An autumn biodiversity survey on heterotrophic and mixotrophic protists along a coast-to-offshore transect in the Gulf of Naples (Italy)

Gabriele Del Gaizo[1,*], Luca Russo[1,2], Maria Abagnale[3], Angela Buondonno[3], Marta Furia[3], Simona Saviano[3], Mauro Vargiu[3], Fabio Conversano[3], Francesca Margiotta[3], Maria Saggiomo[3], Isabella Percopo[4], Domenico D'Alelio[1,*]

[1]Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Naples, Italy; [2]PhD Program in Evolutionary Biology and Ecology, Department of Biology, University of Rome 'Tor Vergata', Rome, Italy; [3]Department of Research Infrastructures for Marine Biological Resources, Stazione Zoologica Anton Dohrn, Naples, Italy; [4]Marine Organism Taxonomy Core Facility – MOTax, Department of Research Infrastructures for Marine Biological Resources, Stazione Zoologica Anton Dohrn, Naples, Italy

ABSTRACT

Plankton communities include both unicellular and multicellular organisms. An important unicellular component is represented by those protists (*i.e*., unicellular eukaryotes) that consume bacteria, other protists, and even small animals. These organisms are an important link between primary producers and metazoans, and they can also be 'mixotrophic', coupling phagotrophic and photoautotrophic behaviours. In this note, we report on the diversity of heterotrophic protists, or protozoo-plankton (from 'protozoan plankton'), and mixotrophic protists, or mixo-plankton, which were sampled at two sites (coastal and offshore stations), at two depths (0 and 10 m), in the Gulf of Naples during the early autumn of 2020. Our survey identified dinoflagellates and oligotrich ciliates as the most abundant groups, while tintinnids (choreotrich ciliates) were less quantitatively relevant. The taxonomic composition of samples investigated herein remarked that reported by previous studies, except for the tintinnid *Ascampbeliella armilla*, which was never reported in the area of study. A coast-to-offshore differentiation in the taxonomical composition of heterotrophic and mixotrophic protists was also observed, with some species more abundant within coastal waters and other better thriving in offshore ones. These differences were associated with distinct environmental and trophic conditions.

INTRODUCTION

Protists are important components of plankton and include many categories of obligate and facultative heterotrophs, which are known, respectively, as protozoo-plankton (from protozoan plankton, including, *e.g*., oligotrich and choreotrich ciliates) and mixoplankton (from mixotrophic plankton, including, *e.g*., dinoflagellates and some oligotrich ciliates) (Flynn *et al*., 2019).

Mixotrophs, being capable of both phagocytosis and photosynthesis within a single cell (Stoecker *et al*., 2009; Flynn *et al*., 2013, 2019), encompass different functional types (Mitra *et al*., 2016), namely: constitutive mixotrophs, bearing innate plastids (*e.g*., dinoflagellates; Stoecker *et al*., 2017); non-constitutive mixotrophs, acquiring plastids from photosynthetic preys (*i.e*., a strategy known as kleptoplasty, which is present in oligotrich ciliates, predating mainly cryptophytic microalgae; Johnson, 2011; Hughes *et al*., 2021); moreover, some non-constitutive mixotrophs can transmit plastids to the progeny (*e.g*., *Mesodinium rubrum*; Johnson *et al*., 2007; Smith and Hansen, 2007), while some other must acquire new plastids after any cell replication (*i.e*., most oligotrich ciliates).

Such a behavioural diversity along with a high trophic plasticity allow mixoplankton to cope with intermittent resources, by easily shifting between autotrophy and heterotrophy (*e.g., Haraguchi et al*., 2018). This ecological property is of much higher relevance in the circulation of matter and energy throughout the plankton food web, in which protists play as connecting nodes between primary producers and metazoan consumers (D'Alelio *et al*., 2016a; Flynn *et al*., 2019; Leles *et al*., 2021).

In this brief communication we report on the diversity of heterotrophic and mixotrophic protists occurring in the Gulf of Naples (Tyrrhenian Sea, Italy) during late summer/early autumn; this period sets in the middle between the spring/summer and mid-autumn phytoplankton blooms and it is characterized by similar amounts of hetero- and mixotrophic protists, based on information collected at the Long-Term Ecological Research station MareChiara (LTER-MC; Ribera d'Alcalà *et al*., 2004; Zingone *et al*., 2019). As the planktonic system generally shifts from autotrophy to heterotrophy during the late summer/early autumn phase, the relevance of mixotrophy is expected as highest in the period under investigation.

By analysing water samples collected from two oceanographic stations in the Gulf of Naples, we provide abundance data for planktonic protists (mainly focusing on dinoflagellates, oligotrich and choreotrich ciliates), identify their trophic modes (either mixo- or heterotrophic) based on information available in the literature, report on compositional differences at spatial scale and discuss these latter by considering the standing environmental and trophic conditions. Though preliminary, our survey provides useful biodiversity data about mixo- and protozooplankton at local scale, as taxonomical information about these organisms have not been updated in the Gulf of Naples in the last two decades (Modigh, 2001; Modigh and Castaldo, 2002; Ribera d'Alcalà *et al*., 2004).

MATERIALS AND METHODS

The Gulf of Naples (Southern Italy) is a coastal embayment open to the Tyrrhenian Sea (Figure 1a). Water sampling was carried out at two hydrographic stations named FE_11 (coordinates: 40°47.285, 14°20.883; distance from the coast: 0.3 NM; maximum depth: 26 m) and FE_12 (coordinates: 40°46.706, 14°19.858; distance from the coast: 1.3 NM; maximum depth: 100 m). These stations were integrated in a basin-scale oceanographic

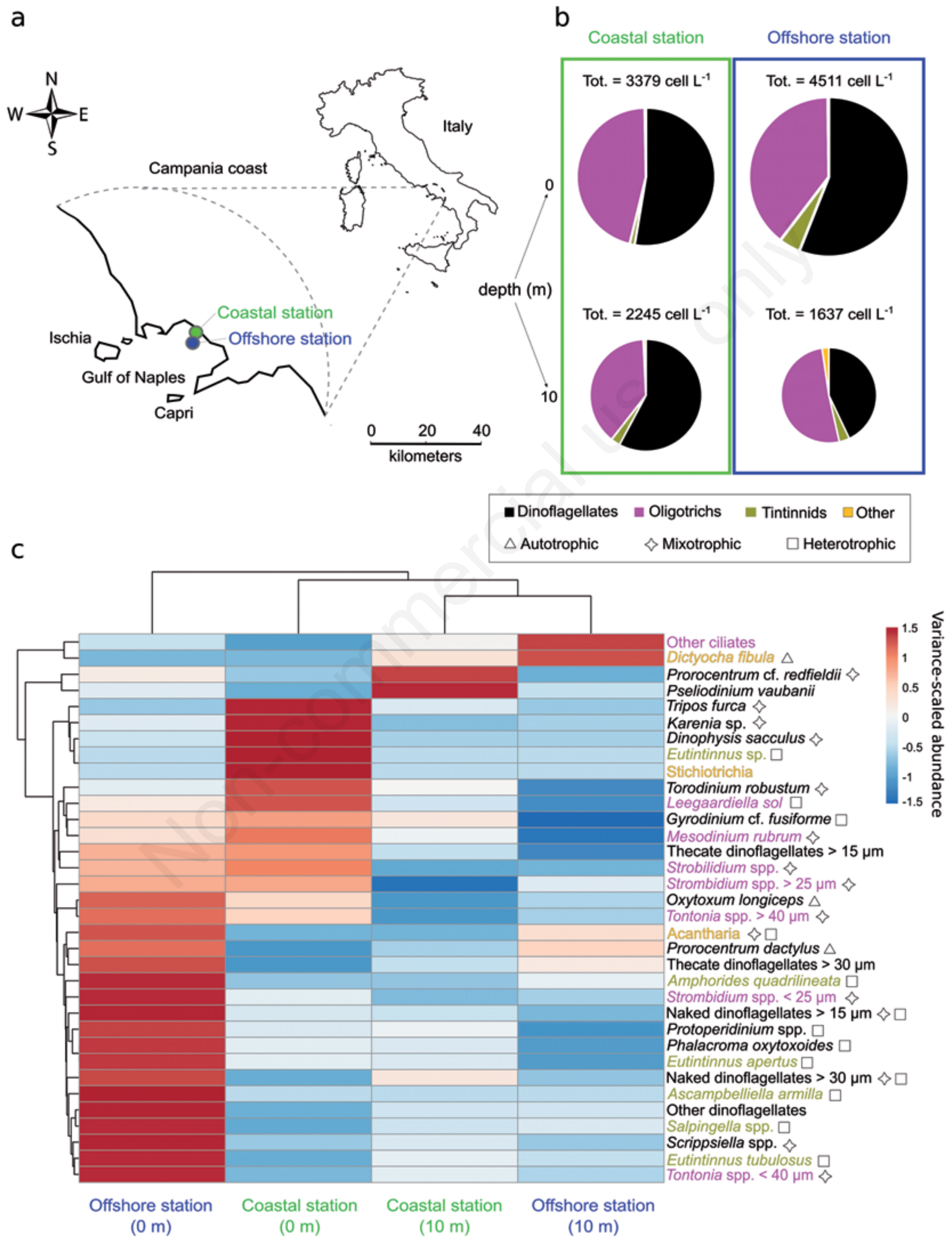

Figure 1. Diversity of protistan grazers in the Gulf of Naples. (a) map of the area and localization of sampling sites. (b) relative abundance of the main taxonomical groups at the two samplings sites and depths. (c) heatmap showing the community composition in different samples. Heatmap's colour scale is indicated on the right. Unit variance scaling is applied, *i.e.*, a difference of +1 or -1 means that the local abundance value for a taxon is one standard deviation away (+ or -) from the average abundance of the taxon considering the whole row.

cruise run on 17 September 2020 and focusing on the characterization of the costal system in the eastern sector of the Gulf of Naples (project FEAMP-ISSPA, funded by the government of Regione Campania, Italy). As these stations were located at different distances from the coast, for more simplicity, we herein refer to FE_11 and FE_12 as coastal station and offshore station (CS and OS), respectively. Both CS and OS were ~5 NM away from LTER-MC (Ribera d'Alcalà *et al.*, 2004; Zingone *et al.*, 2019), which was not part of the cruise mentioned above.

Water column characterization and sampling. At both CS and OS stations, the structure of the water column was described by the acquisition of CTD data, which were obtained with a SeaBird 911 Plus multi-parametric probe with sensors for temperature, conductivity, and other auxiliary proxies, such as dissolved oxygen sensor, fluorescence, water turbidity, and photosynthetically active radiation (PAR). The multi-parametric probe was mounted on a Rosette bearing 12 Niskin bottles with a volume of 10 L. Water samples analysed in the present study were collected from Niskin bottles closed at two depths, 0 and 10 m, in both CS and OS. These depths corresponded to the top and the bottom of the seasonal surface mixed layer, *i.e.*, the water column layer in which a higher concentration of unicellular organisms is expected based on previous research in the study area (*e.g.*, D'Alelio *et al.*, 2015).

Chemical analyses. Samples for dissolved inorganic nutrients were collected directly from the Niskin bottles and immediately stored in 20 ml HDPE vials at -20°C. Chemical analyses were carried out with a continuous flow autoanalyzer (Flow-Sys System), based on an updated version of the protocol developed by Hansen and Grasshoff (1983). For the determination of particulate organic carbon (POC), variable volumes (0,75-1 L) of seawater were filtered on Whatman GF/F pre-combusted (450 °C, 5 h) glass fibre filters immediately stored at -20°C after collection. To remove the inorganic carbonate fraction, filters were exposed overnight to HCl vapours, and then analysed with a Thermo Electron CHN elemental analyser (FlashEA 1112 Series) (Hedges and Stern, 1984). Cyclohexanone-2,4-dinitro[1]phenyl hydrazone was used as a standard. For HPLC pigment analyses, 2-3 L of seawater were filtered (GF/F Whatman - 47 mm diameter) and stored in liquid nitrogen for later analyses, performed with a Hewlett 166 Packard HPLC (1100 Series) according to Vidussi *et al.* (1996). Instrument calibration was carried out with external standard pigments provided by the International Agency for 14C determination-VKI Water Quality Institute. Along with chlorophyll *a* (Chl *a*), some pigments associated to cryptophyte plastids (ß-carotene and alloxanthin) and involved in the kleptoplasty activity by ciliates (Johnson, 2011; Hughes *et al.*, 2021) were analysed.

Biological analyses. Water samples (250 mL) for the identification and enumeration of planktonic protists were fixed with Lugol solution (final concentration 1%), stored in the dark at room temperature, and processed according to Utermöhl's method (Utermöhl, 1958), which is described as follows: i) samples were settled in a graduated cylinder for 48 hours; ii) samples were concentrated to the volume of 100 mL by gently collecting the supernatant through free fall, using a 1 mL pipette connected to a latex tube; iii) after gentle agitation of the cylinder, the concentrated samples were poured into dark bottles and then gently shaken for 150 times; iv) finally, samples (100 mL) were settled in sedimentation chambers for 48 hours. The count was performed over the entire area of the chamber using an inverted optical microscope (Zeiss Axiovert 200) at 20x magnification following standard methods (Utermöhl 1958). Subsamples were considered representative if the count reached 100 individuals. Optical microscopy photographs were taken with ZEISS Axiocam 305 Color. Cell size (only used to categorize broad groups of taxa, see Figure 1c, Supplementary Table 1, and to insert scale

Table 1. Environmental variables of coastal station (CS) and offshore station (OS), at two depths (0 and 10 m).

Environmental variables	Unit	Coastal station (CS)		Offshore station (OS)	
		0 m	10 m	0 m	10 m
Temperature	°C	25.16	24.47	25.37	24.91
Salinity	PSU	37.19	37.87	37.58	38.11
Turbidity	NTU	0.37	0.22	0.33	0.22
Fluorescence	RFU	2.53	1.23	2.04	0.77
Dissolved Oxygen	ml l^{-1}	4.69	4.5	4.95	4.54
PAR	$\mu E\ m^{-2}\ s^{-1}$	84.70	12.31	103.69	31.57
Chl *a*	mg m^{-3}	1.28	0.38	0.55	0.22
POC	mg m^{-3}	223.5	119.21	262.33	99.98
DIN	mmol m^{-3}	4.86	2.22	1.16	0.27
PO_4	mmol m^{-3}	0.14	0.07	0.08	0.04
Bcarotene	mg m^{-3}	0.04	0.004	0.01	0.006
Alloxanthin	mg m^{-3}	0.03	0.01	0.013	0.004

bars into microphotographs) was measured with an eyepiece micrometer. The trophic mode of planktonic protists was identified based on Mitra *et al*. (2014) and Schneider *et al*. (2020) plus many other studies indicated in Supplementary Table 1.

Biodiversity analysis. Multivariate analysis on planktonic protists data matrices was performed using the open-source web platform *ClustVis* (Metsalu and Vilo, 2015). A heatmap was produced to represent differences between the taxonomic composition of planktonic protist community between samples. Both rows and columns in the heatmap were ordered using Euclidean distance and single linkage as a clustering method.

RESULTS

During our sampling, the water column in the Gulf of Naples was moderately stratified, with i) a very shallow freshwater layer driven by land runoff and gliding on seawater at surface, ii) a marked mixed layer exceeding the first 10 m of the water column, and iii) a pycnocline starting at a depth of about 15 m at any sampling station (Supplementary Figure 1). Overall, the water column at CS and OS showed different physical-chemical properties, indicating distinct abiotic and biotic features (Table 1). Specifically, while salinity was higher in offshore and deeper samples, the reverse was true for inorganic nutrients, Chl *a* and other accessory pigments, indicating a resource richer environment towards the coastline and in surface waters. However, the total abundance of planktonic protists with either hetero- or mixotrophic behaviour; complete taxonomical data are shown in Supplementary Table 1 was in the same order of magnitude in all samples, with more similar values between samples taken from the same depth (Figure 1b).

Dinoflagellates were numerically dominant in all samples (relative abundance: 43-58%) except for the one taken from the OS at -10 m, where they were outnumbered by oligotrich ciliates (51.2%). Tintinnids (choreotrich ciliates) reached the highest fraction (4.4%) in the OS at 0 m. Other taxa (*e.g*., *Acantharia*) showed minimum and maximum abundances in surface and deeper waters of the OS (0.2 vs. 2.3%), respectively. As for dinoflagellates, the genera *Protoperidinium*, *Prorocentrum, Gyrodinium*, *Dinophysis* and *Scrippsiella* were dominant. Oligotrichs were mainly represented by the genera *Strombidium*, *Strobilidium*, and *Tontonia*, and by the species *Mesodinium rubrum.* Specimens detected for taxonomical groups mentioned above are shown in Figures 2-3, and their trophic habit is synthesized in Figure 1c and fully referenced in Supplementary Table 1.

According to multivariate analysis performed on taxa abundance data (Figure 1c), a coast-to-offshore differentiation of the protist community investigated was relevant at surface, while deeper samples were very similar to each other and distinct from surface ones. Coastal samples included mostly mixotrophic taxa, while offshore samples encompassed a higher fraction of obligate heterotrophs. Overall, the ratio between potentially photosynthetic and fully heterotrophic protists in the coastal station accounted for 5.0 and 2.3, at 0 and -10 m, respectively; in the offshore station, this ratio accounted for 2.4 and 2.8, at 0 and -10 m, respectively.

Data indicated a substantial reshuffling of the planktonic protist community between different environmental/trophic conditions. Indeed, the Chl *a*/POC ratio near the coast was two folds higher than offshore (Table 1), indicating that the microbial compartment was richer in autotrophic cells in the first than in the second. A similar pattern was found for photosynthetic pigments associated with kleptoplasty (*i.e*., ß-carotene and alloxanthin from cryptophytes) and therefore mixotrophic ciliates.

DISCUSSION

In our preliminary survey, based on one sampling, in one date, during the late summer/early autumn season in the Gulf of Naples, the mixo-/protozoo-plankton community was quantitatively dominated by dinoflagellates and oligotrich ciliates, most of which included mixotrophic genera and species. Tintinnids, which are obligate heterotrophs, were far less abundant, while other minor taxa, such as Radiolaria, were extremely less represented. With the sole exception of the tintinnid *Ascampbeliella armilla*, all taxa we detected have been already reported in the Gulf of Naples during the period of interest (Modigh, 2001; Modigh and Castaldo, 2002; Ribera d'Alcalà *et al*., 2004; Piredda *et al*., 2017). Though never reported before in the Gulf of Naples, *A. armilla* was already found in subtropical/temperate waters, *e.g*., in the Mediterranean Sea, since 1951 (Duran, 1951) and more recently from the Bay of Villefranche (France) (Dolan, 2017) and from the Bay of Mersin (Turkey) (Polat *et al*., 2019).

Most taxa detected in our study were reported as mixotrophic, and these latter were often associated with mature aquatic ecosystems (Mitra *et al.*, 2014; Schneider *et al*., 2020), in which these organisms contribute to a big part of organic nitrogen flows (Gutiérrez-Rodríguez *et al*., 2014). The potential access of mixotrophs to more resources than those that are available to strictly phototrophic or heterotrophic protists is crucial during non-bloom phases, when the aquatic system shifts from net autotrophy to net heterotrophy (Haraguchi *et al*., 2018). Such conditions are typical of late summer/early autumn in the Gulf of Naples, with September being a 'non-bloom month' of transitions between i) the late spring/summer peaks of phytoplankton occurring in a condition of fully stratified water, and ii) the mid-autumn and more ephemeral phytoplankton peak occurring in the surface layer of a partially mixed water column during the so-called Saint Martin's summer (Zingone *et al*., 1995; Ribera d'Alcalà *et al*., 2004; Zingone *et al*., 2019).

We are sure to have sampled planktonic protists in the above-mentioned non-bloom phase, based on the physical-chemical data we collected as well. Firstly, the seasonal thermo-/pycnocline (*i.e*., the water layer showing the largest variation of temperature and density) started around -15 m, *i.e*., the same pattern as that reported at LTER-MC in the Gulf of Naples over the last four decades (Ribera d'Alcalà *et al.*, 2004; Kokoszka *et al*., 2021). Secondly, Chl *a* values (*i.e.*, 0.22-1.28 mg m^{-3}) in all samples were significantly below the minimum associated to phytoplankton blooms (i.e., > ~1.7 mg m^{-3}) in the area (Margiotta *et al*., 2013; D'Alelio *et al*., 2015). Based on the consideration above, the overall dominance of mixotrophs in the surface layer of the Gulf of Naples during the late summer/early autumn appears as motivated by the dominance of non-bloom conditions.

Non-bloom conditions may favour mixotrophic dinoflagellates and ciliates, for which phototrophy and phagotrophy contribute in somewhat equal extent to the biomass production (Jeong *et al.*, 2010; plus estimations made by D'Alelio *et al*., 2016b). Most mixotrophic dinoflagellates, despite bearing innate plastids, can ingest a variety

Figure 2. Dinoflagellates from the Gulf of Naples. (a) *Prorocentrum gracile*; (b) *Prorocentrum dactylus*; (c) *Tripos furca*; (d) *Protoperidinium* sp.; (e) *Dinophysis sacculus*; (f) *Phalacroma oxytoxoides*; (g) *Karenia* sp.; (h) *Pselodinium vaubanii*; (i) *Gyrodinium* sp. Scale bars: 50 µm in (c); 20 µm in (a, e, h, i); 10 µm in (b, d, f, g).

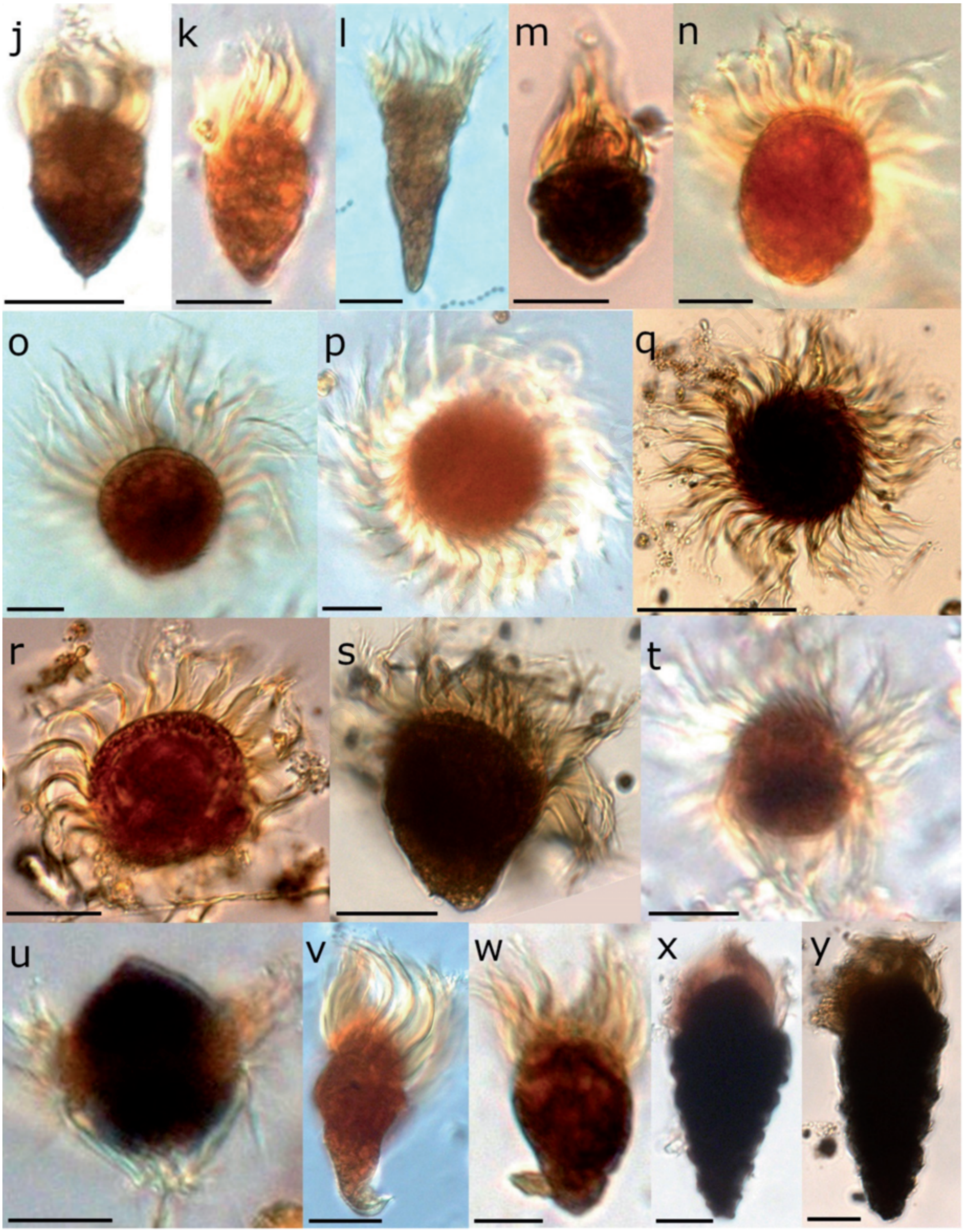

Figure 3. Oligotrich ciliates from the Gulf of Naples. (j-m) *Strombidium* spp.; (n-o) *Strobilidium* spp.; (p-r) *Leegardiella sol*; (s) *Pelagostrobilidium neptuni*.; (t-u) *Mesodinium rubrum*; (v-w) *Tontonia* spp.; (x-y) *Laboea strobila*. Scale bars: 50 µm in (q); 20 µm in (j, m, r, s, v, x, y); 10 µm in (k, l, n, o, p, t, u, w).

of nanoplankton preys as well, such as prymnesiophytes, small dinoflagellates, cryptophytes and raphidophytes (Jeong *et al.*, 2005). Concerning mixotrophic ciliates, since both photosynthesis and phagotrophy sustain their growth, they will get a benefit over completely heterotrophic ciliates in the photic zone of areas with relatively low microbial preys but sufficient amounts of inorganic nutrients (Maselli *et al*., 2020). Mixotrophic ciliates sequester chloroplasts from ingested phytoflagellates as they are non-constitutive mixotrophs (Dolan and Pérez, 2000; Esteban *et al.*, 2010). Most of them are unable to induce plastid replication and must acquire new plastids after cellular division (Stoecker *et al.*, 2009). Pico- and nano-eukaryotes (cell size = 2-12 µm), more abundant in absence of blooms of larger phytoplankton, can be easily captured, and their photosynthetic organelles retained, by mixotrophic ciliates (Haraguchi *et al.*, 2018; Maselli *et al.*, 2020).

In our study and based on compositional features of the community investigated, we also detected a coast-to-offshore differentiation in mixo-/protozooplankton groups (Figure 1c). In the coastal and nutrient richer station, we found a prevalence of mixotrophic dinoflagellates like those in the genera *Prorocentrum* (Jeong et al., 2005), *Dinophysis* (Caroppo, 2001), *Pseliodinium* (Figure 2h) (Gomez, 2018), and *Torodinium* (Gómez *et al.*, 2016), and of the species *Tripos furca* (Figure 2c) – this latter being a photoautotroph that can also feed on ciliates if constrained by limited nutrients conditions (Smalley *et al.*, 2003; Hansen, 2011). On the contrary, the less nutrient-rich offshore waters showed higher abundances of strictly heterotrophic organisms, like (i) phagotrophic dinoflagellates, *e.g.*, *Phalacroma oxytoxoides* and *Protoperidinium* sp. (Figure 2f and 2d; trophic modes identified by, respectively, Park *et al*., 2011 and Jeong *et al*., 2010)*,* and (ii) tintinnids, *e.g.*, *Eutintinnus tubulosus* and *Salpingella* spp. (for trophic modes, see Dolan, 2010; Dolan *et al.*, 2019).

The pattern described above can be explained by the presence, along the transect investigated, of different trophic environments (see *e.g*., D'Alelio *et al.*, 2016a); this hypothesis is further supported by the spatial distribution of *M. rubrum* (Figure 3 t-u), which shows higher abundance at the coastal and nutrient richer site. This species depends heavily upon phototrophy, as up to 90% of its biomass production relies on photosynthesis (Stoecker *et al*., 2009; plus estimation made by D'Alelio *et al.*, 2016b). Being a photoautotroph, it prefers coastal waters, characterized by higher nutrient concentration and lower salinity (Johnson *et al*., 2013). As a non-constitutive mixotroph, *M. rubrum* must acquire plastids from cryptophytes (Smith and Hansen, 2007), whose characteristic pigments in our study peaked at coast as well (Table 1). *M. rubrum* cannot grow indefinitely in the light without its preferred cryptophyte prey and it cannot grow in the dark even if supplied with copious food (Johnson *et al*., 2007): even though we collected a limited number of samples, it is remarkable that we found the highest abundance of *M. rubrum* at the top of the mixed layer (Figure 1b), where light is not limiting, while we found its lowest abundance at depth in the offshore station, where both light and pigments of plastid-providing cryptophytes were at the lowest.

In conclusion, our study reports further evidence of the multifaceted nature of planktonic protists in temperate coastal areas such as the Gulf of Naples. Adding to a high taxonomical diversity, we detected signs of community structuring shaped by the different abundance of distinct functional types, in relation with environmental and trophic conditions. Our study calls for further investigation of the ecology of mixo-/protozoo-plankton at basin and regional scales, due to the relevance of facultative and obligate heterotrophic protists, including mixotrophs, in the circulation of matter and energy in the planktonic food web of coastal systems characterized by intermittent resources.

Corresponding author: gabriele.delgaizo@szn.it; domenico.dalelio@szn.it

Keywords: Plankton; protozoa; dinoflagellates; ciliates; tintinnids; mixotrophy.

Authors' contributions: DDA and IP supervised research. FM and FC planned oceanographic sampling. GDG counted plankton samples, with the help of IP. LR collected information pertaining to trophic modes. MA, AB, MF, MV, FM, MS analysed chemical samples. SS and FC provided physical data. DDA and GDG wrote the paper, with the help of LR and IP. All the authors have read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

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

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

Acknowledgments: This study was supported by the project PO FEAMP 2014/2020 (Misura 2.51) funded by Regione Campania, Italy. The authors thank the Stazione Zoologica Unit for the Access to Marine Ecosystems and Environmental Analysis for organizing and carrying out water sampling. Two anonymous reviewers are gratefully acknowledged for their precious comments that helped to improve the quality of this paper.

Received: 10 August 2021.
Accepted: 22 November 2021.

Licensee PAGEPress, Italy
Advances in Oceanography and Limnology, 2021; 12:10018
DOI: 10.4081/aiol.2021.10018

REFERENCES

Caroppo C, 2001. Autoecology and morphological variability of *Dinophysis sacculus* (Dinophyceae: Dinophysiaceae) in a mediterranean lagoon. Journal of the Marine Biological Association of the United Kingdom 81:11–21.

D'Alelio D, Mazzocchi MG, Montresor M, Sarno D, Zingone A, Di Capua I, et al., 2015. The green-blue swing: plasticity of plankton food-webs inresponse to coastal oceanographic dynamics. Marine Ecology 36.4: 1155-1170.

D'Alelio D, Libralato S, Wyatt T, Ribera d'Alcalà M, 2016a. Ecological-network models link diversity, structure and function in the plankton food-web. Scientific Reports 6:1–13.

D'Alelio D, Montresor M, Mazzocchi MG, Margiotta F, Sarno D, Ribera d'Alcalà M, 2016b. Plankton food-webs: to what extent can they be simplified? Advances in Oceanography and Limnology 7:67–92.

Dolan JR, 2010. Morphology and ecology in tintinnid ciliates of the marine plankton: correlates of lorica dimensions. Acta Protozoologica 49:235–244.

Dolan JR, 2017. Historical trends in the species inventory of tintinnids (ciliates of the microzooplankton) in the Bay of Villefranche (NW Mediterranean Sea): shifting baselines. European journal of protistology 57:16-25.

Dolan JR, Ciobanu M, Marro S, Coppola L, Ji R, 2019. An exploratory study of heterotrophic protists of the mesopelagic Mediterranean Sea. ICES Journal of Marine Science 76:616–625.

Dolan JR, Pérez MT, 2000. Costs, benefits and characteristics of mixotrophy in marine oligotrichs. Freshwater Biology 45:227–238.

Durán M, 1951. [Contribución al estudio de los tintínidos del plancton de las costas de Castellón (Mediterráneo occidental)].[Article in Spanish].

Esteban GF, Fenchel T, Finlay BJ, 2010. Mixotrophy in ciliates. Protist 161:621–641.

Flynn KJ, Mitra A, Anestis K, Anschütz AA, Calbet A, Ferreira GD, 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 KJ, Stoecker DK, Mitra A, Raven JA, Glibert PM, Hansen PJ, 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.

Gomez F, 2018. Redefinition of *Ceratoperidinium* and *Pseliodinium* (Ceratoperidiniaceae, dinophyceae) including reassignment of *Gymnodinium fusus*, *Cochlodinium helix* and *C. pirum* to *Pseliodinium*. CICIMAR Oceánides 33:1–11.

Gómez F, Takayama H, Moreira D, López-García P, 2016. Unarmoured dinoflagellates with a small hyposome: *Torodinium* and *Lebouridinium* gen. nov. for *Katodinium glaucum* (Gymnodiniales, Dinophyceae). European Journal of Phycology 51:226–241.

Gutiérrez-Rodríguez A, Décima M, Popp BN, Landry MR, 2014. Isotopic invisibility of protozoan trophic steps in marine food webs. Limnology and Oceanography 59:1590–1598.

Hansen HP, 1983. Automated chemical analysis, p.347-395. In: Grasshoff K, Kremling K, Ehrhardt M (eds.), Methods of seawater analysis.

Hansen PJ, 2011. The role of photosynthesis and food uptake for the growth of marine mixotrophic dinoflagellates. Journal of Eukaryotic Microbiology 58:203–214.

Haraguchi L, Jakobsen HH, Lundholm N, Carstensen J, 2018. Phytoplankton community dynamic: a driver for ciliate trophic strategies. Frontiers in Marine Science 5:1–16.

Hedges JI, Stern JH, 1984. Carbon and nitrogen determinations of carbonate□containing solids. Limnology and oceanography 29:657-663.

Hughes EA, Maselli M, Sørensen H, Hansen PJ, 2021. Metabolic reliance on photosynthesis depends on both irradiance and prey availability in the mixotrophic ciliate, *Strombidium* cf. *basimorphum*. Frontiers in Microbiology 12:1577.

Jeong HJ, Du Yoo Y, Kim JS, Seong KA, Kang NS, Kim TH, 2010. Growth, feeding and ecological roles of the mixotrophic and heterotrophic dinoflagellates in marine planktonic food webs. Ocean Science Journal 45:65–91.

Jeong HJ, Yeong DY, Jae YP, Jae YS, Seong TK, Seung HL, et al., 2005. Feeding by phototrophic red-tide dinoflagellates: five species newly revealed and six species previously known to be mixotrophic. Aquatic Microbial Ecology 40:133–150.

Johnson MD, 2011. Acquired phototrophy in ciliates: a review of cellular interactions and structural adaptations. Journal of Eukaryotic Microbiology 58:185-195.

Johnson MD, Oldach D, Delwiche CF, Stoecker DK, 2007. Retention of transcriptionally active cryptophyte nuclei by the ciliate *Myrionecta rubra*. Nature 445:426–428.

Johnson MD, Stoecker DK, Marshall HG, 2013. Seasonal dynamics of *Mesodinium rubrum* in Chesapeake Bay. Journal of Plankton Research 35:877–893.

Kokoszka F, Iudicone D, Zingone A, Saggiomo V, Ribera d'Alcalà M, Conversano F, 2021. Advances in Oceanography and Limnology (AIOL Journal) (in press).

Leles SG, Bruggeman J, Polimene L, Blackford J, Flynn KJ, Mitra A, 2021. Differences in physiology explain succession of mixoplankton functional types and affect carbon fluxes in temperate seas. Progress in Oceanography 190:102481.

Margiotta F, Mangoni O, Santarpia I, Saggiomo M, Saggiomo V, Casotti R, 2013. Biologia Marina Mediterranea 20:214.

Maselli M, Altenburger A, Stoecker DK, Hansen PJ, 2020. Ecophysiological traits of mixotrophic *Strombidium* spp. Journal of Plankton Research 42:485–496.

Metsalu T, Vilo J, 2015. ClustVis: a web tool for visualizing clustering of multivariate data using principal component analysis and heatmap. Nucleic Acids Research 43:566–570.

Mitra A, Castellani C, Gentleman WC, Jónasdóttir SH, Flynn KJ, 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 KJ, Tillmann U, Raven JA, Caron D, Stoecker DK, et al., 2016. Defining planktonic protist functional groups on mechanisms for energy and nutrient acquisition: incorporation of diverse mixotrophic strategies. Protist 167:106–120.

Modigh M, 2001. Seasonal variations of photosynthetic ciliates at a Mediterranean coastal site. Aquatic Microbial Ecology 23:163–175.

Modigh M, Castaldo S, 2002. Variability and persistence in tintinnid assemblages at a Mediterranean coastal site. Aquatic Microbial Ecology 28:299–311.

Park MG, Lee H, Kim KY, Kim S, 2011. Feeding behavior, spatial distribution and phylogenetic affinities of the heterotrophic dinoflagellate *Oxyphysis oxytoxoides*. Aquatic Microbial Ecology 62:279–287.

Piredda R, Tomasino MP, D'Erchia AM, Manzari C, Pesole G, Montresor M, et al., 2017. Diversity and temporal patterns of planktonic protist assemblages at a Mediterranean long-term ecological research site. FEMS Microbiology Ecology 93:fiw200.

Polat S, Kurt TT, Tuğrul S, 2019. Spatial and temporal variations of tintinnids (Ciliata: Protozoa) in the Bay of Mersin, Northeastern Mediterranean Sea. Mediterranean Marine Science 20:342-356.

Ribera d'Alcalà M, Conversano F, Corato F, Licandro P, Mangoni O, Marino D, et al., 2004. Seasonal patterns in plankton communities in pluriannual time series at a coastal Mediterranean site (Gulf of Naples): an attempt to discern recurrences and trends. Scientia Marina 68:65–83.

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

Smalley GW, Coats DW, Stoecker DK, 2003. Feeding in the mixotrophic dinoflagellate *Ceratium furca* is influenced by intracellular nutrient concentrations. Marine Ecology Progress Series 262:137–151.

Smith M, Hansen PJ, 2007. Interaction between *Mesodinium rubrum* and its prey: importance of prey concentration, irradiance and pH. Marine Ecology Progress Series 338: 61–70.

Stoecker DK, Hansen PJ, Caron DA, Mitra A, 2017. Mixotrophy in the marine plankton. Annual Review of Marine Science 9:311-335.

Stoecker DK, Johnson MD, De Vargas C, Not F, 2009. Acquired phototrophy in aquatic protists. Aquatic Microbial Ecology 57:279–310.

Utermöhl H, 1958. Toward the improvement of the quantitative phytoplankton method. Mitteilungen-Internationale Vereiningung fur Limnologie 9:1–38.

Vidussi F, Claustre H, Bustillos-Guzmàn J, Cailliau C, Marty JC, 1996. Determination of chlorophylls and carotenoids of marine phytoplankton: separation of chlorophyll a from divinylchlorophyll a and zeaxanthin from lutein. Journal of plankton research 18:2377-2382.

Zingone A, Casotti R, Ribera d'Alcalà M, Scardi M, Marino D, 1995. 'St Martin's Summer': the case of an autumn phytoplankton bloom in the Gulf of Naples (Mediterranean Sea). Journal of Plankton Research 17:575-593.

Zingone A, D'Alelio D, Mazzocchi MG, Sarno D, 2019. Time series and beyond: multifaceted plankton research at a marine Mediterranean LTER site. Nature Conservation 310:273–310.