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John R. Dolan[a], Maria Ciobanu[a], and Laurent Coppola[a]

a. Laboratoire d'Océanographie de Villefranche-sur-Mer, Sorbonne Université, CNRS UMR 7093, Station Zoologique, 06230 Villefranche-sur-Mer, France

Correspondence
John R. Dolan, LOV, CNRS, Station Zoologique, 06230 Villefranche-sur-Mer, France
Telephone number: +33 4 93 76 38 22; FAX number +33 4 93 76 38 34; e-mail: dolan@obs-vlfr.fr

ABSTRACT
The deep sea has long been a mysterious and attractive habitat for protistologists. However logistical difficulties severely limit sampling opportunities. Consequently, our knowledge of the protists in the deep sea, (arguably the largest habitat on earth), is relatively sparse. Here we present a unique time-series concerning 3 different protist taxa that share only the characteristics of being relatively large, robust to sampling, and easily identifiable to species level using light microscopy: tintinnid ciliates, phaeogromid cercozoans (e.g. Challengerids) and amphisolenid dinoflagellates. We sampled a near-shore deep water site in the N.W. Mediterranean Sea at 250 m depth over a two-year period at approximately weekly intervals from January 2017 to December 2018. To our knowledge, no previous studies have employed sampling on a similar time scale. We found taxa that appear to be restricted to deep waters, distinct seasonal patterns of abundance in some taxa, and in others non-seasonal successional patterns. Based on data from sampling following a flash flood event, the Challengerid population appeared to respond positively to a pulse of terrigenous input. Some of the distinct mesopelagic tintinnid ciliates and amphisolinid dinoflagellates were also found in 2 samples from the North Atlantic mesopelagic gathered from near the Azores Islands in September 2018. We conclude that there are a variety of protist taxa endemic to the mesopelagic, that the populations are dynamic, and they may be widely distributed in the deep waters of the world ocean.

Keywords: Amphisolinidae; Deep-Sea; Phaeogromidae; Plankton; Time-series; Tintinnida

[1]Article based on a Past-President's address given at the annual meeting of the International Society of Protistologists held jointly with the Phycological Society of America at the University of British Columbia in Vancouver, July 29- August 2, 2018.

INTRODUCTION

The search for protists in the deep sea has a long and distinguished history. Likely the first publication was Ehrenberg's "On microscopic life in the Ocean at the South Pole and at Considerable Depth" in which he described apparently living organisms from surface water (diatoms) in samples taken from 300 - 500 m depth (Ehrenberg 1844). Interestingly, this finding of diatoms in good shape at great depths was re-discovered just a few years ago (Agusti et al. 2015). A few years after Ehrenberg's discoveries, Bailey reported "vast numbers of Globigerina" as well as diverse diatoms from material recovered from soundings in depths down to about 200 m on the North Atlantic coast of the U.S. (Bailey 1851). Ehrenberg later published descriptions of new forms, some now known as tintinnids and radiolarians, from bottom material brought up from deep waters in the North Atlantic (Ehrenberg 1854). Shortly after, Bailey described new "microscopic forms" from soundings taken at depths ranging from 1600 - 2700 m in the North Pacific (Bailey 1856) and from across the Atlantic between the Canada coast and Ireland (Bailey 1857). From Mediterranean deep water mud samples, Ehrenberg described more new forms (Ehrenberg 1858). South Pacific deep water bottom samples were investigated by Ehrenberg (1861) and Harting (1863). Over about 20 years, protists were catalogued from a very wide range of deep sea sites.

Not long after the early protistological studies of Ehrenberg, Bailey and Harting was the Challenger Expedition (1872-1876). It famously opened the era of modern oceanography. Haeckel spent 10 years working on the deep water samples of the Challenger Expedition to produce his iconic and massive monographs of the Radiolaria (Haeckel 1887). The reports of diverse organisms found in the deep water trawls of the Challenger Expedition clearly established the suspected existence of a benthic fauna endemic to the deep sea. However, the existence of pelagic taxa (protist and multicellular), endemic to deep waters remained a debated matter well into the 20th century. Haeckel believed that there were radiolarians specific to certain deep sea depth strata but acknowledged that in the absence of closing plankton net samples (which did not exist at the time of the Challanger) the question of a deep sea pelagic radiolarian fauna was an open question (Haeckel, 1887, pg. cliii). The strongly opposing view was the school of Louis Agassiz, arguing that there was a vast life-less "azoic zone" between the surface waters and the deep-sea benthos as the devices he used failed to bring much up from deep water . Carl Chun and Karl Brandt championed the existence of a diverse and unique deep sea fauna, including protists (e.g., Fowler 1898). The arguments of Agassiz that alleged deep water forms were simply contaminants from surface waters were finally put to rest with the development of oceanographic devices allowing sampling at discrete depths with certainty of no contamination from surface waters (Mills 1980). Those first devices used were closing plankton nets and they were extensively employed during the Valdivia deep sea expedition 1898-1899 (Chun 1903) vindicating Haeckel's 1887 opinion. Chun's popular account of the expedition, "From the Depths of the Sea" (Chun 1903) included not only illustrations of deep sea fish but radiolarians as well (Fig. 1).

Fig. 1. The cover of the 1903 edition of Carl Chun's popular account of the Valdivia Expedition 1898-1899 "*Aus den Tiefen des Weltmeeres*" (From the Depths of the Ocean). Inset shows the illustration of radiolairians from the Indian Ocean on page 314.

The proposition that there are several vertical faunal zones in the sea, (from the surface down to abyssal depths of 1000-5000m), was first based on the distributions of radiolarians by Haecker (Russell 1927). Previous schemes simply divided the ocean into an illuminated surface layer, a twilight layer and the dark depths (e.g. Haeckel 1887; Lobianco 1903). Haecker (1908), based on collections made during the Valdivia expedition, defined several oceanic depth strata as Collodarian ("Collidenschicht" 0-50 m), Challengerid ("Challengeridentschicht" 50-400 m), etc. Now it is generally recognized that there are planktonic protists that are found in more or less specific depth strata in deep marine waters. This has been especially well documented in radiolaria (e.g., Boltovskoy 2017) as well as foraminifera (Rebotim et al. 2017 and references therein). Molecular studies have shown in at least one species of foraminifera, genetically distinct, 'cryptic species', inhabit different strata of deep waters (Weiner et al. 2012).

Outside the world of protistology, currently there is growing interest in the general ecology of the mesopelagic zone, the depth strata between 200 and 1000 m separating the illuminated water of the photic zone wherein photosynthesis occurs, and the completely dark, cold, and immense bathypelagic realm. Firstly, this reflects renewed interest in the "biological carbon pump" (the transit to, and long-term sequestration of organic carbon in, the bathypelagic) and more specifically, how the "pump" may be altered by climate change (e.g., Honjo et al. 2014; Boyd et al. 2017). Secondly, there is the overt recognition that our knowledge of the ecology of mesopelagic realm is actually sketchy (Costello & Breyer 2017; St John et al. 2016). Questions raised decades ago concerning

exactly how the mesopelagic food web is fueled (e.g., Marshall 1979; Kimor 2002) remain unanswered today. For example, measures of the flux of particulate carbon into the mesopelagic appear to be largely insufficient to meet estimates of respiratory energy demands of mesopelagic organisms (Burd et al. 2010; Robinson et al. 2010). Large uncertainty exists even with regard to the biomasses in the mesopelagic. New estimates of the biomass of myctophids, the small mesopelagic fish, the most numerous vertebrates on the planet, are an order of magnitude higher than previous estimates (Irigoien et al. 2014).

In recent years the protist fauna of the mesopelagic has not been neglected by protistologists (e.g., Edgcomb 2016). A few studies have even provided data on specific species of nanoflagellates (Arndt et al. 2003), radiolarians (Ikenoue et al. 2015); tintinnids (Krsinic & Grbec 2006), and rates of bactivory (Pachiadaki et al. 2016) in the mesopelagic. However, for the most part, studies have generated data of two basic types. The first basic type is cell count data of large aggregate heterogeneous groups of diverse ecologies or phylogenetic affinities (i.e., "nano-sized flagellates", "ciliates", "dinoflagellates", "sarcodines"). The second type is rRNA sequence occurrence data, with the vast majority of sequences assigned to high-level taxa as many taxa are not well-represented in sequence databases.

Abundances (cell counts) of aggregate groups have been reported for a very wide range of localities. From across the world ocean between 30°N and 30°S from the Malaspina program (Pernice et al. 2015), to the subarctic Pacific (Fukada et al. 2007), the North West Pacific (Yamaguchi et al. 2002; 2004), the Tropical N Pacific to Arctic (Sohrin et al. 2010), the subtropical & tropical Atlantic (Morgan-Smith et al. 2011), sites around the Canary Islands (Boras et al. 2010), the Tropical & South Atlantic (Rocke et al. 2015), the Eastern and Western Mediterranean Sea (Rocke et al. 2015; Tanaka & Rassoulzadegan 2002), and the Ross Sea in Antarctica (Safi et al. 2012).

Genetic surveys have also included a variety of mesopelagic sites ranging from the pioneering work of Lopez-Garcia et al (2001) in Antarctic waters, the Antarctic Ross Sea (Zoccarato et al. 2016) to the Sargasso Sea (Not et al. 2007), the North Atlantic (Grattepanche et al. 2016), the Eastern Tropical Pacific (Duret et al. 2015), Norwegian Sea deep water coral reefs (Jensen et al. 2012), submarine canyons of the N. W. Mediterranean Sea (Celussi et al. 2018), the South China Sea (Xu et al. 2017), and the West Pacific (Zhao et al. 2017). Taken as a whole, the studies, whether reporting on count or sequence data, suggest a very large variability in mesopelagic protist communities across various systems with reported concentrations varying by over an order of magnitude. Differences in general patterns such as changes in diversity with depth have been reported (Grattepanche et al. 2016). Notably, all of these studies are 'snapshots' sampling a given site a single time.

Time-series studies have long been acknowledged as invaluable in plankton studies as evidenced by the remarks of Ernst Haeckel (1891):

> *"To obtain a complete and more certain survey of the temporary variations of plankton composition requires an unbroken series of observations, carried on at one and the same place at least for the space of a full year—still better for several successive years—to obtain from the yearly and monthly oscillations a general average.*

[...] As there are good and bad wine and fruit years, so there are rich and barren plankton years".

To our knowledge, only a few studies have examined temporal variability in mesopelagic protists. Sequence-based studies employing sampling from monthly to quarterly intervals have been conducted in the San Pedro Channel of the California coast (Countway et al. 2010; Kim et al. 2014; Hu et al. 2016) and in the Canadian Arctic on several dates in different seasons (Terrado et al. 2009). The Arctic study found large seasonal differences in the composition of the mesopelagic protist assemblage. In contrast, the three San Pedro Channel studies all reported that variability was minimal in deep water communities (150, 500m depth) compared to the surface water communities which showed large seasonal changes. A study by Gowing et al. of mesopelagic protists in the Arabian Sea, based on microscopic examination of material, found relatively small differences in the abundance and biomass in different monsoonal seasons (Gowing et al. 2003). These time-series studies present then conflicting views of mesopelagic protist communities as either seasonally variable or fairly invariant.

In a previous paper, our "exploratory study" (Dolan et al. 2017), we presented data showing remarkable changes in particular groups of protists related to seasonal changes in the structure of the water column in a near-shore deep water site in the N.W. Mediterranean Sea. We sampled at weekly intervals from early January 2017 to early June 2017 in both surface and deep waters. We focused on 3 groups in which species identification is relatively easy using light microscopy: tintinnid ciliates, phaeogromid cercozoans (Challengerids), and amphisolenid dinoflagellates. These protists of the microzooplankton are all not only relatively large, but also occur in very low concentrations, compared to those of nano and pico-plankton typically targeted in sequence-based studies.

We found that the mesopelagic tintinnid assemblage was composed of species found in the surface layer as well species apparently restricted to the mesopelagic. The deep-water species were a minor component of the assemblage during the winter mixing of the water column but dominated the assemblage when the water column stratification separates the surface and mesopelagic strata. The phaeogromids, nearly absent from surface waters, reacted negatively to winter water column mixing, declining to very low concentrations. We found a shift in the species composition of the amphisolinid dinoflagellates from dominance by species of *Amphisolenia* in the winter to dominance by *Triplosolenia* with water column stratification.

Our preliminary study showed then the existence of a temporally variable deep-water protist fauna. Consequently, we continued sampling and here report on data collected over 24 months, from early January 2017 to late December 2018, representing 85 sampling dates (the previous report was based 20 dates). Our goals were 1) to confirm apparent seasonal changes in composition reported in our preliminary study, 2) to obtain a more complete catalogue of the mesopelagic forms, and 3) to assess both the seasonality of species compositions as well as the inter-annual variability of mesopelagic assemblages. We also present data on mesopelagic protists from 2 sites near the Azores Islands (North Atlantic) that allow a preliminary comparison of protist assemblages from distant sites to evaluate the hypothesis that deep water taxa we encountered may have wide geographic ranges.

MATERIALS AND METHODS

Details of our sampling protocol, sample processing and analysis, as well as data analyses are given in Dolan et al. (2017). Here we will but briefly summarize the method and materials previously described. Our study site is "Point C", a deep water standard sampling site of the French SOMLIT monitoring program ("Service d'Observation en Milieu LITtoral", SOMLIT/ILICO, the French Coastal Monitoring Network). The site is located about 1 km off the Cap de Nice ((43°51'00''N, 07°19'00''E), near the entrance to the Bay of Villefranche (NW Mediterranean Sea). The SOMLIT program includes weekly water column profiles of salinity, temperature and oxygen down to depth of 300m at the sampling site obtaining using a CTD probe. Data from the CTD profiles were used to characterize the water column at the sampling site in terms of water column stratification to distinguish periods of mixis from stratification, as detailed in Dolan et al. 2017. Briefly, a stratification index was calculated based on the difference in potential density between 10m and 300m following Behrenfeld et al. (2006), Dave and Lozier (2010), and Lozier et al. (2011); if the difference is <0.125 kgm^3, the upper 300m can be considered as non-stratified or mixed (de Boyer Montégut et al., 2004).

Our weekly sampling was conducted on the same or following day as the SOMILT program. Over the two-year period, we sampled 85 times with missing dates due to bad weather or unavailability of the boat due mechanical problems or maintenance. We obtained water samples using a 30 L Niskin bottle. The Niskin bottle was gently emptied, by prying open the lower cap, into a 20 µm mesh plankton net suspended in a 30 L bucket. For the mesopelagic depth (250 m), 8 bottle casts totaling 240 L were emptied into the plankton net. The net cod end material was fixed immediately with Lugol's (2% final concentration). The net was then thoroughly rinsed, to avoid sample contamination before sampling the surface layer at 30 m depth. In the lab, concentrated material from the plankton net samples of water from 250 m depth was examined in aliquots of 1-3 ml using an inverted microscope until material from an initial volume of approximately 100 L was analyzed. Similarly, material from about 20 liters initial volume from samples taken from 30 m depth was also examined. However, in this report we report only data from the 250 m sampling. The material from 30 m depth sampling was used only to establish co-occurrence in surface layer or restriction to deep water.

We distinguished tintinnid taxa as either endemic to the mesopelagic "deep water" or in contrast forms primarily found in the surface mixed layer "surf" but occur also in deep waters. The assignation of tintinnid taxa broadly followed the criteria given in Dolan et al. (2017). Briefly, deep water species fulfilled 3 conditions: 1) found in deep water samples, 2) not found in surface samples on more than 2 dates, and 3) if found in surface water sample concentrations were trace (i.e., 1 cell). The contrasting species group, those from the surface mixed layer ("surf") found in deep waters, were forms fulfilling 2 conditions: 1) found in higher concentrations in surface compared to deep water samples, and 2) occurred more often in surface water samples than in deep water samples. Amphisolenid dinoflagellates and phaeogromid cercozoans were rarely found in surface waters thus there were no apparent surface layer species.

Oligotrich ciliates larger than 20 µm in longest dimension were also enumerated. Oligotrichs smaller than 20 µm were abundant and frequently encountered but not recorded as water samples were initially concentrated using a 20 µm plankton net. Statistical analysis was restricted to simple t-tests and correlations.

We recognize that examination of material from larger volumes of water taken from 250m compared to 30m results in different detection limits. The possibility exists that mesopelagic forms are not completely absent from the surface mixed layer but were exceedingly rare in the surface mixed layer samples.

Material from 2 sites near the Azores Islands in the North Atlantic was also analyzed. Sampling was graciously done by Hartmut Arndt onboard the Meteor, cruise M150. Niskin bottle samples were obtained from 250 m depth, 100 - 120 L were filtered through a 10 µm plankton net and the net cod end material fixed with Lugol's solution. One site was about 500 m total depth (38°48'43''N, 27°03'06''W) sampled on Sept. 12, 2018, the other was 1000 m total depth (36°53'93''N, 25°07'19''W) and was sampled on Sept. 18, 2018. Concentrated material was examined as described above and all material of the samples was examined.

RESULTS

Tintinnid Ciliates

A total of 81 morphologically distinct forms, putative species, were found in deep water samples. Of the 81, those apparently not endemic to deep waters, i.e., the "surf" taxa found in greater concentrations and more frequently in the samples from 30 m depth, numbered 65. Thus, we found 16 "deep water" species, that is taxa found more often and in greater abundances in the samples from 250m depth. Figure 1 shows the 16 tintinnid forms found to be "deep species". All have hyaline (as opposed to agglutinated) lorica. The 16 deep water forms were of two basic sizes in terms of lorica opening diameter, either small (10 - 25 µm diam.) or large (40 - 60 µm), rather than consisting of a continuous spectrum of morphologies.

Several of the taxa appear to be new, as the lorica morphologies do not conform to any known species. Of the 16 deep water species 6 can be termed 'common', found on at least 50% of the dates. These common forms (noted in the caption of Fig. 2) are a morphologically diverse set with lorica opening oral diameters ranging from about 10 µm to about 40µm. However, none of the relatively large species (*Daturella striata, Parundella lohmanni, Parundella messinsis, Xystonellopsis spicata*) were found on a majority of the dates sampled. The complete data set for the 83 dates of the concentrations estimated for all 85 species is provided in the Supplementary Data File.

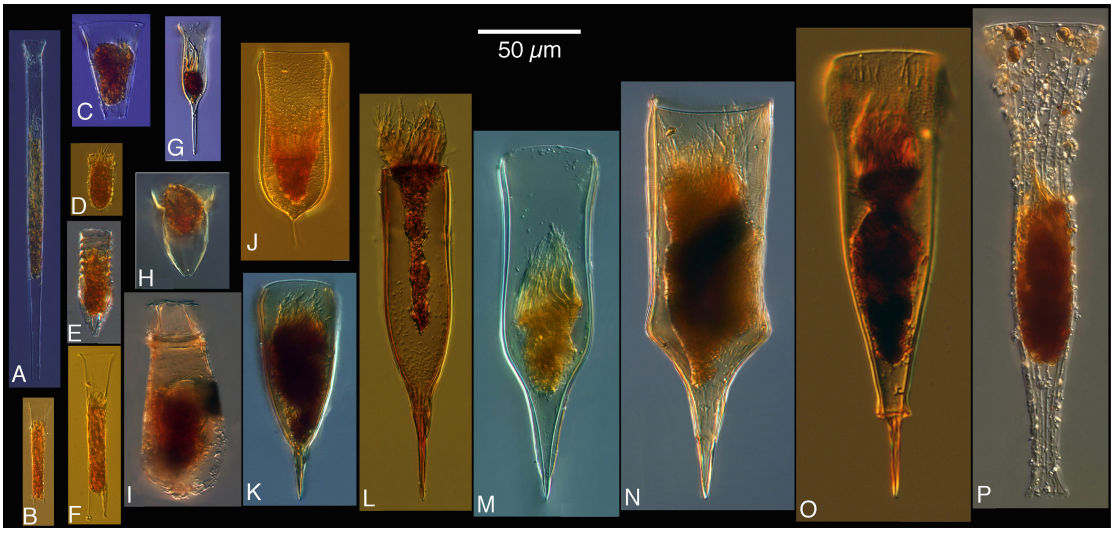

Fig. 2. Deep Sea Tintinnids. A. a new *Salpingella* sp., B. a new *Eutintinnus* spp., C. *Eutintinnus haselae*, D. a new *Undella* sp., E. a new 'ringed' *Amphorellopsis* sp., F. a new *Eutintinnus* sp., G. *Albatrossiella agazzi*, H. a new *Ormosella* sp., I. a new *Brandtiella* sp., J. *Xystonellopsis aciculifera*, K. *Xystonellopsis scyphium*, L. *Parundella longa*, M. *Parundella lohmanni*, N. *Parundella messinensis*, O. *Xystonellopsis spicata*, P. *Daturella striata*. Common species (found in > 50% of sampling dates were *Albatrossiella agassizi* (G), *Amphorellopsis* 'ringy' sp. (E), *Xystonellopsis aciculifera* (J), *Ormosella* sp. (H), *Salpingella* sp. (A), and *Xystonellopsis scyphium* (K). Note that we retain the original, incorrect spelling of *E. hasalae* (see Gomez 2007), as the appellation used most commonly in the literature.

Temporal changes in the total abundances of tintinnids (pooling deep species and forms found in surface waters) paralleled temporal changes in the concentrations of oligotrichs with peak abundances found during the period of winter mixis of the water column (Fig. 3). However most of variability in tintinnid abundances was due to large seasonal changes in the concentrations of "surf" forms, those found in surface waters with "deep species" concentrations varying relatively little (Fig. 4). This pattern is reflected in the coefficients of variation for the two groups (Table 1.). Simple correlation analysis indicated a close correspondence between the concentrations of "surf" tintinnids and oligotrichs rather than concentrations of deep water tintinnids (Fig. 5).

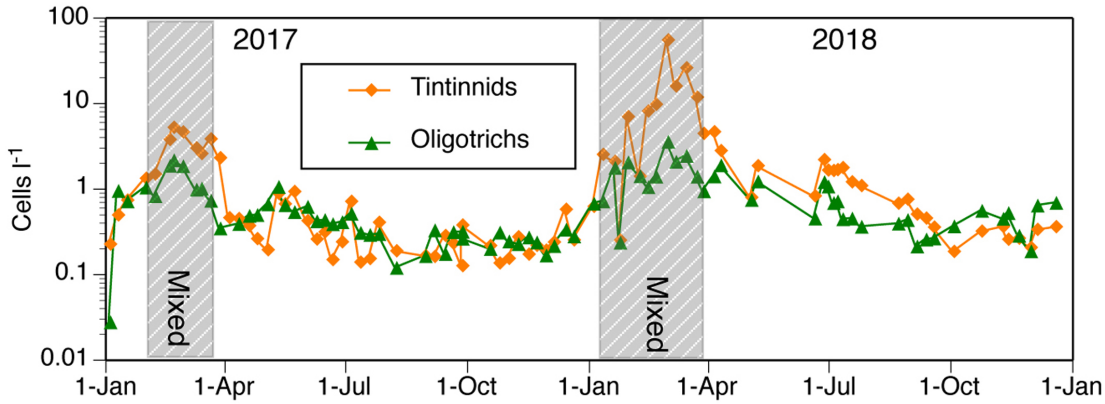

Fig. 3. Temporal changes in the abundance of tintinnids (total = "deep species" + "Surf", those found in higher concentrations and commonly in surface waters) and oligotrich ciliates. Grey striped boxes denote periods of mixed water column conditions (seawater density is homogenous throughout the water column). Note the similar temporal trends of total tintinnids and oligotrichs with marked peaks during the winter.

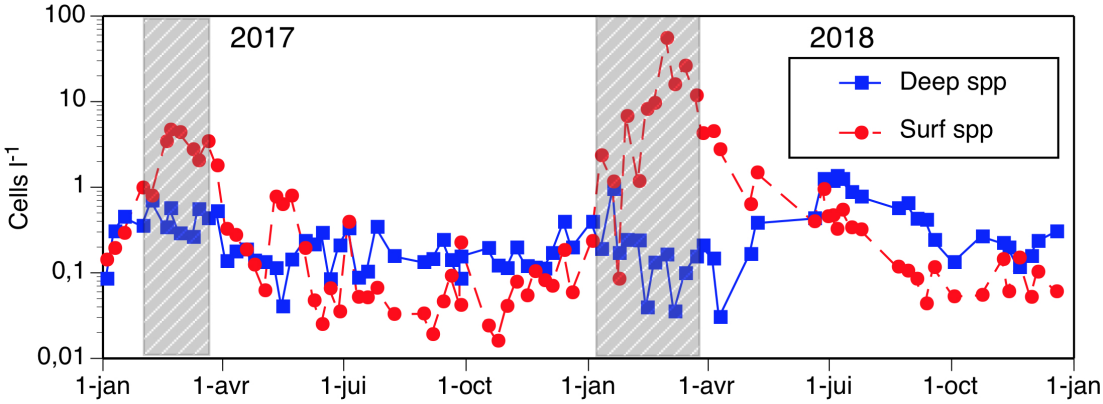

Fig. 4. Temporal changes in the abundance of tintinnids distinguishing "deep species" from "Surf", those tintinnid species also found in surface waters, and oligotrich ciliates. Grey striped boxes denote periods of mixed water column conditions (seawater density is homogenous throughout the water column). Note that "Surf spp" dominated the tintinnid assemblage during periods of water column mixing in both years but were otherwise usually a minority component of the tintinnid assemblage.

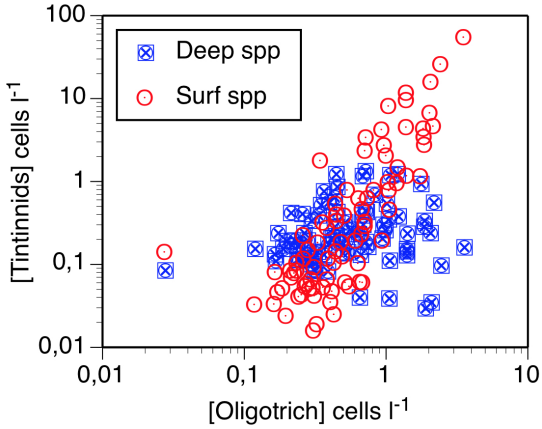

Fig. 5. Correlation of concentrations of oligotrich ciliates (cells > 20 µm diameter) with concentrations of deep water species of tintinnids and concentrations of Surf species of tintinnids. Concentrations of oligotrichs were closely related to those of "Invasive" tintinnids (r = 0.79, n = 84) but not concentrations of "deep" tintinnids (r = 0.03, n = 84).

The most commonly encountered deep sea tintinnids were forms with small lorica oral opening diameters: *Salpingella* sp. (Fig. 1 A.), the 'ringed' *Amphorellopsis* sp. (Fig. 1 E) and *Albatrossiella agassizi* (Fig. 1. G.). None of the three species showed any obvious seasonality. Throughout 2017 the 3 appeared to vary independently of one another but they did peak together in the summer of 2018. (see supplemental Fig. 1). Some deep water tintinnid species did show seasonal shifts in abundance coinciding with changes in the stratification of the water column. *Xystonellopsis aciculifera* and *X. spicata* showed contrasting patterns *with X. spicata* peaking during the winter periods of water column stratification and the relatively common *X. aciculifera* was not detected during those periods (Fig. 6).

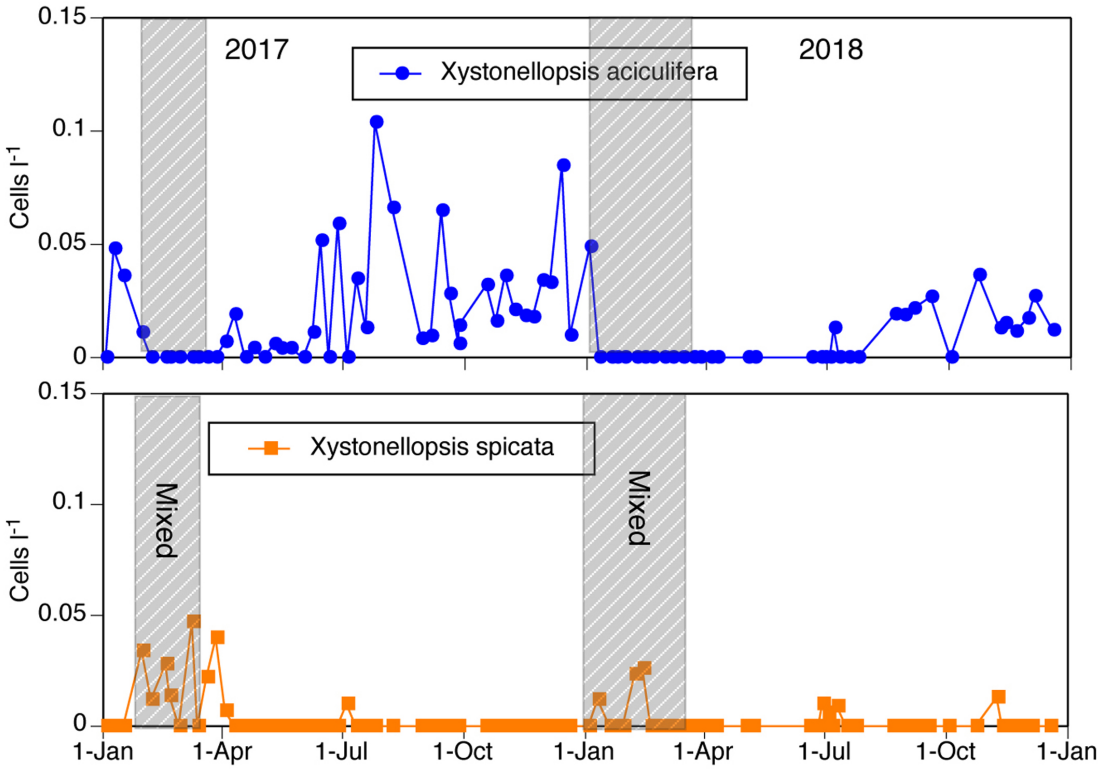

Fig. 6. Temporal variability, suggestive of seasonality, of the deep sea tintinnid species *Xystonellopsis aciculifera* and *X. spicata*. Periods of water column mixing, indicated by grey striped boxes, corresponded to the absence of *X. aciculifera* and the peak abundances *of X. spicata*.

Comparing 2017 and 2018, some differences are readily apparent. Average concentrations were higher and variability greater in 2018 compared to 2017. This applied to both both "Deep Sea" and "Surf" species. The Deep Sea species average abundance in 2017 was 0.23±0.15 cells l^{-1} compared to 0.42 ±0.39 $cells^{-1}$ in 2018. The "Surf" species average abundance was considerably higher in 2018, 4.12±9.99 cells l^{-1} compared to 0.65±1.18 $cells^{-1}$in 2017. However, the trend did not apply to all species. Note that Figure 6 shows that both *Xystonellopsis aciculifera* and *X. spicata* were more abundant and occurred more often in 2017 compared to 2018.

A temporal rarefaction curve showed some expected and unexpected trends. Plotting cumulative number of species encountered as a function of cumulative number of samplings (Fig. 7) shows that most of the deep water species were encountered in the first samplings and only 1 'new' deep water species was added in 2018. The catalogue of "Surf" species, those usually found in the surface layer but encountered in the deep water, samples increased steadily and show no signs of reaching a plateau.

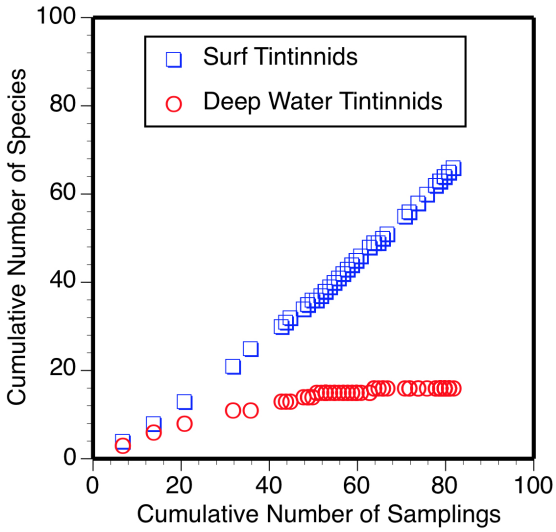

Fig. 7. The cumulative number of tintinnid species encountered as a function of the number of samplings (in chronological order from January 3, 2017 to December 18, 2018). Note that of the 16 species deep water tintinnid species found, most were found in the first few samplings. The majority of the 82 species encountered were "Surf", those found in higher concentrations and more often in surface waters. The plot suggests that the number of Surf species encountered will likely increase with further sampling effort, unlike the list of deep water forms.

Phaeogromid Cercozoans

We encountered 10 taxa of the genera *Challengeranium*, *Challengeron*, *Challengeria*, *Euphysetta*, *Lirella*, and *Medusetta* in samples from 250 m depth (see Supplementary Data File). The 7 most frequently encountered species, displaying a conspicuously wide range of morphologies are shown in Figure 8. Only 3 species could be termed 'common', found on over 50% of the dates, were *Challengeranium diadon*, *Challengeron willemoesii* and *Challengeria xiphodon*.

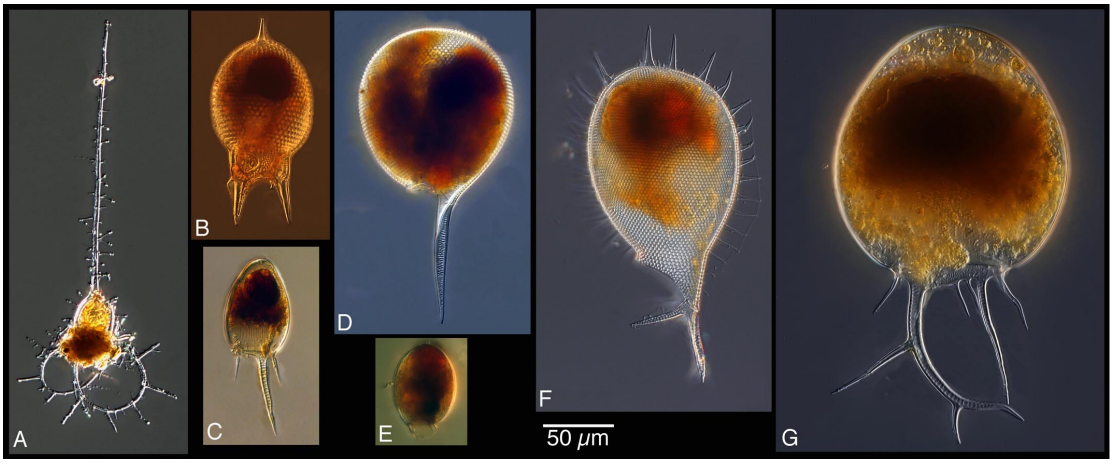

Fig. 8. Species of phaeogromid cercozoans found on at least 10% of the sampling dates. A. *Medusetta parthenopaea*, B. *Challengeranium diadon*, C. *Euphysetta pusilla*, D. *Challengeria xiphodon*, E. *Lirella bullata*, F. *Challengeron willemoesii*, G. *Euphysetta lucani*. Common species (found on > 50% of sampling dates): *Challengeron diadon* (B), *Challengeron willemoesi* (F) and *Challengeria xiphodon* (D).

The three most common species all showed low abundances during the periods of water column mixing but differed with regard to their period of peak abundance (Fig. 9). *Challengeranium diadon*, the most abundant and frequently occurring phaeogromid, had peak abundance in early summer. The large *Challengeron willemoesii* was most abundant in late summer- early autumn and *Challengeria xiphodon* was most abundant in the spring. The distinct periods of peak abundance in the 2 most common forms, *C. diadon* and *C. xiphodon* resembled a successional pattern with *C. xiphodon* peaking shortly before *C. diadon*. Comparing 2017 and 2018, the average abundances of phaeogromids were nearly identical, unlike the tintinnids. Average abundance in 2017 was 0.074 ±0.050 cells l^{-1} compared to 0.065±0.49 cells l^{-1} for 2018.

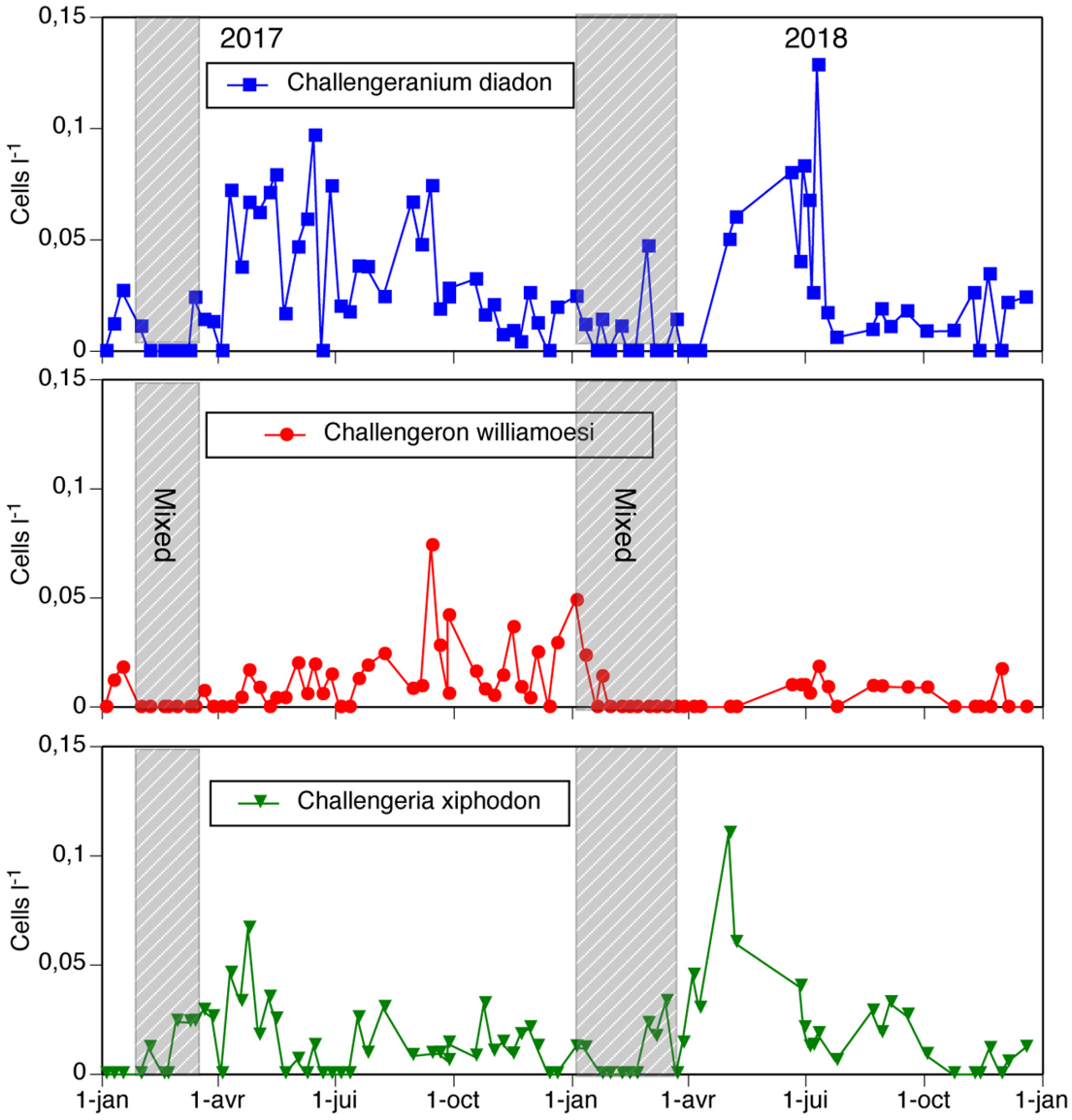

Fig. 9. Temporal variability of the 3 most commonly encountered phaeogromid species: *Challengeranium diadon*, *Challengeron willamoesi*, and *Challengeria xiphodon*. Periods of water column mixing, indicated by grey striped boxes, corresponded to the absence or low abundance of all three species. Note that the periods of maximal concentrations differed for the 3 species. *C. diadon* was most abundant in June-July of 2017 and 2018. *C. williamoesi* peaked in September in 2017 then in July and again in November of 2018. *C. xiphodon* peaked in May in both 2017 and 2018.

Amphisolenid Dinoflagellates

A total of 7 amphisolenid taxa were encountered. The most frequently occurring species (56 of the 85 dates) was *Triposolenia bicornis*, the only *Triposolenia* species found. *Amphisolenia* was represented by 6 taxa with *A. globifera* occurring most often (54 of the 85 dates). The other *Amphisolenia* species (*A. bidentata, A. extensa, A. brevicaudata and A. truncata*) were found on few dates (1- 13). Very occasionally specimens in which the structure of the 'foot' were unclear were encountered and recorded as "*Amphisolenia* sp." in the supplementary data file. The 4 forms most commonly encountered are shown in Figure 10.

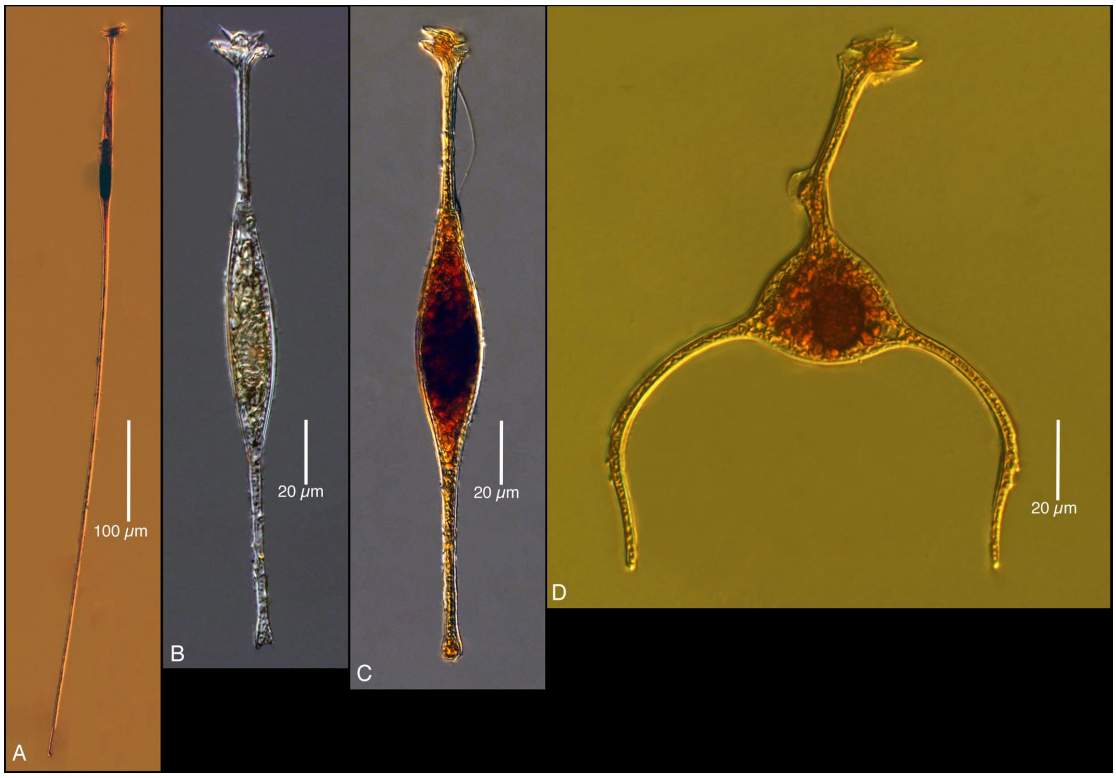

Fig. 10. Species of Amphisolinid dinoflagellates found on at least 8% of the sampling dates. A. *Amphisolenia extensa*, B. *Amphisolenia bidentata*, C. *Amphisolenia globulosa*, D. *Triposolenia bicornis*. Only *A. globulosa* and *T. bicornis* were found on a majority of sampling dates.

Triposolenia and *Amphisolenia* spp. displayed distinct temporal patterns of abundance. (Figure 11) *Triposolenia* was nearly absent during periods of water column mixis and peak abundances were found in late summer. In contrast, *Amphisolenia* spp. were most abundant during the periods of water column mixing and found in lower concentrations than *Triposolenia* during summer months. The average abundance of amphisolenid dinoflagellates throughout 2017 was 0.06 ±0.04 cellsl^{-1}, slightly lower than the 0.04±0.04 cells l^{-1} for 2018.

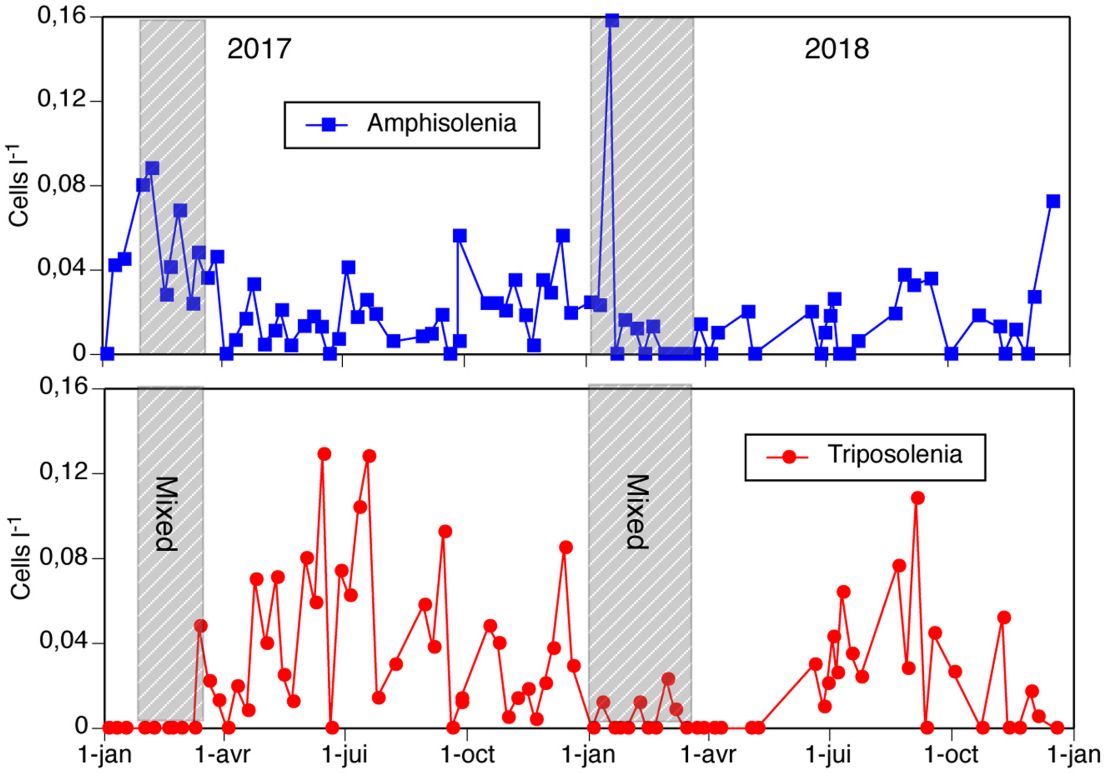

Fig. 11. Temporal variability, suggestive of seasonality, of species of *Amphisolenia* (all forms pooled) and *Triposolenia bicornis*. Periods of water column mixing, indicated by grey striped boxes, corresponded to the apparent absence of *T. bicornis* and the peak abundances of *Amphisolenia* spp.

Observations Following a Flash Flood Type Event

Samples taken on July 6, July 10, and July 17, 2018 permitted observation of the effects of an input of terrigenous matter into deep water. On July 5th 2018 in the early evening an intense, 30 minute, rainstorm occurred ending a period of several weeks without rain in the region. The rainfall was reported as 2 cm (= 20 l per m^2) over the metropolitan region of approximately 100 km^2. Sampling 16 h after the rain event on July 6th we observed a great deal of particulate matter on the sea surface. The quantity and qualities of the material floating at the surface we had not previously nor subsequently seen (see supplemental fig. 2). However, the sample from 250 m depth contained an average or unremarkable concentration of particulate matter (particles ≥ 20 µm retained in the plankton net). In contrast, the sample collected 4 days later was heavily charged with particulate matter and the sample collected 11 days following the rain event showed a return to a 'normal' load of particulate matter (see Figure 12).

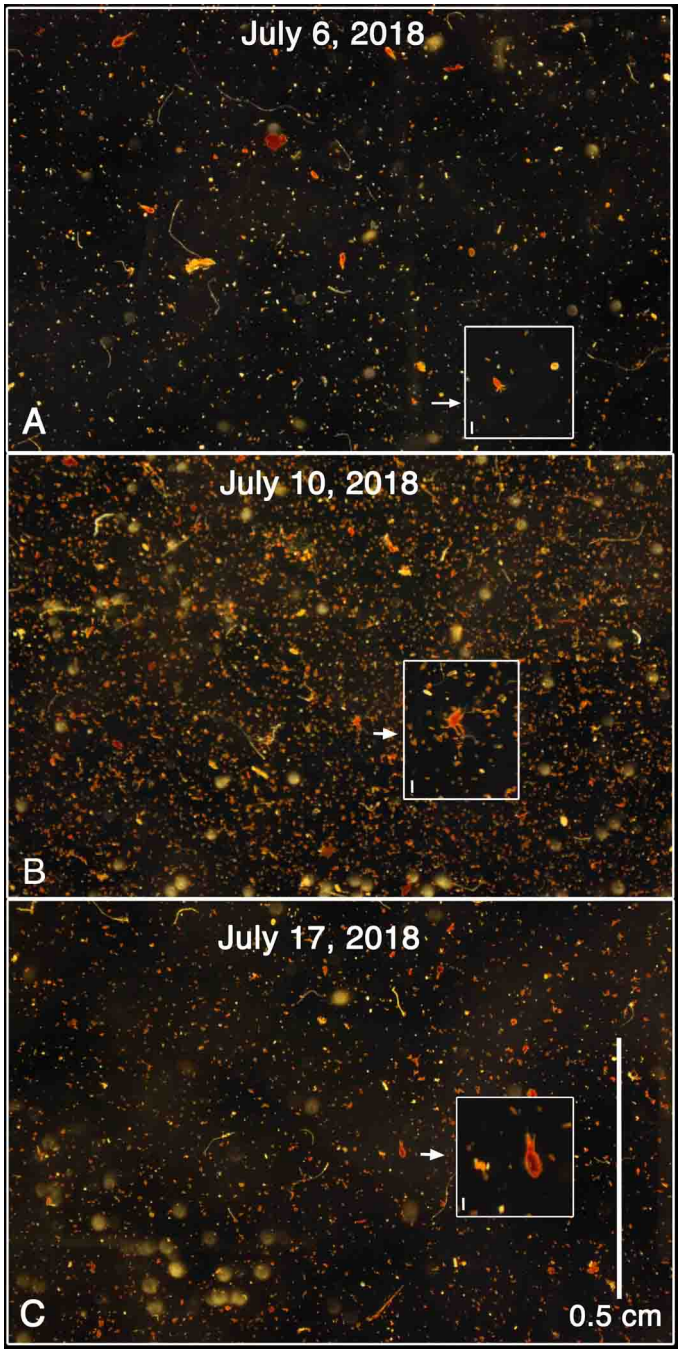

Fig. 12. Particulate matter (particles ≥ 20 µm) load in samples taken at 250 m depth 16 hours following a rainstorm (A- July 6, 2018), 5 days post-storm (B- July 10 2018) and 12 day post-storm (C- July 17 2018). Areas shown contain material from about 5 l of water. Insets show magnified views to show particle sizes relative to copepod nauplii (A, B) or a copepodite (C). Inset scale bars = 100 µm.

The graphs showing temporal changes in organismal concentrations (Figs. 3, 4, 6, 7, 9, 10, 12) show an apparent shift in the abundance of only one taxon on July 10th 2018, relative to the preceding (July 6th 2018) and following dates (July 17 2018). The phaeogromid *Challengerium diodon* showed an abrupt increase in abundance from about 0.03 cells l^{-1} on July 6th to 0.13 cells l^{-1} of July 10th and then a decline to 0.02 cells l^{-1} on July 17th (Fig. 9). The increase in abundance from July 6th to July 10th translates into a generation time of about 2

days. Notably then only one taxon showed an apparent distinct change in abundance coinciding with the detection of large amounts of particulate matter, presumably input from the rainstorm.

Azores Deep Water Samples

We found both similarities and differences in the tintinnid, phaeogromid and amphisolinid faunas of the North Atlantic compared to the Mediterranean site. While the two samples yielded very different estimates of organismal concentrations (see supplemental data file), the average concentrations estimated from the Azores sample were very similar to those encountered at the Mediterranean site, excluding the periods of water column mixis. In the following description the organismal concentrations given represent an average of the 2 Azores samples compared to the average concentrations of the Mediterranean site during the stratified period. In the Azores, samples tintinnids were found in concentrations of about 0.2 cells l^{-1} compared an average of about 0.3 cells l^{-1} in the Mediterranean site. The phaeogromids were less abundant than the tintinnids, 0.07 cells l^{-1}, similar to the concentrations found in the Mediterranean site, 0.08 cells l-1. The Amphisolinid dinoflagellates were more abundant in the Azores samples, 0.14 cells l^{-1} compared to 0.06 cells l^{-1} at the Mediterranean site.

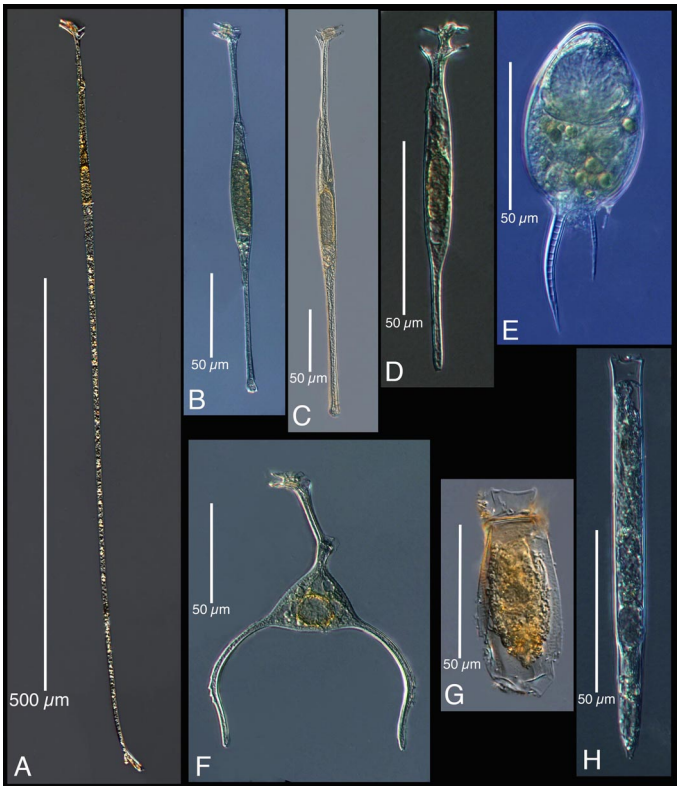

Fig. 13. Examples of forms found in the samples from 250m depth at two sites in the North Atlantic Ocean near the Azores. Amphisolenid dinoflagellates included the large species *A. astralagus* (A), the moderately-sized *A. bidentata* (B) and *A. globulosa* (C) and the small *A. lacintata* (D) and *Triposolenia bicornis* (F). The most abundant phaeogromid was *Euphysetta pusilla* (E). The tintinnids were dominated by a small form of Salpingella (H) differing from the small Mediterranean *Salpingella* (Fig. 1A) as the oral opening of the lorica lacks flared opening of the Mediterranean form. Also found were specimens closely resembling the *Brandtiella* sp (G) found in the Mediterranean samples (Fig. 2I).

Similarities and differences were also found in the taxonomic composition of the assemblages in the Azores samples compared to the Mediterranean site. The most abundant tintinnid species in the Azores samples was a very thin form of *Salpingella* (Fig. 13H), similar to but not identical to, the thin *Salpingella* form dominant among the deep sea forms in the Mediterranean samples (Fig. 2A, Fig. 6). The second most common tintinnid form in the Azores samples was an undescribed *Branditiella* form (Fig. 13G) found also in the Mediterranean samples (Fig. 2I). In the Azores samples the composition of the Phaeogromid assemblage was similar to that found in the Mediterranean site but the relative importance of species differed. The phaeogromids were dominated by *Euphysetta pusilla* (Fig. 13E), also found in the Mediterranean site (Fig. 9C) but as a minor component, and the dominant phaeogromid form of the Mediterranean site, *Challengerianum diadon* (Fig. 8A, Fig. 9) while present, was a minor component of the assemblage in the Azores samples. The most common amphisolinid dinoflagellate was a small form, *Amphisolenia lacintata* (Fig. 13D). Nearly as abundant were two forms common in the Mediterrean site, *A. bidentata* (Fig. 10B) and *A. globulosa* (Fig. 10C). Also found in the Azores sample was *Triposolenia bicornuta* (Fig. 13F) a dominant form in the Mediterranean samples, and a large, apparently rarely seen, *A. astragalus* (Fig. 13A) resembling in it large size *A. extensa* found in the Mediterranean samples (Fig. 10A).

DISCUSSION

In our previous exploratory study (Dolan et al. 2017), we showed changes in the abundances of different groups of deep water protists related to seasonal changes in the structure of the water column at our site in the N.W. Mediterranean Sea. The study concerned the period from early January 2017 to early June 2017. Having found organisms and patterns of interest, we continued our sampling and the data presented here are from sampling 85 dates over a 2-year period. With the extended time series, we set out 1) to obtain a more complete catalogue of the mesopelagic forms, 2) to confirm apparent seasonal changes in composition reported in our preliminary study, and 3) to assess possible inter-annual variability of mesopelagic assemblages. In addition, sampling just after a flash flood event allowed evaluation of the response of the deep water protists to an apparent input of terrigenous matter. Material from a pair of deep water samples from a distant site, the North Atlantic near the Azores Islands, afforded a chance to determine if the forms found in the N.W. Mediterranean Sea might be widely distributed.

With regard to the catalogue of mesoplelagic forms, our temporally extensive sampling uncovered only a few confirmed deep water forms (i.e., not found in surface layer samples) that were not seen in the first 5 months of sampling. Continued sampling for another 18 months recovered but a single additional deep water tintinnid ciliate species, the *Brandtiella* sp.. The vast majority of additional tintinnid species encountered were forms primarily inhabiting surface waters. Among the phaeogromid cercozoans, only two forms not detected in the first 5 months were found: *Challengeron channeri* and *Lirella bullata*. For the amphisolenid dinoflagellate, a single species was added to the catalogue: *Amphisolenia brevicauda* (for details see supplementary data file). These findings suggest that the deep water forms are stable sets, composed of

relatively few species, with little inter-annual variation in species composition at least at the site sampled.

The samples provided by Hartmut Arndt of deep water sites near the Azores Islands allowed an opportunity, albeit quite limited, to compare the Mediterranean assemblages to geographically distant deep water assemblages. Notably some of the same forms were found as those in the Mediterranean site and the average concentrations were similar. Thus, the mesopelagic protist assemblages of tintinnid ciliates, phaeogromid cercozoans and amphisolenid dinoflagellates we found in the Mediterranean may be of wide geographic distribution. However we can not exclude the possibility of geographically distant populations being genetically distinct.

Admittedly, some of the forms we encountered have long been known to be widely distributed in deep sea waters. For example *Challengeranium diodon*, was described as *Challengeron diadon* by Haeckel (1887) from a deep water sample in the Southwest Pacific. It is known from deep water from Norwegian fjords (Jorgensen 1905) to the North Pacific (Gowing 1993) and the Adriatic Sea (Krsinic & Krsinic 2012). Likewise, *Xystonellopsis aciculifera*, was originally described by Jorgensen as *Favella aciculifera* from deep water net tows in both the Eastern and Western Mediterranean (Jorgensen 1924). It is considered a deep sea species in the Adriatic Sea (Krsinic & Grbec 2006) and is also known from deep water sampling in the Gulf of Mexico (Balech 1968). *Amphisolenia* and *Triposolenia* species, although relatively rare, are also widely distributed in tropical and warm water systems and have been described as "shade species" because they are found usually deep in the water column (Balech 1967). Also we should point out that some of our mesopelagic forms have been reported from shallower waters. For example, *Amphisolenia bidentata* has been reported in the Bay of Villefranche (Gomez & Gorsky 2003) at 50 m depth and *Triplosolenia bicornta* also been reported in shallow (< 30m) waters of the Eastern Mediterranean (Balkis 2009). *Eutintinnus haslae* was described from samples taken a variety of depths in the Tropical Pacific and Indian Oceans (Taniguchi & Hada 1981). *Albatrosiella agasszi* was found in the South Atlantic in plankton net tows taken between 100 and 10 m depth (Fernandes 2004).

The general patterns of seasonality we observed in 2017 were repeated in 2018. The tintinnid forms most abundant in the surface layer ('invasive' to deep waters) markedly dominated during the period of water column mixing but were otherwise a minority component of the tintinnid assemblage (Fig. 4). Within the tintinnid assemblage of deep forms, the 2017 pattern of *Xystonellopsis spicata* appearing during the period of water column mixing while *X. aciculifera*, generally present the rest of the year, declined to undetectable concentrations was repeated in 2018 (Fig. 6). The phaeogromids were nearly absent during the period of water column mixing (Fig. 10). *Amphisolenids* were dominated by Amphisolenia spp. during the period of water column mixing but otherwise usually dominated by *Triposolenia* (Fig. 12).

While seasonal patterns were quite similiar in 2017 and 2018, there were some obvious differences in concentrations, at least with regard to tintinnid and oligotrich ciliates. Summary statistics of the protist assemblages, given in Table 1, show that average concentrations and relative temporal variability, as indicated coefficient of variation of average concentration, of all of the ciliate groups was higher in 2018 than in 2017. In contrast, the summary statistics of

both the phaeogromids and amphisolonids for 2017 closely resemble those for 2018. Using standard statistical tests (e.g., t-test, correlation analyses of both transformed and raw data) differences between abundances in 2017 and 2018 were not significant nor were there any significant relationships among the groups (data not shown) other than that shown in Fig. 5 between oligotrich abundance and abundance of invasive tintinnids.

Fortuitous sampling allowed us to assess the response of mesopelagic assemblages to a large increase in particulate matter, presumably material washed into the sea from a flash flood type event, in early July 2018 (Fig. 13). Only one assemblage, the phaeogromids, showed an abrupt change in concentration corresponding with a high concentration of particulate matter, abrupt increase in concentration largely due to one species, *Challengeria diodon* (Fig. 10). As challengerids are thought to feed on organic aggregates, based on contents of food vacuoles (Gowing, 1986; Gowing & Bentham 1994), perhaps it is not surprising that a challengerid would respond positively to a transient increase in particulate matter. The lack of any apparent response among tintinnid or oligotrich ciliates or amphisolinids leads to the suggestion that they, in contrast to the phaeogromids, may not participate in the transformation of particulate organic matter that rapidly travels through the mesopelagic, in the form of large, > 20 µm, particles. Thus, their roles in "the biological carbon pump" may differ from that of the phaeogromids. Interestingly, the response time we found of about two weeks for the mesopelagic assemblage to return to pre-flash flood concentrations is quite similar to that known for the microbial communities of coastal lagoons (Pecqueur et al. 2011).

CONCLUSION

Our study focused on groups of protists that have been largely neglected in recent years. The forms are relatively large, removed during pre-screening employed in most sequence-based studies, and found in relatively low concentrations requiring examination of material from 10^1 to 10^2 liters, which is rarely attempted. However, their distinctive morphologies permit species identification with relative ease. A uniquely intensive sampling over a two year period permitted detection of distinct, species-specific, temporal dynamics. We found seasonal patterns of abundance in some taxa, and in others non-seasonal successional patterns. Some of the peculiar forms we found in the mesopelagic Mediterranean were also found in sample obtained from the North Atlantic supporting the idea that the organisms may be wide spread. Fortuitous sampling around a flash flood type event associated with the appearance of high concentrations of particulate matter showed an apparent reaction restricted to a single species. We conclude that there are a variety of protist taxa endemic to the mesopelagic, that the populations are dynamic, have distinct ecologies, and they may be widely distributed in the deep waters of the world ocean.

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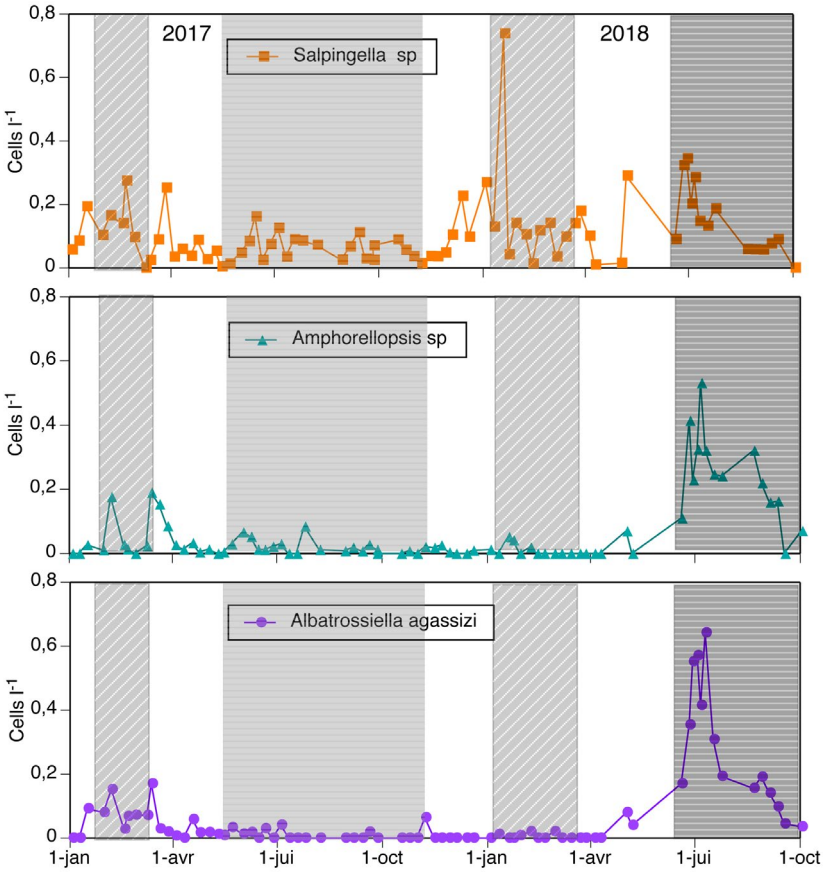
SUPPORTING MATERIAL

1. Supplementary Figures (2) with legends.

2. Supplementary Excel Data File

JEM ms Supplementary Figures (2) with Legends



Supplemental Figure 1. Temporal variability in the concentrations of the 3 most commonly found deep sea tintinnids. All three are characterized by small lorica opening diameters (10 - 25 µm diam.) suggestive of a diet of small nano and large pico-sized prey: *Salpingella* sp. (Fig. 1 A.), the 'ringed' *Amphorellopsis* sp. (Fig. 1 E) and *Albatrossiella agassizi* (Fig. 1.G.). Note that the three co-varied in the later 6 months of 2018.

Supplemental Figure 2. Photo of sea surface at sampling site, approximately 1 Km offshore, on the morning of July 6 2018 following the flash flood type rain event during the evening of July 5, 2019.

Note

Your are perhaps viewing a PDF file.

This Supplementary Data File is an Excel File.
to be available for readers to easily manipulate the data.

Unfortunately, some publishers
transform everything into a PDF!

The original Excel file is available on request (dolan@obs-vlfr.fr)

Taxon	Dec	Jan	Jar	Ja	Jan	30	Fe	Fe	Fe	Fe	M	M	Ma	Mi	Ap	Ap	Ap	Ap	M	Ma
Tintinnid Ciliates																				
Albtrossiella agassizi				0	0,08		0	0	0	0	0	0	0	0	0		0	0	0	0
Amphorellopsis sp 'small sac'			0	0	0,03		0		0				0	0	0	0	0	0	0	
Amphorellopsis "ringy sp."				0	0,01		0	0	0		0	0	0	0	0	0	0	0	0	
Brandtiella "new sp."																				
Daturella striata					0,02		0						0	0		0	0	0	0	0
Eutintinnus haslae			0	0			0		0							0	0		0	0
Eutintinnus sp. "tiny trumpet ø15"																0			0	0
Eutintinnus sp. " tiny tube ø9"											0	0	0	0						
Xystonellopsis aciculifera	0		0	0	0,01										0	0		0		0
Ormosella sp. "small stemless"	0	0	0	0	0,03		0	0				0	0	0	0					
Parundella lohmannii								0	0		0			0	0		0			
Parundella longa								0	0	0										
Parundella messinensis					0,01		0		0			0	0				0		0	
Salpingella sp. "thin sp"	0	0	0	0	0,1		0	0	0	0		0	0	0	0	0	0	0	0	0
Xystonellopsis spicata					0,03		0	0	0		0		0	0	0					
Xystonellopsis scyphium		0	0	0	0,01		0		0	0		0	0	0	0	0	0	0		0
Acanthostomella conicoides																				
Amphorides laackmanni									0					0		0				
Amphorellopsis tetragona					0,11		0	0	1	0	0	0	0		0					
Amphorellopsis tropica												0								
Amphorellopsis turbinea																				
Amphorides quadrilineata			0	0	0,15		0	0	0	0	0		0	0	0		0	0		1
Ascampbelliella torulata			0		0,01															
Canthariella pyramidata					0,03		0		0	0	0	0	0	0						
Climacocylis scalaria								0						0						
Codonaria cistelllula											0									
Codonella amphorella																				
Codonella elongata	0				0,01			0	0				0	0		0	0		0	0
Codonella nationalis								0												
Codonellopsis lusitanica															0					
Condonellopsis morchella										0		0	0		0		0			
Codonellopsis orthoceras	0										0									
Condonellopsis pusilla			0	0	0,03		0	0	0	1	0	0	1	0	0	0		0		0
Cyttarocylis ampulla								0		0			0	0						
Dadayiella ganymedes				0					0											0
Dadayiella pachytoecus								0		0	0									
Dictyocysta elegans				0																
Dictyocysta entzi																				
Dictyocysta lepida		0		0	0,02		0							0						
Dictyocysta mitra	0	0	0	0																
Epiplocylis undella																				
Eutintinnus apertus																	0			0

Eutintinnus fraknoii																			
Eutintinnus lusus-undae					0,01														
Eutintinnus perminutus																			
Eutintinnus pinguis																			
Eutintinnus stramentus																			
Eutintinnus tubulosus							0												
Odontophorella serrulata	0																		
Ormosella apsteini	0				0,01							0							
Ormosella bresslaui																			
Ormosella trachelium		0						0											
Parundella aculeata					0,02		0	0	0	0		0	0		0	0	0		
Proplectella fastigata	0					0													0
Protorhabdonella curta																			
Rhabdonella spiralis																			
Salpingella acuminata	0	0	0	0	0,17	0	1	1	1	1	1	0	0	0	0	0			0
Salpingella curta	0				0,16	0	1	1	1	1	0	0	0	0	0	0	0		
Salpingella decurtata			0		0,02	0	0	0	0	0	0	0	0	0	0	0		0	
Salpingella faurei			0	0	0,17		0	0	0	0	0	0	0	0	0	0	0	0	0
Salpingella liminata																			
Salpingella rotundata																			
Steenstrupiella steenstrupii						0	0												0
Stenosomella nivalis				0	0,02	0	0	0	0	0	0	2	0	0	0	0	0	0	
Stenosomella ventricosa							0			0									
Tintinnidium sp. "ø50"					0,01		0	0		0									
Tintinnopsis angulata																			
Tintinnopsis beroidea																	0		
Tintinnopsis baltica						0													
Tintinnopsis campanula																			
Tintinnopsis cylindrica																			
Tintinnopsis minuta																			
Tintinnopsis nana																			
Tintinnopsis radix																			
Undella clevei	0			0		0		0					0						
Undella hyalina																			
Undella sp. "tiny Ø1"1							0	0	0										
Undellopsis marsupialis																			
Xystonella lanceolata																			
Xystonella treforti							0												
Xystonellopsis paradoxa																			
Oligotrich Ciliates																			
30 µm Oligotrich		0	0	0	0,22	0	1	1	1	0	0	0	0		0	0	0	0	0
40 µm Oligotrich	0		1	0	0,57	0	1	1	1	0	0	0	0		0	0	0	0	0
50µm Oligotrich	0			0	0,24	0	1	1	1	0	0	0	0		0	0	0	0	0

40 µm Tontonid oligotrich			0																
70 µm Tontonid oligotrich			0		0,02			0	0	0	0		0		0				0
Laboea strobila																			
Amphisolenid Dinoflagellates																			
Triposolenia bicornis	0										0	0	0		0	0	0	0	0
Amphisolenia bidentata			0	0			0		0										
Amphisolenia extensa																			
Amphisolenia globifera					0,08	0		0	0	0	0	0	0		0	0	0	0	0
Amphisol brevicaudata																			
Amphisolenia truncata					0,01														
Amphisolenia sp.																			
Phaeogromids																			
Medusetta sp						0							0			0		0	0
Medusetta parthenopaea								0	0	0	0	0	0		0	0	0	0	0
Lirella bullata																			
Challengeron channeri																			
Challengeranium diadon	0		0	0	0,01						0	0	0		0	0	0	0	0
Challengeron willemoesii			0	0								0				0	0	0	
Challengeria xiphodon	0					0			0	0	0	0	0		0	0	0	0	0
Protocystis tritonis																		0	0
Euphysetta lucani				0				0								0	0		0
Euphysetta pusilla																0	0	0	0
Liters equiv. examined =>	93	70	82	#	87,2	#	#	#	#	#	#	#	#	#	#	#	#	#	#
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Red denotes deep water taxa
absent or rare in surface water samples

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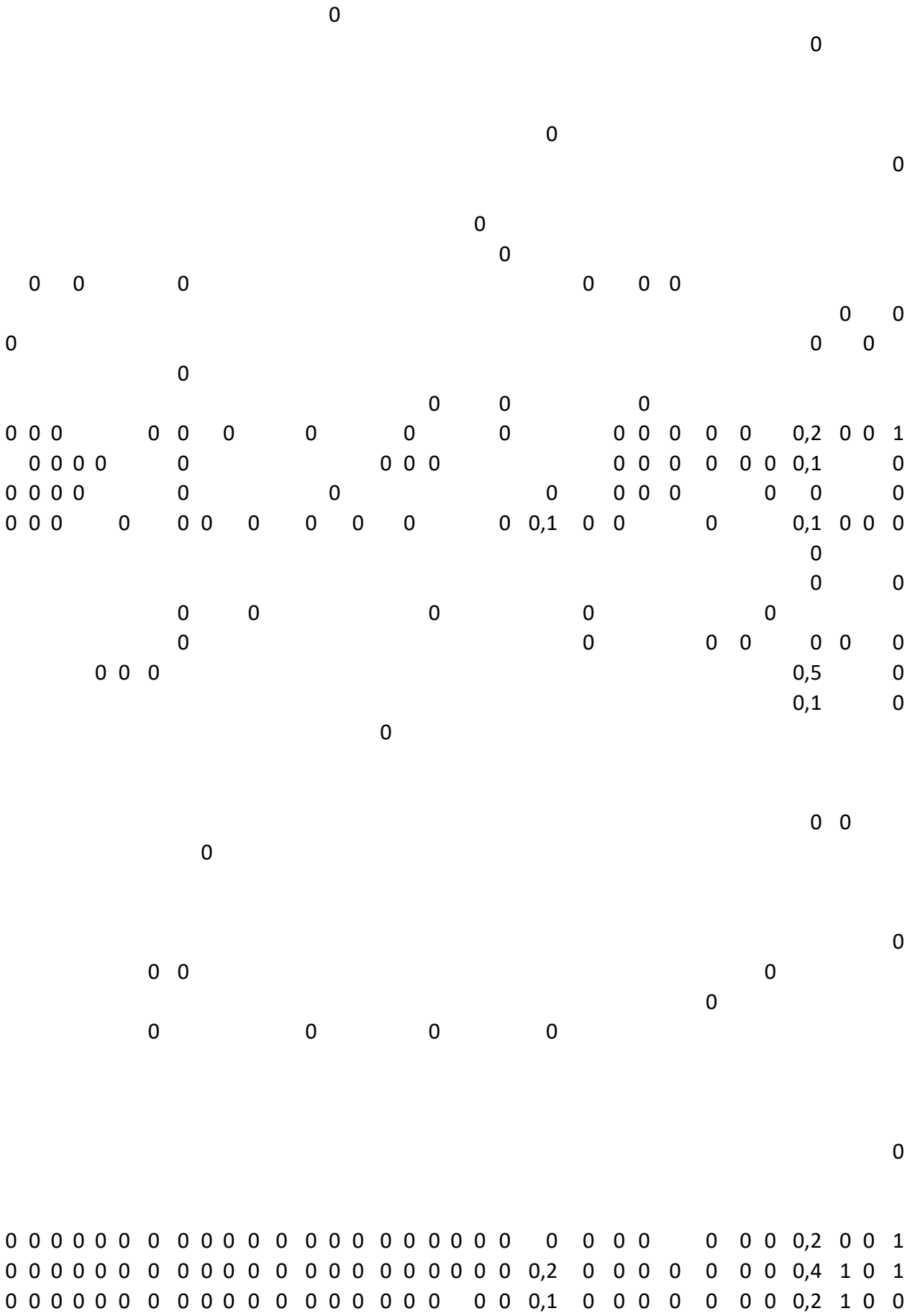

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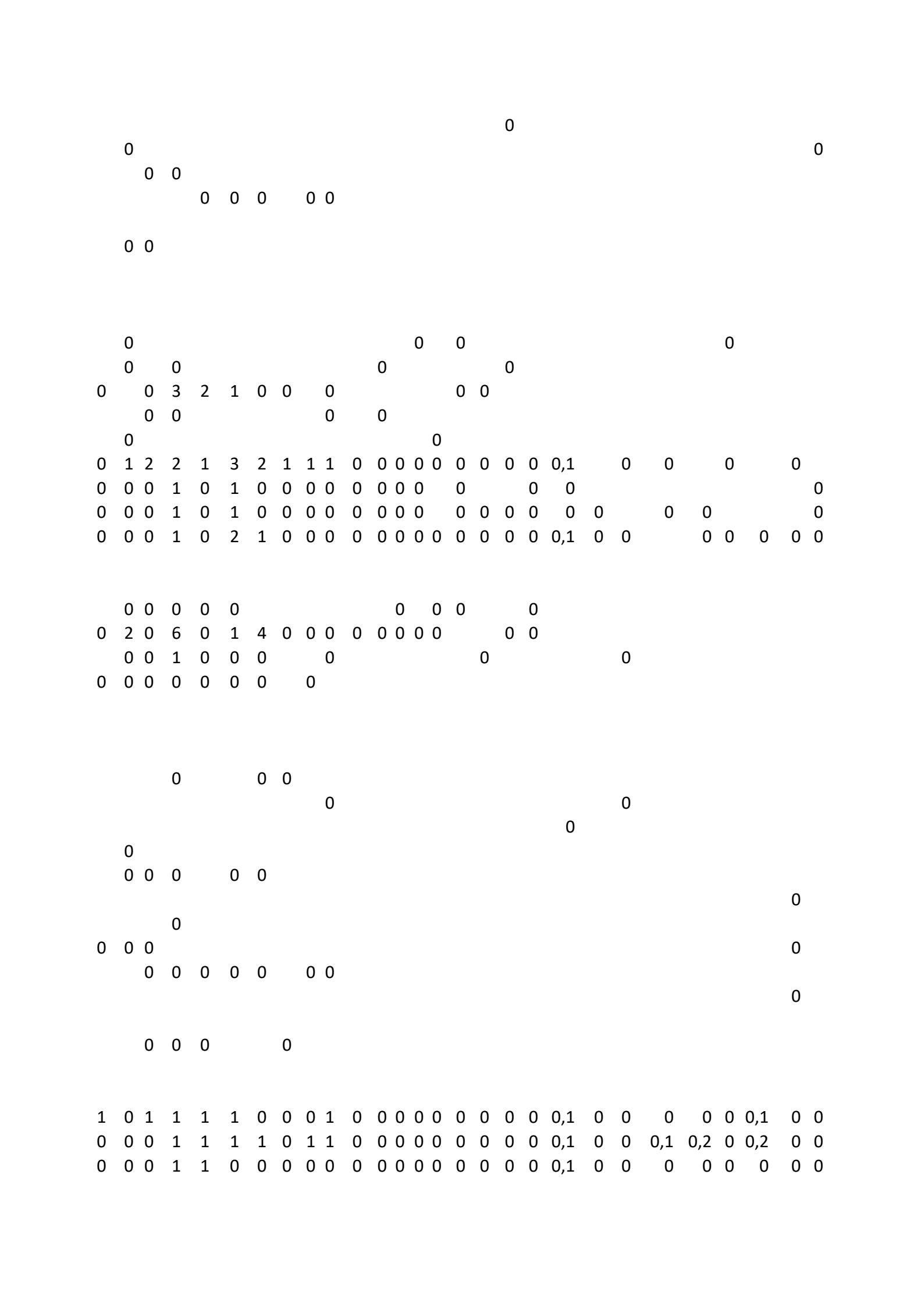

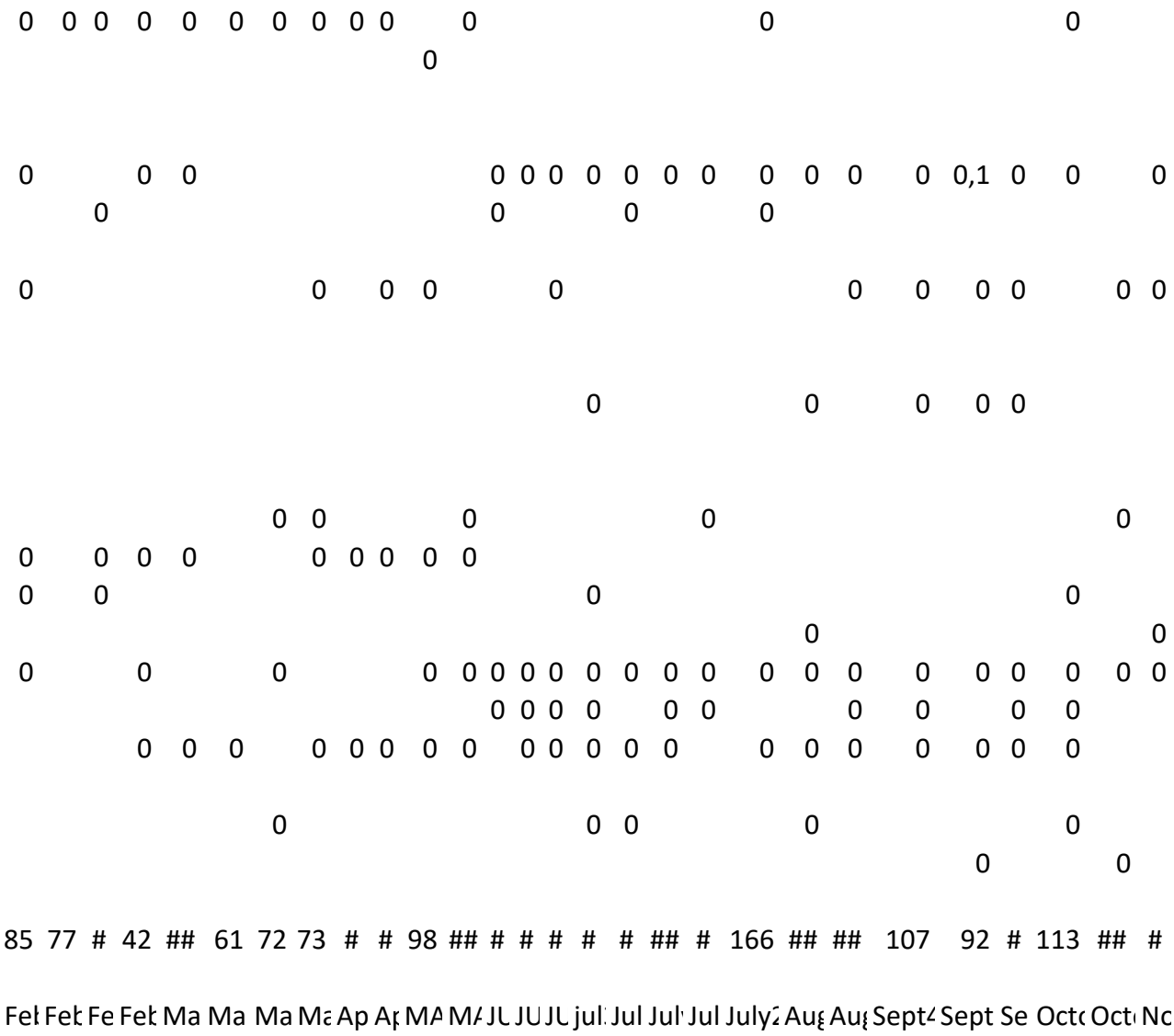
85 77 # 42 ## 61 72 73 # # 98 ## # # # # # ## # 166 ## ## 107 92 # 113 ## #
Feb Feb Fe Feb Ma Ma Ma Ma Ap Ap MA MA JU JU JU jul Jul Jul Jul July Aug Aug Sept Sept Se Oct Oct Nc

Nov	Nov	Nov	Dec	Dec	Taxon		
					Tintinnid Ciliates		
0			0	0	*Albtrossiella agassizi*	48	@
		0	0		*Amphorellopsis sp 'small sac'*	19	
0	0	0	0	0	*Amphorellopsis "ringy sp."*	60	@
					Brandtiella "new sp."	6	
			0		*Daturella striata*	37	
0	0		0	0	*Eutintinnus haslae*	31	
0	0		0	0	*Eutintinnus sp. "tiny trumpet ø15"*	27	
					Eutintinnus sp. " tiny tube ø9"	9	
0	0	0	0	0	*Xystonellopsis aciculifera*	46	@
	0		0	0	*Ormosella sp. "small stemless"*	46	@
				0	*Parundella lohmannii*	26	
					Parundella longa	6	
					Parundella messinensis	34	
0	0	0	0	0	*Salpingella sp. "thin sp"*	83	@
					Xystonellopsis spicata	16	
		0	0		*Xystonellopsis scyphium*	41	@
					Acanthostomella conicoides		
					Amphorides laackmanni		
					Amphorellopsis tetragona		
					Amphorellopsis tropica		
					Amphorellopsis turbinea		
	0				*Amphorides quadrilineata*		
					Ascampbelliella torulata		
					Canthariella pyramidata		
					Climacocylis scalaria		
					Codonaria cistelllula		
					Codonella amphorella		
					Codonella elongata		
					Codonella nationalis		
					Codonellopsis lusitanica		
	0				*Condonellopsis morchella*		
					Codonellopsis orthoceras		
		0			*Condonellopsis pusilla*		
					Cyttarocylis ampulla		
					Dadayiella ganymedes		
					Dadayiella pachytoecus		
					Dictyocysta elegans		
					Dictyocysta entzi		
			0		*Dictyocysta lepida*		
	0		0	0	*Dictyocysta mitra*		
				0	*Epiplocylis undella*		
					Eutintinnus apertus		

					Eutintinnus fraknoii
					Eutintinnus lusus-undae
					Eutintinnus perminutus
					Eutintinnus pinguis
					Eutintinnus stramentus
					Eutintinnus tubulosus
					Odontophorella serrulata
					Ormosella apsteini
					Ormosella bresslaui
			0		*Ormosella trachelium*
					Parundella aculeata
			0		*Proplectella fastigata*
					Protorhabdonella curta
					Rhabdonella spiralis
0		0	0	0	*Salpingella acuminata*
	0				*Salpingella curta*
0	0	0	0		*Salpingella decurtata*
0	0		0	0	*Salpingella faurei*
					Salpingella liminata
					Salpingella rotundata
	0		0		*Steenstrupiella steenstrupii*
					Stenosomella nivalis
					Stenosomella ventricosa
					Tintinnidium sp. "ø50"
					Tintinnopsis angulata
					Tintinnopsis beroidea
					Tintinnopsis baltica
					Tintinnopsis campanula
					Tintinnopsis cylindrica
	0				*Tintinnopsis minuta*
					Tintinnopsis nana
					Tintinnopsis radix
					Undella clevei
					Undella hyalina
					Undella sp. "tiny Ø1"1
					Undellopsis marsupialis
					Xystonella lanceolata
					Xystonella treforti
					Xystonellopsis paradoxa

Oligotrich Ciliates

0	0	0	0	0	30 µm Oligotrich
0	0	0	0	0	40 µm Oligotrich
0	0	0	0	0	50µm Oligotrich

				40 µm Tontonid oligotrich		
				70 µm Tontonid oligotrich		
				Laboea strobila		
				Amphisolenid Dinoflagellates		
	0	0		*Triposolenia bicornis*	56	
0		0		*Amphisolenia bidentata*	13	
		0		*Amphisolenia extensa*	7	
		0	0	*Amphisolenia globifera*	54	
				Amphisol brevicaudata	3	
				Amphisolenia truncata	1	
				Amphisolenia sp.	5	
				Phaeogromids		
				Medusetta sp	18	
				Medusetta parthenopaea	31	
		0		*Lirella bullata*	12	0,14457831
				Challengeron channeri	2	
0		0	0	*Challengeron diadon*	65	
	0			*Challengeron willemoesii*	45	
0		0	0	*Protocystis xiphodon*	56	
				Protocystis tritonis	3	
				Euphysetta lucani	18	
0				*Euphysetta pusilla*	14	
66	87	58	##	83 <= Liters equiv. examined		
No	No	No	De	Dec <= Dates		

Taxon	Sample 1 raw count	Sample 1 cells/L
Tintinnid Ciliates		
Brandtiella sp. "new sp"	3	0,034562212
Parundella difficillus	2	0,023041475
Proplectella subcaudata		
Salpingella acuminata		
Salpingella sp. " thin sp"	22	0,253456221
Xystonella acus		
Xystonella treforti		
Oligotrichs		
40 µm Oligotrich	1	0,011520737
Phaeogromids		
Challengeria xiphodon	2	0,023041475
Euphysetta pussila	1	0,011520737
Lirella bullata		
Medusetta sp		
Amphisolenid Dinoflagellates		
Amphisolenia astragulus	1	0,011520737
Amphisolenia bidentata	5	0,057603687
Amphisolenia globulosa	6	0,069124424
Amphisolenia lacintata	5	0,057603687
Amphisolenia sp		
Triplosolenia bicornis	3	0,034562212

Sample 1 DATA

100 l=> 60 ml
site depth = 1060 m
Meteor event # M150_423
Septem18_2018
36°53,935N
25°7,185W
Total vol ex = 52 ml
Vol eq = 86.8 L

Sample 2 DATA

120L => 80 ml
site depth = 439m
Meteor event # M150_308
Septem12_2018
38°48,35N

27°3,063W
Total Vol Ex = 64 ml
Vol Eq Ex = 96 L

Red denotes deep water taxa
absent or rare in surface water samples
in time-series data from NW Medit.

Sample 1 tins/l	0,311059908	0,197196621
Sample 2 tins/L	0,083333333	
Sample 1 Phaeos/l	0,034562212	0,074572773
Sample 2 Phaeos/l	0,114583333	
Sample 1 Amphis/l	0,230414747	0,136040707
Sample 2 Amphis/L	0,041666667	

Sample 2 raw count	Sample 2 cells/L
2	0,020833333
1	0,010416667
1	0,010416667
2	0,020833333
1	0,010416667
1	0,010416667
5	0,052083333
1	0,010416667
5	0,052083333
3	0,03125
1	0,010416667