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1 **Oceanic protists with different forms of acquired phototrophy display**
2 **contrasting biogeographies and abundance**
3

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31

32 **Abstract**

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

51

52 **Keywords**

53 biogeography, mixotrophy, acquired phototrophy, kleptoplasty, photosymbiosis, marine
54 protists

55 Main Text

56 1. Background

57 Primary production in the oceans is critical for life on Earth, with almost half of global
58 photosynthesis undertaken by marine plankton [1]. This production is traditionally viewed as
59 mediated by “plant-like” phytoplankton, which are in turn grazed by “animal-like”
60 zooplankton. However, it is now recognized that a large proportion of marine plankton do not
61 follow this traditional “plant-animal” dichotomy but are actually mixotrophic protists -
62 single-celled organisms that can perform both photosynthesis and phagocytosis
63 simultaneously [2–5]. In reflection of this shift in our understanding of the marine trophic
64 paradigm, a new functional group classification of marine planktonic protists has been
65 proposed [6] in which mixotrophic protists are broadly divided into constitutive mixotrophs
66 (i.e., those that have a constitutive ability to photosynthesize; CMs), and non-constitutive
67 mixotrophs (i.e., those that do not possess an innate ability to photosynthesize; NCMs).

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

77 Here we report the first analysis of the global biogeography of non-constitutive
78 mixotrophs – marine predatory plankton that exploit/use/recycle the photosynthetic
79 machinery of their prey. Our analysis shows that these organisms are not only ubiquitous and

80 abundant, but their biogeography differs markedly between the different groups. Our study
81 establishes NCMs as important members of marine planktonic food webs across different
82 biogeographic provinces at large spatial and temporal scales.

83 **2. Methods**

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

91 Qualitative data were obtained mainly from the Ocean Biogeographic Information System
92 (OBIS) database; over 110,000 distribution records of >60 species were obtained (table S2).
93 Quantitative data were obtained through a bibliographic survey of published literature (1970 -
94 present) using the ISI Web of Science database on 13 March 2017. We targeted works from
95 which the quantitative contribution of mixotrophs to the microzooplankton assemblage could
96 be estimated. Over 180 articles were examined (table S3), of which ~45 articles provided
97 quantitative data for mixotrophic oligotrich ciliates (GNCMs) and the mixotrophic
98 *Mesodinium* spp. (pSNCM), hereafter referred to as *Mesodinium* (table S4). The relative
99 contribution of mixotrophic Rhizaria (eSNCMs) to the planktonic assemblage within the
100 topmost 100 m was estimated from recent research that used a non-destructive *in situ* imaging
101 system [18].

102 Non-metric multidimensional scaling (NMDS) was used to explore dissimilarities
103 between non-constitutive mixotrophic species according to their spatial distributions. For this

104 analysis, we used the qualitative data (table S2) to build a presence-absence matrix of species
105 occurrences across the different provinces and biomes (table S1). The quantitative
106 distribution of the NCMs across the seven biomes was also analysed, as was the seasonal
107 progression of biomass for mixotrophic ciliates (both GNCMs and pSNCMs) and Rhizaria
108 (eSNCMs). Two-Way ANOVAs were conducted to compare mixotrophic biomass (relative
109 and absolute values) across time and space. All analyses were carried out using R software
110 [19].

111 3. Results

112 Our analysis revealed that acquired phototrophy is ubiquitous in the global oceans;
113 however, the biogeography of the three functional groups differed markedly (figure 1). While
114 the eSNCMs were observed to be widely distributed, GNCMs and pSNCMs were more
115 restricted spatially (figure 1a and 1b versus 1c). Indeed, each of the three major plankton taxa
116 within the eSNCM functional grouping (Dinoflagellates, Radiolaria, and Foraminifera) has a
117 wider distribution than the GNCMs and the pSNCMs (figure S2).

118 The NMDS analysis based on the dissimilarities amongst species distributions (derived
119 from their presence or absence within the biogeographic provinces) revealed species
120 clustered together according to the NCM functional groups (figure 2a). Notably, pSNCMs
121 were positioned between the GNCMs and eSNCMs; certain pSNCMs species (e.g., *Amylax*
122 *triacantha* and *Dinophysis mitra*) were closer to the GNCMs, while others (e.g., *Mesodinium*
123 *rubrum* and *Dinophysis acuminata*) were closer to the eSNCMs. Spatially, while GNCMs
124 were mainly associated with the Temperate Seas, Polar Seas, and Mediterranean biomes,
125 eSNCMs were primarily associated with the Oligotrophic Gyres, Coastal Upwelling, and
126 Equatorial Upwelling biomes (figure 2a). The eSNCM distribution was observed to follow
127 two distinctly diverse patterns; one group was closely related to the Equatorial Upwelling

128 biome, while a second cosmopolitan group, occurred in the intersection between most biomes
129 (figure 2a). The Coastal Sea biome was not associated with any single functional group; all
130 the NCM functional groups occurred at least in one biogeographic province within this
131 biome. Our results suggest that the contribution of mixotrophic ciliates increases towards
132 more productive and less variable systems, while the opposite pattern is expected for
133 mixotrophic Rhizaria (figure 2b and c; see also ESM).

134 The quantitative data analysis revealed that the biomass contribution of acquired
135 phototrophy across biomes varied according to the functional groups in a similar fashion as
136 did their presence/absence (figures 3, S4-S6 and tables S5-S6). The absolute biomass of
137 GNCMs was highest during summer within the Mediterranean Sea biome (figure 3a), making
138 up 70% of total ciliate biomass (figure S6). The absolute pSNCM biomass (represented by
139 *Mesodinium*) was highest during spring in the Coastal Seas biome (figure 3b), encompassing
140 up to 80% of total ciliate biomass (figure S6). The highest absolute biomass values of
141 mixotrophic Rhizaria (eSNCMs) were observed during autumn within the Equatorial
142 Upwelling biome (figure 3c). Lower absolute biomass values were observed within the
143 Coastal Seas and Mediterranean Sea biomes (figure 3c); the mixotrophic Rhizaria contributed
144 up to 65% of total Rhizaria biomass within these regions (figure S6).

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

152 4. Discussion

153 From our analysis we conclude that mixotrophic plankton with acquired phototrophy are
154 prevalent across all ocean biomes, from polar to tropical regions, and from coastal to oceanic
155 environments, in both hemispheres (figure 1). However, the distribution patterns of the three
156 functional types (i.e., GNCMs, pSNCMs, and eSNCMs) differed markedly amongst
157 provinces and also displayed seasonal variation. There was no obvious latitudinal constraint
158 on the occurrence of GNCMs and pSNCMs (figure 1a and-b). However, most records for
159 these functional groups were from studies of coastal environments; there are very few reports
160 of these groups within oceanic systems, particularly from within oligotrophic gyres [5, 20,
161 21]. In contrast, eSNCMs were present across all latitudinal and coastal-oceanic gradients [5,
162 18, 22], with Radiolaria and Foraminifera dominating oceanic waters at low latitudes (0°-30°
163 N & S) (figure 1c). Quantitatively, the relative contribution of GNCMs to total ciliate
164 assemblage (abundance and biomass) was lower in low latitudes (figure S7) while the
165 contribution of mixotrophic Rhizaria to total Rhizaria assemblage has been observed to
166 decrease towards the higher latitudes [18].

167 Protists with acquired phototrophy occur in an ecophysiological continuum from species
168 that have low or moderate control over the acquired phototrophic machinery (GNCMs and
169 pSNCMs, respectively) to those that can strongly regulate phototrophy by control of their
170 endosymbiont populations (eSNCMs) [5, 6, 11]. Our results suggest that these differences are
171 reflected in their biogeography (figure 2a). For example, pSNCM species with lower
172 physiological control over their acquired plastids (e.g., *A. triacantha* and *D. mitra*) were
173 closer in the NMDS analysis to the GNCMs while those with a higher level of control (e.g.,
174 *M. rubrum* and *D. acuminata*) were closer to the eSNCMs from coastal regions (figure 2a)
175 [23, 24].

176 While both GNCMs and pSNCMs share biogeographies that are similar and somewhat
177 restricted to neritic regions, temporal and spatial differences can be drawn between them.
178 Within temperate seas, GNCMs tend to dominate after the phytoplankton bloom, particularly
179 in summer, under stratified water column conditions, while pSNCMs are more commonly
180 encountered during spring, on nutrient-replete conditions (figure 3a and b). *Mesodinium* also
181 thrive in upwelling zones, largely due to their high rates of phototrophic growth in nutrient
182 rich waters [12, 23]. In contrast, GNCMs are usually outnumbered by heterotrophic ciliates
183 (mainly tintinnids) in upwelling regions [25]. The striking dominance of eSNCMs amongst
184 mixotrophs in less productive ecosystems with low abiotic variability (figure 2b vs c), such as
185 within the oligotrophic open ocean, underscores the importance of resource partitioning and
186 symbiosis in these low nutrient environments, and further helps to explain the anomalous
187 preponderance of large cells there [26]. The limited success of GNCMs in these conditions
188 could be attributed to the low availability of prey which is detrimental to growth of the
189 GNCMs which rely on a near-constant supply of prey for acquired phototrophy as well as for
190 essential nutrients [5, 11] (figure 2b and c).

191 Acquired phototrophy among microzooplankton has been typically neglected in field and
192 modelling studies; NCMs have hitherto not been considered a major component of the
193 microzooplankton. According to our analysis, when we calculated the average contribution of
194 mixotrophs across temporal and spatial scales, the mixotrophic ciliates (GNCMs + the
195 pSNCM *Mesodinium*) contribute ~45% to the total ciliate numeric abundance, and ~40% of
196 total ciliate biomass (figure S4). A previous study estimated that ~30% of the numeric
197 abundance of marine oligotrich ciliates globally were mixotrophic (i.e., GNCMs) [27].
198 Amongst the eSNCMs, nearly half of total Rhizaria biomass is comprised of mixotrophic taxa
199 across all temporal and spatial scales (figure S5). Importantly, our analysis reveals great
200 variation not only in the presence of mixotrophs temporally and spatially but also on which

201 mixotroph functional group dominates which biome during specific seasons. While for
202 simplicity, one may wish to refer to a single numeric value defining mixotroph abundance,
203 our study indicates the danger of doing so especially when conducting modelling studies in
204 support of ecosystem management (e.g., HABs, fisheries, biogeochemistry etc.).

205 The proposed new mixotroph-centric paradigm for marine ecology [6, 28] envisions a
206 high proportion of marine planktonic protists expressing mixotrophy, with a consequential
207 more fully embedded, intertwined set of interactions within the microbial loop [29] than
208 previously appreciated. There is an important contrast in physiology, and thence ecology,
209 amongst protists with acquired phototrophy (NCMs; i.e., “animals that photosynthesize”) and
210 also between these and the better-studied constitutive mixotrophs (i.e., “plants that eat”; the
211 CMs). In the upper water column, activity by GNCMs can shorten and thus potentially
212 increase the efficiency of energy transfer along pelagic food webs [5, 6, 11]. In the open
213 ocean, eSNCMs include giant photosynthetic protists that contribute significantly to vertical
214 carbon flux, influencing the biological carbon pump [18, 30, 31]. In eutrophic coastal regions,
215 some pSNCMs and eSNCMs form extensive blooms. Blooms of the pSNCM ciliate
216 *Mesodinium* spp. are of particular concern because they can act as biogeochemical hotspots
217 and are the source of the phototrophic capability of the toxicogenic pSNCM, *Dinophysis* spp.
218 [32, 33]. *Dinophysis*, an organism colloquially considered as an alga rather than a
219 microzooplankter with acquired phototrophy, causes Diarrhetic Shellfish Poisoning, which
220 can be responsible for closures of shellfish aquaculture operations [14, 16]. In the Arabian
221 Sea, shifts from diatom blooms to those of the eSNCM “green *Noctiluca*” are
222 circumstantially associated with hypoxia, and may adversely affect fisheries in a coastal
223 ecosystem supporting 120 million people [15].

224 In conclusion, diverse physiological and ecological properties are associated with various
225 modes of acquired phototrophy. Our biogeographic analysis of plankton communities across

226 the world's oceans highlights the prevalence of planktonic protists with acquired phototrophy
227 through symbiotic associations with prey or enslavement of their organelles. Thus it is
228 critically important that appropriate NCM functional subgroups are represented within
229 conceptual and mathematical models supporting marine research across all spatial and
230 temporal scales of observation. To support such action it is necessary for surveys and
231 monitoring studies to routinely document the presence of these organisms, to expect to find
232 them rather than consider them *a priori* as novelties.

233 **Competing interests:** We have no competing interests.

234 **Authors' Contributions:** The concept of this work arose from a workshop involving all
235 authors excepting SGL. SGL compiled the data and conducted the analysis guided by all
236 authors. SGL drafted the original manuscript under the guidance of AM and KJF. All authors
237 contributed to building the manuscript to its final state.

238 **Data accessibility:** The original (raw) data are available as referenced. Processed data are
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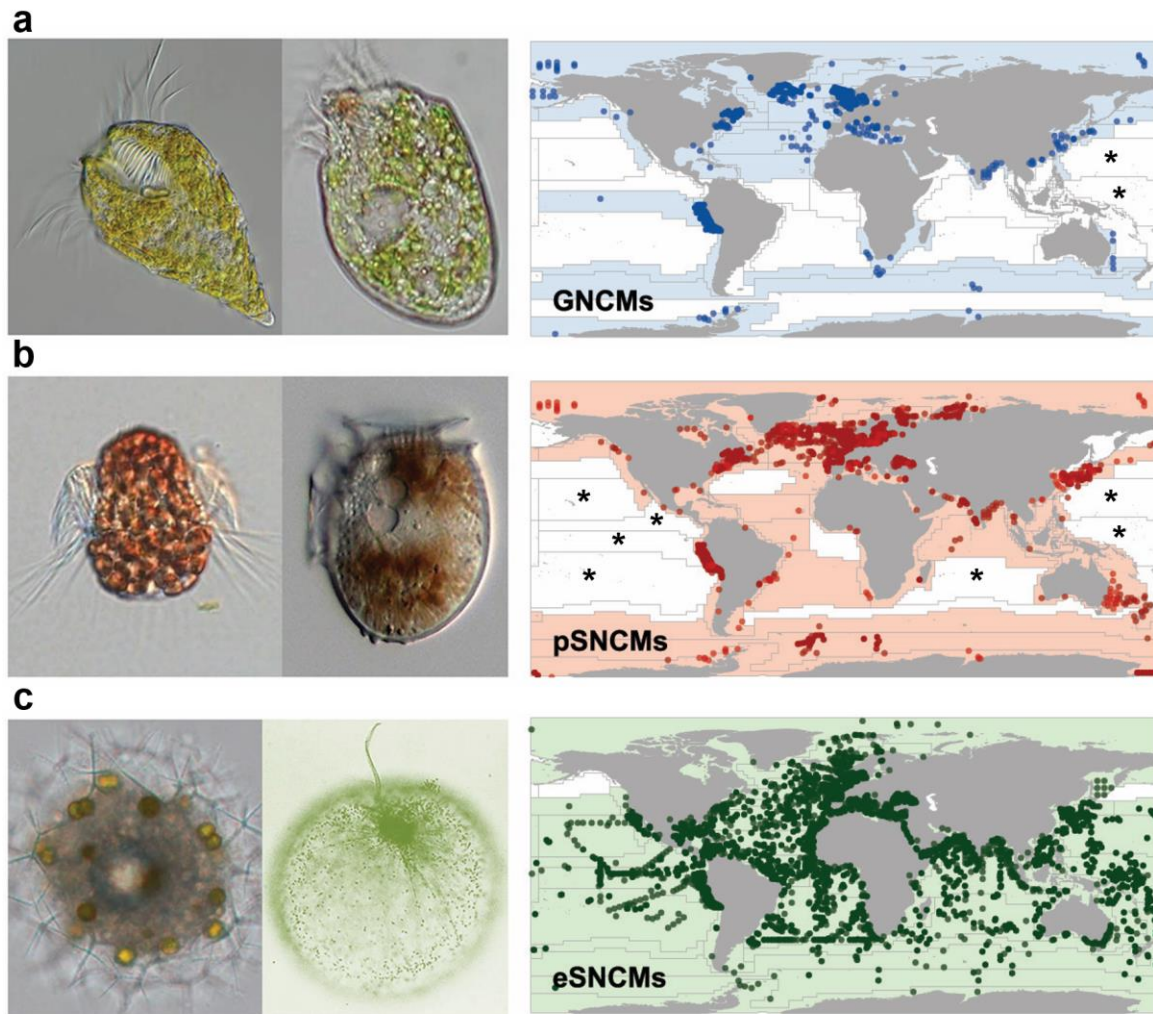
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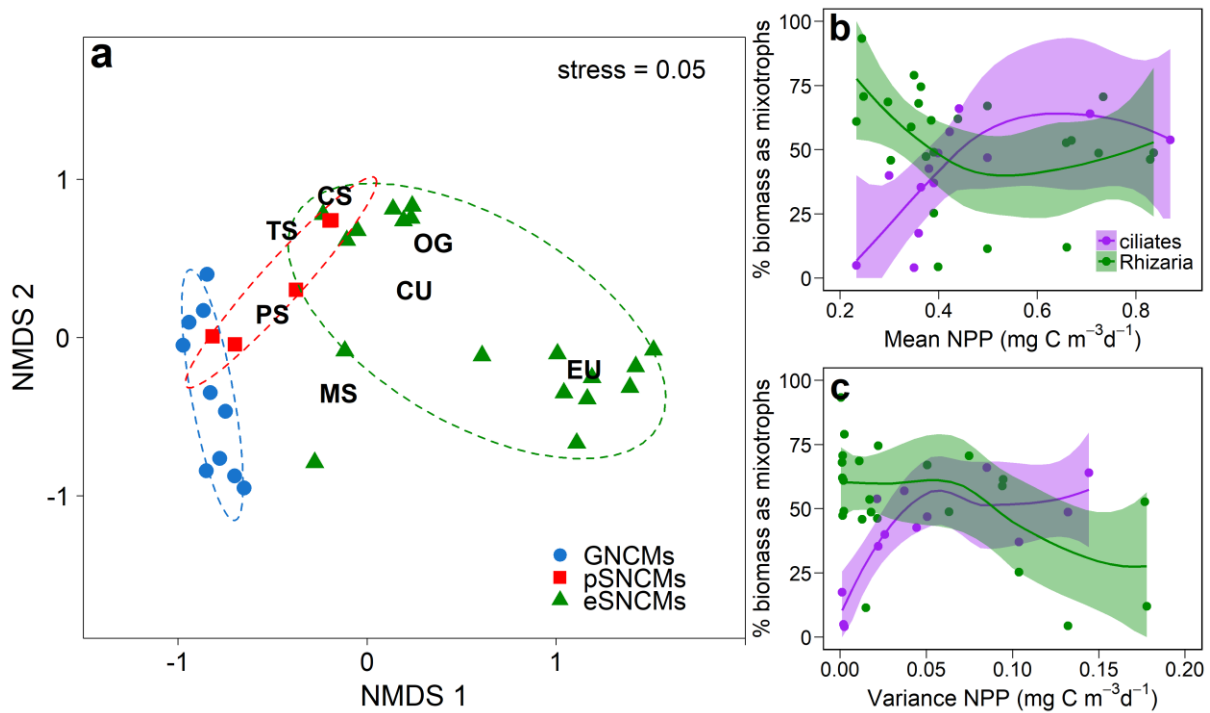
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336 *Mesodinium rubrum*. *J. Phycol.* **48**, 569–579. (doi:10.1111/j.1529-8817.2012.01150.x)



338

339 **Figure 1.** Global distribution of protists with acquired phototrophy. Functional groups
 340 identify protists which acquire plastids from a variety of prey (GNCMs; blue, panel a), or
 341 from specific prey (pSNCMs; red, panel b), or enslave entire specific autotrophic prey as
 342 symbionts (eSNCMs; green, panel c). Images next to each map provide protist genus
 343 examples within each functional group. From left to right (size as length): panel a, GNCMs –
 344 *Laboea* (100 μm) and *Strombidium* (50 μm); panel b, pSNCMs – *Mesodinium* (60 μm) and
 345 *Dinophysis* (40 μm); panel c, eSNCMs – *Sphaerozoum* (200 μm) and *Noctiluca* (500 μm).
 346 On maps, symbols correspond to the exact location where mixotrophic species/taxa were
 347 found (from > 110,000 records); the grid indicates biogeographic provinces. Colour-cast

348 provinces indicate the presence of NCMs and white provinces correspond to absence.
349 Provinces marked with * indicate that studies conducted in these areas did not record
350 presence of mixotrophic species; unmarked white provinces indicate a lack of field studies
351 providing information on acquired phototrophy among microzooplankton.
352



354

355 **Figure 2.** Differences in the biogeography of non-constitutive mixotrophs (NCMs). Panel a:

356 results from the NMDS analysis showing the ordination of species and biomes in a two-

357 dimensional space. Species were classified according to the non-constitutive mixotroph

358 functional groups (GNcMs, pSNcMs, and eSNcMs). Each symbol represents a NCM

359 species; different symbols and dashed ellipses (at 80% confidence interval) represent

360 different functional groups. The different biomes are: MS, Mediterranean Sea; PS, Polar

361 Seas; TS, Temperate Seas; CS, Coastal Seas; OG, Oligotrophic Gyres; CU, Coastal

362 Upwelling; EU, Equatorial Upwelling (see also figure S3). Panels b and c: relative

363 contribution of mixotrophs (% biomass) as a function of nutrient load (b) and system

364 variability (c). Contribution of mixotrophic ciliate biomass (GNcMs + the pSNcM

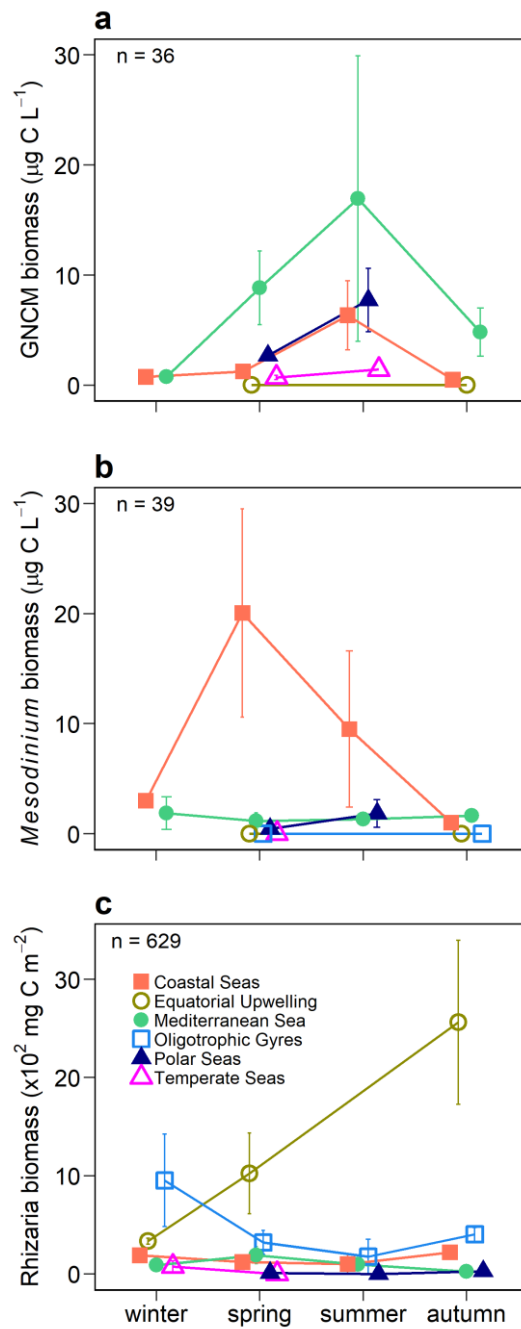
365 *Mesodinium*; purple) is plotted relative to total ciliate biomass while contribution of

366 mixotrophic Rhizarian biomass (green) is presented relative to total Rhizarian biomass.

367 Annual average and variance of net primary productivity (NPP) were used as a proxy for

368 nutrient load and system variability, respectively (data obtained from [17]); each symbol
369 represents a biogeographic province (see also ESM). Loess regressions were fitted to data
370 using R package ggplot2; 95% confidence intervals shown.

371



373

374 **Figure 3.** Spatial and temporal distribution of protists with acquired phototrophy. Panel a,
 375 GNCM ciliates; panel b, pSNCM *Mesodinium* spp.; panel c, eSNCM Rhizaria. Seasonal
 376 biomass abundance for each group is shown across different biomes (note that biomass unit
 377 in panels a and b are different from panel c); n, indicates the total number of observations
 378 used. No published data were available for the Coastal Upwelling biome.