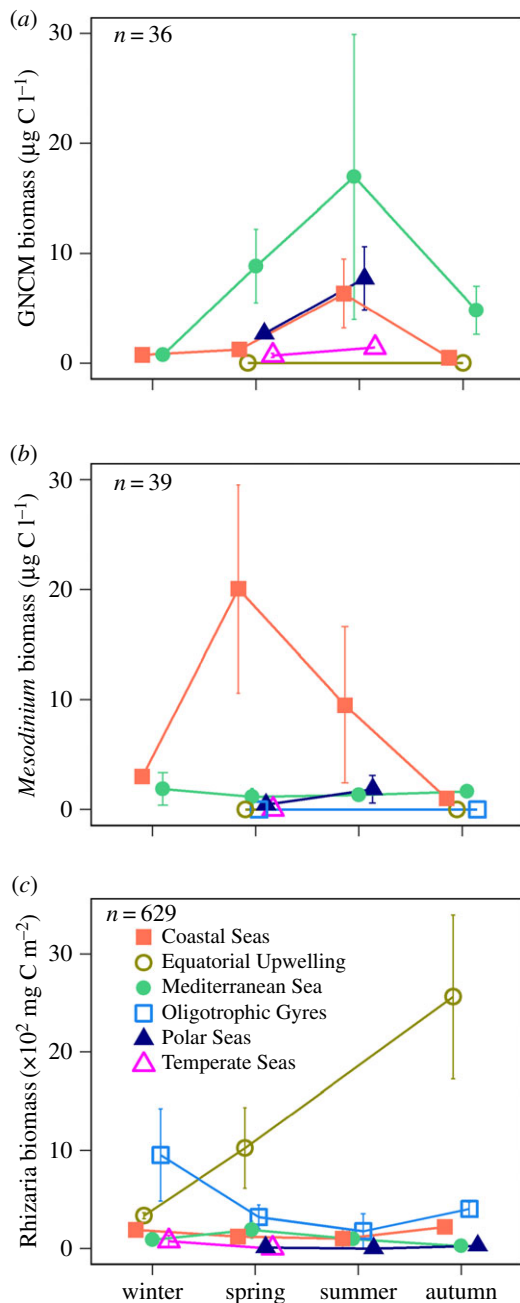
Figure 3. Spatial and temporal distribution of protists with acquired phototrophy. (a) GNCM ciliates, (b) pSNCM *Mesodinium* spp., (c) eSNCM Rhizaria. Seasonal biomass abundance for each group is shown across different biomes (note that biomass units in (a,b) are different from (c)); *n* indicates the total number of observations used. No published data were available for the Coastal Upwelling biome.

biomes (figure 3c); the mixotrophic Rhizaria contributed up to 65% of total Rhizaria biomass within these regions (electronic supplementary material, figure S6).

While there is a paucity of data for GNCMs and *Mesodinium* within certain oceanic regions and/or periods of the year (electronic supplementary material, table S5), we carried out the ANOVAs to gain a basic understanding of the impact of seasonality and/or biomes on their biogeographies. These ANOVAs (electronic supplementary material, table S7) suggest that the combined effect of biome and season on *Mesodinium* (absolute biomass) and mixotrophic Rhizaria (absolute and relative biomass) was significant. The ANOVA of relative biomass of GNCMs showed a significant effect only when considering seasonality.

4. Discussion

From our analysis, we conclude that mixotrophic plankton with acquired phototrophy are prevalent across all ocean biomes, from polar to tropical regions, and from coastal to oceanic environments, in both hemispheres (figure 1). However, the distribution patterns of the three functional types (i.e. GNCMs, pSNCMs and eSNCMs) differed markedly among provinces and also displayed seasonal variation. There was no obvious latitudinal constraint on the occurrence of GNCMs and pSNCMs (figure 1a,b). However, most records for these functional groups were from studies of coastal environments; there are very few reports of these groups within oceanic systems, particularly from within oligotrophic gyres [5,20,21]. By contrast, eSNCMs were present across all latitudinal and coastal-oceanic gradients [5,18,22], with Radiolaria and Foraminifera dominating oceanic waters at low latitudes (0°–30° N and S; figure 1c). Quantitatively, the relative contribution of GNCMs to total ciliate assemblage (abundance and biomass) was lower in low latitudes (electronic supplementary material, figure S7), while the contribution of mixotrophic Rhizaria to total Rhizaria assemblage has been observed to decrease towards the higher latitudes [18].

Protists with acquired phototrophy occur in an ecophysiological continuum from species that have low or moderate control over the acquired phototrophic machinery (GNCMs and pSNCMs, respectively) to those that can strongly regulate phototrophy by control of their endosymbiont populations (eSNCMs) [5,6,11]. Our results suggest that these differences are reflected in their biogeography (figure 2a). For example, pSNCM species with lower physiological control over their acquired plastids (e.g. *A. triacantha* and *D. mitra*) were closer in the NMDS analysis to the GNCMs, while those with a higher level of control (e.g. *M. rubrum* and *D. acuminata*) were closer to the eSNCMs from coastal regions (figure 2a) [23,24].

While GNCMs and pSNCMs share biogeographies that are similar and somewhat restricted to neritic regions, temporal and spatial differences can be drawn between them. Within temperate seas, GNCMs tend to dominate after the phytoplankton bloom, particularly in summer, under stratified water column conditions, while pSNCMs are more commonly encountered during spring, in nutrient-replete conditions (figure 3a,b). *Mesodinium* also thrive in upwelling zones, largely due to their high rates of phototrophic growth in nutrient-rich waters [12,23]. By contrast, GNCMs are usually outnumbered by heterotrophic ciliates (mainly tintinnids) in upwelling regions [25]. The striking dominance of eSNCMs among

according to the functional groups in a similar fashion as did their presence/absence (figure 3; electronic supplementary material, S4–S6 and tables S5–S6). The absolute biomass of GNCMs was highest during summer within the Mediterranean Sea biome (figure 3a), making up 70% of total ciliate biomass (electronic supplementary material, figure S6). The absolute pSNCM biomass (represented by *Mesodinium*) was highest during spring in the Coastal Seas biome (figure 3b), encompassing up to 80% of total ciliate biomass (electronic supplementary material, figure S6). The highest absolute biomass values of mixotrophic Rhizaria (eSNCMs) were observed during autumn within the Equatorial Upwelling biome (figure 3c). Lower absolute biomass values were observed within the Coastal Seas and Mediterranean Sea

mixotrophs in less productive ecosystems with low abiotic variability (figure 2b versus figure 2c), such as within the oligotrophic open ocean, underscores the importance of resource partitioning and symbiosis in these low nutrient environments, and further helps to explain the anomalous preponderance of large cells there [26]. The limited success of GNCMs in these conditions could be attributed to the low availability of prey, which is detrimental to growth of the GNCMs as they rely on a near-constant supply of prey for acquired phototrophy as well as for essential nutrients [5,11] (figure 2b,c).

Acquired phototrophy among microzooplankton has been typically neglected in field and modelling studies; NCMs have hitherto not been considered a major component of the microzooplankton. According to our analysis, when we calculated the average contribution of mixotrophs across temporal and spatial scales, the mixotrophic ciliates (GNCMs + the pSNCM Mesodinium) contribute approximately 45% to the total ciliate numeric abundance, and approximately 40% of total ciliate biomass (electronic supplementary material, figure S4). A previous study estimated that approximately 30% of the numeric abundance of marine oligotrich ciliates globally were mixotrophic (i.e. GNCMs) [27]. Among the eSNCMs, nearly half of total Rhizaria biomass is composed of mixotrophic taxa across all temporal and spatial scales (electronic supplementary material, figure S5). Importantly, our analysis reveals great variation not only in the presence of mixotrophs temporally and spatially, but also on which mixotroph functional group dominates which biome during specific seasons. While, for simplicity, one may wish to refer to a single numeric value defining mixotroph abundance, our study indicates the danger of doing so, especially when conducting modelling studies in support of ecosystem management (e.g. HABs, fisheries and biogeochemistry).

The proposed new mixotroph-centric paradigm for marine ecology [6,28] envisions a high proportion of marine planktonic protists expressing mixotrophy, with a consequential more fully embedded, intertwined set of interactions within the microbial loop [29] than previously appreciated. There is an important contrast in physiology, and thence ecology, among protists with acquired phototrophy (NCMs; i.e. 'animals that photosynthesize'), and also between these and the better-studied constitutive mixotrophs (i.e. 'plants that eat'; the CMs). In the upper water column, activity by GNCMs can shorten and thus potentially increase the efficiency of energy transfer along pelagic food webs [5,6,11]. In the open ocean, eSNCMs include giant photosynthetic protists that contribute significantly to vertical carbon flux, influencing the biological carbon pump [18,28,30,31]. In eutrophic coastal regions, some pSNCMs and eSNCMs form extensive blooms. Blooms of the pSNCM ciliate Mesodinium spp. are of particularly concern, because they can act as biogeochemical hotspots and are the source of the phototrophic capability of the toxicogenic pSNCM, Dinophysis spp. [32,33]. Dinophysis, an organism colloquially considered as an alga rather than a microzooplankter with acquired phototrophy, causes diarrhetic shellfish poisoning, which can be responsible for closures of shellfish aquaculture operations [14,16]. In the Arabian Sea, shifts from diatom blooms to those of the eSNCM 'green Noctiluca' are circumstantially associated with hypoxia, and may adversely affect fisheries in a coastal ecosystem supporting 120 million people [15].

In conclusion, diverse physiological and ecological properties are associated with various modes of acquired phototrophy. Our biogeographic analysis of plankton communities across the world's oceans highlights the prevalence of planktonic protists with acquired phototrophy through symbiotic associations with prey or enslavement of their organelles. Thus, it is critically important that appropriate NCM functional subgroups are represented within conceptual and mathematical models supporting marine research across all spatial and temporal scales of observation. To support such action, it is necessary for surveys and monitoring studies to routinely document the presence of these organisms, to expect to find them rather than consider them a priori as novelties.

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