In situ associations between marine photosynthetic picoeukaryotes and potential parasites – a role for fungi?

Cécile Lepère[1-3*], Martin Ostrowski[4], Manuela Hartmann[5], Mike V. Zubkov[5], David J. Scanlan[1*]

[1] School of Life Sciences, University of Warwick, Coventry CV4 7AL, UK

[2] Université Clermont Auvergne, Université Blaise Pascal, Laboratoire "Microorganismes : Génome et Environnement", BP 10448, F-63000 Clermont-Ferrand, France

[3] CNRS, UMR 6023, LMGE, F-63171 Aubière, France

[4] Department of Chemistry and Biomolecular Sciences, Macquarie University, North Ryde, NSW 2109, Australia

[5] National Oceanography Centre, Southampton, Hampshire SO14 3ZH, UK

* Corresponding authors

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1758-2229.12339

Summary

Photosynthetic picoeukaryotes (PPEs) are important components of the marine picophytoplankton community playing a critical role in CO_2 fixation but also as bacterivores, particularly in the oligotrophic gyres. Despite an increased interest in these organisms and an improved understanding of the genetic diversity of this group we still know little of the environmental factors controlling the abundance of these organisms. Here, we investigated the quantitative importance of eukaryotic parasites in the free-living fraction as well as in associations with PPEs along a transect in the South Atlantic. Using TSA-FISH (Tyramide Signal Amplification - Fluorescence *in situ* hybridization) we provide quantitative evidence of the occurrence of free-living fungi in open ocean marine systems, while Perkinsozoa and Syndiniales parasites were not abundant in these waters. Using flow cytometric cell sorting of different PPE populations followed by a dual-labelled TSA-FISH approach we also demonstrate fungal associations, potentially parasitic, occurring with both pico-Prymnesiophyceae and pico-Chrysophyceae. These data highlights the necessity for further work investigating the specific role of marine fungi as parasites of phytoplankton to improve understanding of carbon flow in marine ecosystems.

Introduction

Photosynthetic picoeukaryotes (PPEs), herein defined as cells <5 µm in diameter, are gaining recognition as significant contributors to CO_2 fixation in many marine ecosystems (Jardillier *et al*., 2010; Cuvelier *et al*., 2010). Whilst long considered as obligate autotrophs, PPEs are now known to include active bacterivores i.e. they exhibit mixotrophic behaviour (Hartmann *et al*., 2012, 2013; Unrein *et al*., 2014). Hence, these organisms are not only key CO_2 fixers but also play a role in controlling bacterioplankton abundance, acting as producers of organic matter and predators at the same time. Interest in these organisms has thus increased dramatically in recent years, and we now have a relatively good knowledge of their molecular diversity, largely through surveys of nuclear and plastid-encoded small subunit rRNA genes (e.g. Vaulot *et al*., 2008; Kirkham *et al*., 2013). In contrast, factors controlling the abundance of PPEs remain poorly understood. Recent ship-board nutrient addition experiments suggest that, at least over the short term (10-11 h), nutrient availability does not limit CO_2 fixation by these organisms (Grob *et al*., 2015) suggesting top-down regulation the most likely controlling factor of open ocean PPE CO_2 fixation. Here, we investigated the impact of eukaryotic parasitism on PPEs, which remains largely unexplored in ocean ecosystems.

Eukaryotic parasites are characterized by complex life cycles. They can include developmental stages comprising free-living zoospores 2-6 µm in size that are well represented in molecular studies (Guillou *et al*., 2008; Chambouvet *et al*., 2008, 2014). As a result they can infect hosts belonging to various trophic levels (Marcogliese and Cone, 1997). For a long time they have been neglected in mathematical models of aquatic trophic networks (Lafferty *et al*., 2008). However, their introduction into such models can

have important qualitative and quantitative impacts on ecosystem functioning, e.g. by extending the length of food chains and/or modulating the transfer of carbon (Niquil *et al*., 2011).

In oceanic environments eukaryotic parasites are mainly representatives of the "superphylum" Alveolata - a polyphyletic group including ciliates, Apicomplexa, Perkinsozoa and Dinoflagellata (Chambouvet *et al*., 2008, 2014; Gachon *et al*., 2010; Guillou *et al*., 2008; Leanders and Keeling, 2003). Many Fungi, especially those belonging to the Chytridiomycota, are also known to be parasitic in a wide range of habitats (e.g. see Sime-Ngando, 2011). However, very few fungal lineages have been detected or isolated from oceanic environments (Massana and Pedrós-Alió, 2008), perhaps due to the limited number of geographic regions that have been analysed so far. Fungal diversity has been investigated by both conventional culture-dependent methods (Le Calvez *et al*., 2009; Burgaud et al. 2009; Jebaraj et al. 2010;) and culture-independent methods (Bass *et al*., 2007; Lopez-Garcia *et al*., 2007; Jebaraj *et al*., 2010; Nagano *et al*., 2010; Sauvadet *et al*., 2010; Nagahama *et al*., 2011). Deep-sea environments, including hydrothermal vents, are the best studied in terms of fungal composition (for a review see: Nagano and Nagahama, 2012). Fungi reported from these environments mostly belong to the phylum Ascomycota. However, Chytridiomycota have also been detected as one of the major fungal components in several deep-sea environments, such as hydrothermal vents and methane cold-seeps, but only by culture-independent methods (Nagano and Nagahama, 2012). In contrast, very few species have been detected in surface marine waters using both culture and culture-independent approaches (Massana and Pedrós-Alió, 2008; Gleason *et al*., 2011, Richards *et al*., 2012; Lepelletier *et al*., 2014). Therefore,

culture-independent molecular probing of potentially infected organisms could reveal important new information about interactions with marine fungi.

Here, we employed a sensitive dual-label (parasite-host) TSA-FISH (Tyramide Signal Amplification-Fluorescence *in situ* Hybridisation) analysis to begin to evaluate the potential impact of eukaryotic parasitism by members of the Syndiniales, Perkinsozoa and a wide range of Fungi including Chytridiales (i.e. the largest group of the true-fungal division of Chytridiomycota) on PPEs. Samples used in this study were collected along a transect in the Atlantic Ocean from October 13th- December 1st 2009 during the AMT19 cruise (Figure 1) aboard the Royal Research Ship James Cook. Ten stations encompassing the southern subtropical gyre (SG) and southern temperate (ST) region of the Atlantic Ocean were sampled from the surface mixed layer. Filtered samples were analysed to evaluate the abundance and distribution of free-living members of the Syndiniales, Perkinsozoa and Fungi along AMT19. We also combined flow cytometric cell sorting and dual-label TSA-FISH to determine interactions between PPEs and potential parasites for two different PPE size fractions that are easily distinguishable populations on flow cytograms: small, plastidic eukaryotes (Plast-S, 2±0.1 µm in size) and large, plastidic eukaryotes (Plast-L, 3.1±0.3 µm in size). For more details on the materials and methods please refer to the Supporting Information.

Results and Discussion

PPE composition along AMT19

To determine which photosynthetic classes were potentially susceptible to parasitism we first assessed the composition of the Plast-S and Plast-L populations along AMT19. The contribution of different classes to the total eukaryotic community (<5 µm) is expressed

as a percentage of all positively hybridised eukaryotic cells targeted by the probe EUK1209 (Giovannoni *et al*., 1988). At all stations, the Plast-S fraction was dominated by Pelagophyceae (30±11%) and Chrysophyceae (20%±14) whereas Prymnesiophyceae were the principal component of the Plast-L cells (48±18%) (Supplementary Table 1). Cryptophyceae were detected at some stations, but where detected represented only 2±1% of the total eukaryote population in both fractions (Supplementary Table 1). The composition of these PPE size classes is similar to those obtained previously in Atlantic waters (Jardillier *et al*., 2010; Grob *et al*., 2011). At most of the stations the three classes Prymnesiophyceae, Chrysophyceae and Pelagophyceae encompassed the majority of PPEs. However, for some stations (i.e. JC039056; JC03970), the percentage of PPEs targeted by these FISH probes was rather low, suggesting other PPE classes, perhaps with more sporadic distributions, dominate in such locations. Indeed, previous molecular characterization found for example some Prasinophyceae clades (e.g. 16S Clades VI and VIII) can constitute a large part of the PPE community in some oceanic regions (Kirkham *et al*., 2013).

Parasite abundance and distribution along AMT19

Sequences affiliated to Syndiniales have been regularly observed in 18S rRNA gene libraries from marine ecosystems (Guillou *et al*., 2008). However, their quantitative distribution has rarely been studied in oceanic waters (Siano *et al*., 2011). The abundance of the free living stage of Syndiniales (dinospores, 3-7 µm diameter, Figure 2a) assessed using the general Syndiniales group II probe (ALV01) was highly variable along the AMT19 transect, though generally cell numbers were low. No Syndiniales dinospores were detected at six stations (Table 1), principally in the Subtropical Gyre (SG), whereas

in Southern Temperate (ST) areas they reached a maximum concentration at station JC03972 (800 cells ml^{-1}) and contributed up to 26% of total eukaryote cells (<5µm; targeted by the probe EUK1209). However, the total abundance of Syndiniales may be underestimated because of the specificity of the ALV01 probe, which targets only 33 of the 44 described clades. Unfortunately, oligonucleotide probes for FISH analyses could not be designed to cover the entire genetic diversity of marine alveolates group 2 (MALV II, Siano *et al*., 2011). Nonetheless, our FISH data is consistent with Syndiniales dinospores being more abundant in coastal waters compared with open ocean sites, especially when considering oligotrophic systems (Guillou *et al*., 2008; Chambouvet *et al*., 2008; Siano *et al*., 2011). Indeed, these latter authors found a positive correlation between zoospore occurrence and higher nutrient concentrations.

The phylum Perkinsozoa is part of the Alveolata "super-phylum" and comprises a diverse group of aquatic parasites infecting a wide range of species such as molluscs, amphibians and phytoplankton (Bråte *et al*., 2010, Lepelletier *et al*., 2014). However, FISH analysis using the PERKIN_01 and PERKIN_02 probes gave no positive signals, except for stations JC03970 (1-2 cells ml^{-1}) and JC03972 (3-4 cells ml^{-1}) (Figure 2b). This is consistent with Perkinsozoa being largely absent from the water column but rather being preferably found in sediments (Chambouvet *et al*., 2014).

Recent environmental surveys of lacustrine microbial eukaryotes have revealed a wide species diversity and major role of fungal parasites in these systems, consisting primarily of chytrids (Chytridiomycota). In contrast, 18S rRNA gene surveys focusing on the small eukaryotic fraction (<5 µm) in surface ocean waters have shown <1% of the sequences to be affiliated with fungi (Massana and Pedrós-Alió, 2008). However,

whether this low abundance of fungal sequences is real or due to copy number bias when using the 18S rRNA gene (Zhu *et al*., 2005) is unclear. To potentially get around the copy number problem, herein we assessed the distribution and abundance of free living fungal stages along AMT19 using three FISH probes targeting (1) all divisions of the Eumycota (MY1574; Baschien *et al*., 2008), (2) fungal species of the order Chytridiales (Chyt1061; Jobard *et al*., 2010), and (3) a subsection of environmental fungal sequences branching within the Cryptomycota clade which forms one of the deepest branches within the fungi (LKM11_01; Mangot *et al*., 2009). Members of Chytridiales and Cryptomycota are known to be parasites of phytoplankton in freshwater ecosystems (Sime-Ngando *et al*., 2011; Jones *et al*., 2011).

Free-living stages of fungi (mostly zoospores) were observed at all stations with probe MY1574, representing on average 9.3% of the total eukaryote community (Table 1 and Figure 2c). The maximum abundance of Eumycota (14% of all eukaryote cells targeted by the probe EUK1209) was recorded at station JC03974 in the ST region. Chytridiales (Chyt1061 probe) were less abundant, representing on average 3.5% of the total eukaryote community, and was absent from surface waters at three stations (Table 1). The LKM11-01 probe gave no positive signals at station sampled along AMT19, suggesting members of the Cryptomycota are not abundant in ocean surface waters (Jones *et al*., 2011), although environmental sequences corresponding to this group have been retrieved from deep-sea ecosystems, ocean sediments and freshwater lakes (Nagano *et al*., 2012; Jones *et al*., 2011; Lepère *et al*., 2008).

Associations between PPEs and potential parasites

Using dual labeling TSA-FISH on sorted Plast-S and Plast-L cells, no association was

detected between Syndiniales dinospores and PPEs. This observation is consistent with their known parasitism of larger cell types e.g. dinoflagellates (Siano *et al.*, 2011). An increase in abundance of larger eukaryotic cells may be the reason for the comparatively high Syndiniales cell counts at station JC03972, though unfortunately we did not perform dinoflagellate cell counts here.

Studies of fungal pathogens of marine algae have mostly focused on macroalgae (Kohlmeyer & Kohlmeyer 1979; Küpper et al. 2006) and tend to rely on cultivation-based methods. In this study, dual-labeled TSA-FISH combined with wheat germ agglutinin (WGA) chitin staining allowed us to detect associations between fungi and PPEs. The fungal structures that were identified correspond to the chitin positive sporangia life stage. Sporangia, which are larger than zoospores, appear attached to the surface of their algal hosts (Figure 3 and 4). The use of oligonucleotide probes that target rRNA allows to visualize active cells, which helps to reject the hypothesis of saprotrophic nutrition by the attached fungi.

No fungal associations were observed for any PPE class within the Plast-S population. However, dual TSA-FISH demonstrated fungi in association with Prymnesiophyceae and Chrysophyceae within Plast-L populations from several stations along the transect (Figures 3 and 4, Table 2). We would argue here that the significant difference ($p<0.05$) in associations between Plast-S and Plast-L populations is not due to sampling and sorting issues since the same method was used. Where positive signals were detected, on average 3±0.6% of Chrysophyceae cells were associated with fungi (detected by probes MY1574 and Chyt1061; Table 2, Figure 4). In contrast, an average of 6.4±0.9% Plast-L Prymnesiophyceae cells were identified with attached fungal structures

detected by the Eumycota probe MY1574, and 3.5% (on average) with the Chytrid probe Chyt1061. These associations were observed at stations all along the AMT19 transect studied here, including both ST and SG regions (Table 2), the numbers corresponding fairly well to observed maximum abundances of Prymnesiophyceae in the flow sorted Plast-L population (Supplementary Table 1). Moreover, we were able to see putative different stages of a fungal infection, highlighted by positive signals with the MY1574 probe combined with WGA (Wheat Germ Agglutinin) staining detecting the presence of fungal chitin (Figure 3).

Conclusions

This work suggests the quantitative importance of fungi in open ocean pelagic marine systems. Our direct microscopy observations complement phylogenetic data (for a review see: Richards *et al*., 2012) which suggested that marine fungi are more abundant and taxonomically diverse than previously thought. Thus, they are known to include a number of novel groups, the majority of which branch below the Dikarya radiation, close to the chytrid branches (Le Calvez *et al*., 2009; Richards *et al*., 2012), and are suspected to be parasitic.

Indeed, here, for the first time, we demonstrate potentially parasitic fungal associations with picophytoplankton, particularly members of the Prymnesiophyceae, one of the most abundant members of the PPE community globally (Liu *et al*., 2009; Kirkham *et al*., 2013). Further investigation of the diversity and specific roles of marine fungi is therefore warranted, particularly to better understand carbon flow in pelagic ecosystems. Besides viral and grazing pressure, our data suggests that picophytoplankton may be subjected to parasitism across vast tracts of the global ocean. We propose that future

investigation of eukaryotic parasitism will provide important new insights essential for measuring and modeling microbial food webs and biogeochemical cycles.

Acknowledgements

The authors would like to thank the Captain and crew aboard the RRS James Cook during AMT19. We thank Sophie Mazard for her helpful comments on the manuscript. This study was supported by the UK Natural Environment Research Council through Research Grants NE/F004249/1 and NE/G005125/1. C.L. was supported by the FP7- IEF Marie Curie program. This is Atlantic Meridional Transect Publication no 203.

References

Baschien, C., Manz, W., Neu, T.R., Marvanova, L., and Scewzyk, U. (2008) In situ detection of freshwater fungi in an alpine stream by new taxon-specific fluorescence in situ hybridization probes. *Appl Environ Microbiol* **74:** 6427-6436.

Bass, D., Howe, A., Brown, N., Barton, H., Demidova, M., *et al*. (2007) Yeast forms dominate fungal diversity in the deep oceans. *Proc Biol Sci* **274:** 3069–3077.

Burgaud, G., Le Calvez, T., Arzur, D., Vandenkoornhuyse, P., Barbier, G. (2009) Diversity of culturable marine filamentous fungi from deep-sea hydrothermal vents. *Environ Microbiol* **11:** 1588–1600.

Chambouvet, A., Morin, P., Marie, D., and Guillou, L. (2008) Control of toxic marine dinoflagellate blooms by serial parasitic killers. *Science* **322:** 1254-1257.

Chambouvet, A., Berney, C., Romac, S., Audic, S., Maguire, F., de Vargas, C., and Richards T.A. (2014) Diverse molecular signatures for ribosomally 'active' Perkinsea in marine sediments. *BMC Microbiol*. **14:** 110.

Cuvelier, M.L, Allen, A.E., Monier, A., McCrow, J.P., Messié, M., Tringe, S.G., *et al*. (2010) Targeted metagenomics and ecology of globally important uncultured eukaryotic phytoplankton. *Proc Natl Acad Sci USA* **107:** 14679–14684.

Gachon, C.M., Sime-Ngando, T., Strittmatter, M., Chambouvet, A., and Kim, G.H. (2010) Algal diseases: spotlight on a black box. *Trends Plant Sci* **15:** 633-640.

Gleason, F.H., and Marano, A.V. (2011) The effects of anti-fungal substances on some zoosporic fungi (Kingdom Fungi). *Hydrobiologia* **659:** 81-92.

Grob, C., Hartmann, M., Zubkov, M.V., and Scanlan, D.J. (2011) Invariable biomass specific primary production of taxonomically discrete picoeukaryote groups across

the Atlantic Ocean. *Environ Microbiol* **12:** 3266-3274.

Grob, C., Jardillier, L., Hartmann, M., Ostrowski, M., Zubkov, M.V., and Scanlan, D.J. (2015) Cell-specific CO_2 fixation rates of two distinct groups of plastidic protists in the Atlantic Ocean remain unchanged after nutrient addition. *Environ Microbiol Rep* **7:** 211-218.

Giovannoni, S. J., E. F. Delong, G. J. Olsen, and N. R. Pace. **(**1988) Phylogenetic group-specific oligodeoxynucleotide probes for identification of single microbial cells. J. Bacteriol. **170:**720–726.

Guillou, L., Viprey, M., Chambouvet, A., Welsh, R.M., Kirkham, A.R., Massana, R., Scanlan, D.J., and Worden, A.Z. (2008) Widespread occurrence and genetic diversity of marine parasitoids belonging to Syndiniales (Alveolata). *Environ Microbiol* **10:** 3349-3365.

Hartmann, M., Zubkov, M.V., Scanlan, D.J., and Lepère, C. (2013). *In situ* interactions between photosynthetic picoeukaryotes and bacterioplankton in the Atlantic Ocean: evidence for mixotrophy. *Environ Microbiol Rep* **5:** 835-840.

Hartmann, M., Grob, C., Tarran, G.A., Martin, A.P., Burkill, P.H., Scanlan, D.J., and Zubkov, M.V. (2012) Mixotrophic basis of Atlantic oligotrophic ecosystems. *Proc Natl Acad Sci USA* **109:** 5756-5760.

Jardillier, L., Zubkov, M.V., Pearman, J., and Scanlan, D.J. (2010). Significant CO_2 fixation by small prymnesiophytes in the subtropical and tropical northeast Atlantic Ocean. *ISME J* **4:** 1180–1192.

Jebaraj, C.S., Raghukumar, C., Behnke, A., and Stoeck, T. (2010) Fungal diversity in oxygen-depleted regions of the Arabian Sea revealed by targeted environmental

sequencing combined with cultivation. *FEMS Microbiol Ecol* **71:** 399-412.

Jones, M.D.M., Forn, I., Gadelha, C., Egan, M.J., Bass, D., *et al*., (2011) Discovery of novel intermediate forms redefines the fungal tree of life. *Nature* **474:** 200–203

Kirkham, A., Lepère, C., Jardillier, L., Mead, A., and Scanlan, D.J. (2013) A global perspective on marine photosynthetic picoeukaryote community structure. *ISME J* **7:** 922-936.

Kohlmeyer, J., and Kohlmeyer, E. (1979) *Marine Mycology: The Higher Fungi*. New York: Academic Press.

Kupper, F.C., Maier, I., Müller, D.G., Loiseaux-de Goer, S., and Guillou, L. (2006) Phylogenetic affinities of two eukaryotic pathogens of marine macroalgae, *Eurychasma dicksonii* (Wright) Magnus and *Chytridium polysiphoniae* Cohn. *Cryptogam Algol* **27:** 165–84.

Lafferty, K.D., Allesina, S., Arim, M., Briggs, C.J., de Leo, G., Dobson, A.P. *et al*., (2008) Parasites in food webs: the ultimate missing links. *Ecol Letts* **11:** 533-546.

Leander, B. S. and Keeling, P.J. (2003) Morphostasis in alveolate evolution. *Trends Ecol Evol* **18:** 395–402.

Le Calvez, T., Burgaud, G., Mahé, S., Barbier, G., and Vandenkoornhuyse, P. (2009) Fungal diversity in deep-sea hydrothermal ecosystems. *Appl Environ Microbiol* **75:** 6415–6421.

Lepelletier F., Karpov, S. A., Alacid, E., Le Panse, S., Bigeard, E., Skovgaard, A., Jeanthon, C., and Guillou, L. (2014). *Parvilucifera rostrata* sp. nov. (Perkinsozoa), a novel parasitoid that infects planktonic dinoflagellates. *Protist* 165: 31-49.

Lepère, C., Domaizon, I., and Debroas, D. (2008) Unexpected importance of potential parasites in the composition of the freshwater small-eukaryote community. *Appl Environ Microbiol* **74:** 2940-2949.

Liu, H., Probert, I., Uitz, J., Claustre, H., Aris-Brosou, S., Frada, M., *et al*., (2009). Extreme diversity in non-calcifying haptophytes explains a major pigment paradox in open oceans. *Proc Natl Acad Sci USA* **106:** 12803-12808.

Lopez-Garcia, P., Vereshchaka, A., and Moreira, D. (2007) Eukaryotic diversity associated with carbonates and fluid seawater interface in Lost City hydrothermal field. *Environ Microbiol* **9:** 546–554.

Marcogliese, D.J., and Cone, D.K. (1997) Food webs: a plea for parasites. *Trends Ecol Evol* **12:** 320-325.

Massana, R., and Pedrós-Alió, C. (2008) Unveiling new microbial eukaryotes in the surface ocean. *Curr Opin Microbiol* **11:** 213-218.

Nagahama, T., Hamamoto, M., Nakase, T., Takami, H., and Horikoshi, K. (2001). Distribution and identification of red yeasts in deep-sea environments around the northwest Pacific Ocean. *Antonie van Leeuwenhoek* **80:** 101–110.

Nagano, Y., and Nagahama, T. (2012) Fungal diversity in deep-sea extreme environments. *Fungal Ecol* **5:** 463-471.

Nagano, Y., Nagahama, T., Hatada, Y., Nunoura, T., Takami, H., Miyazaki, J., Takai, K., and Horikoshi, K. (2010) Fungal diversity in deep-sea sediments -the presence of novel fungal groups. *Fungal Ecol* **3:** 316-325.

Niquil, N., Kagami, M., Urabe, J., Christaki, U., Viscogliosi, E., and Sime-Ngando, T. (2011) Potential role of fungi in plankton food web functioning and stability: a simulation analysis based on Lake Biwa inverse model. *Hydrobiologia* **659:** 65-79.

Richards, T.A., Jones, M.D.M., Leonard, G., and Bass, D. (2012) Marine fungi: their ecology and molecular diversity. *Ann Rev Mar Sci* (2012) **4:** 495–522.

Sauvadet, A.L., Gobet, A., and Guillou, L. (2010) Comparative analysis between protist communities from the deep-sea pelagic ecosystems and specific deep hydrothermal habitats. *Environ Microbiol* 12: 2946-2964.

Siano, R., Alves-de-Souza, C., Foulon, E., El Bendif, M., Simon, N., Guillou, L., and Not, F. (2011) Distribution and host diversity of *Amoebophryidae* parasites across oligotrophic waters of the Mediterranean Sea. *Biogeosciences* **8:** 267–278.

Sime-Ngando, T., Lefevre, E., and Gleason, F.H. (2011) Hidden diversity among aquatic heterotrophic flagellates: ecological potentials of zoosporic fungi. *Hydrobiologia* **659:** 5-22.

Unrein, F., Gasol, J.M., Not, F., Forn, I. and Massana, R. (2014) Mixotrophic haptophytes are key bacterial grazers in oligotrophic coastal waters. *ISME J* **8:** 164–176.

Vaulot, D., Eikrem, W., Viprey, M., and Moreau, H. (2008) The diversity of small eukaryotic phytoplankton (<3μm) in marine ecosystems. *FEMS Microbiol Rev* **32:** 795-820.

Zhu F, Massana R, Not F, Marie D, Vaulot D. (2005) Mapping of picoeucaryotes in marine ecosystems with quantitative PCR of the 18S rRNA gene. *FEMS Microbiol Ecol* (2005) 52: 79-92.

Tables:

Table 1. The percentage contribution of Syndiniales and Fungi, targeted by the ALVO1, MY1574 and Chyt1061, probes respectively, to the total eukaryotic community (<5 µm).

Table 2. The percentage association between PPEs and fungi along AMT19.

Figures:

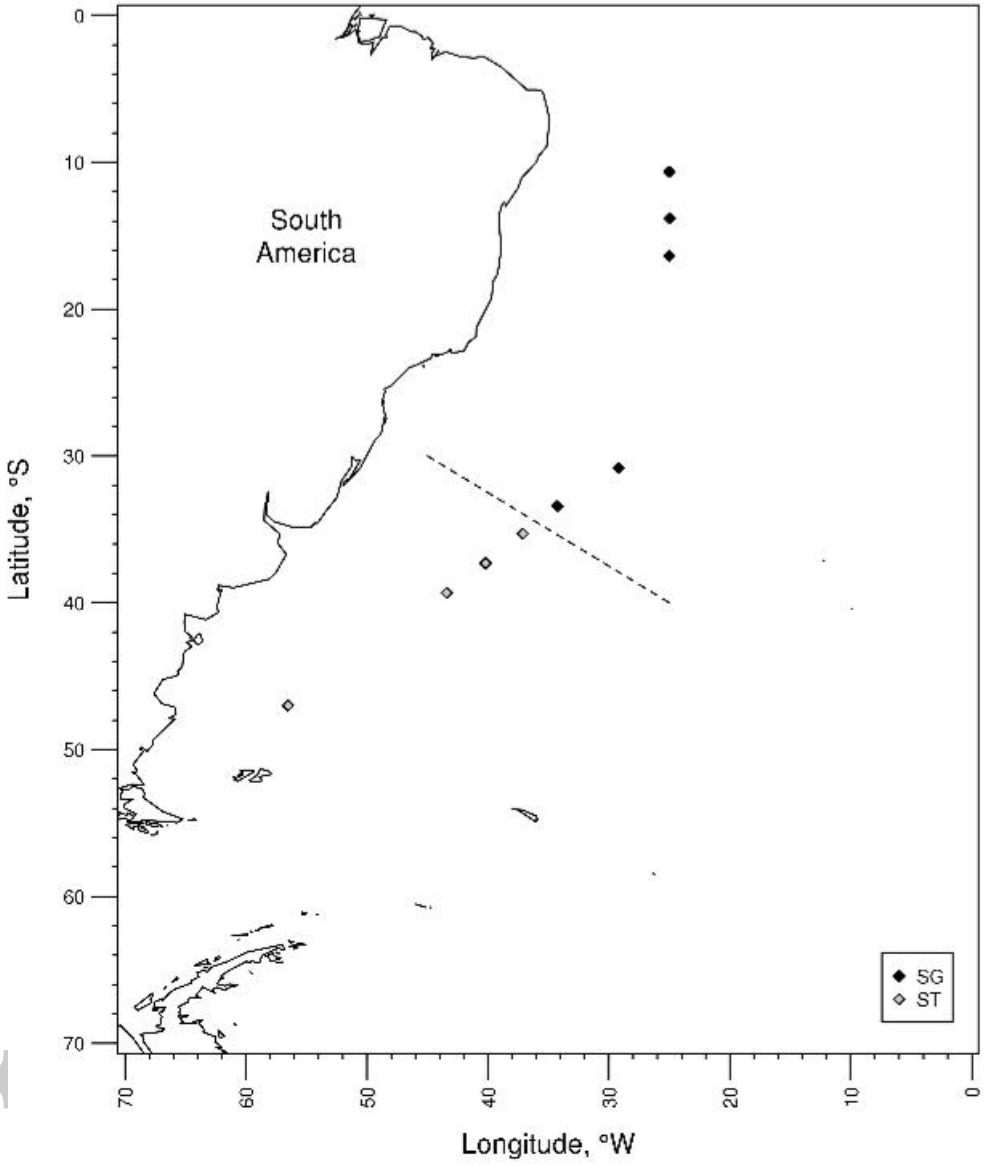
Figure 1. A schematic map of the South Atlantic Ocean showing the area sampled along AMT19 in 2009. ST: Southern temperate region; SG: Southern subtropical gyre; Dotted line represents the separation between ST and SG.

Figure 2. Epifluorescence micrographs of a) free living Syndiniales (targeted by the ALV01 probe b) Perkinsozoa (targeted by the PERKIN-01 probe), and c, d) fungi (targeted by probes MY1574, and Chyt1061) (the green colour shows the positive signal of the horseradish peroxidase (HRP)-labeled probes).

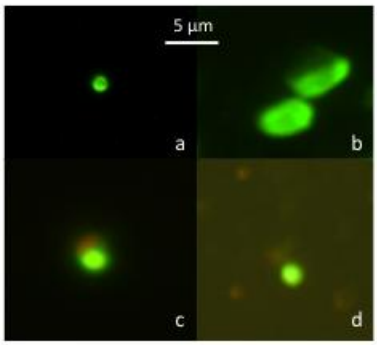
Figure 3. Epifluorescence micrographs of the potentially different stages of fungal infection of Prymnesiophyceae cells. The green colour shows the positive signal of the horseradish peroxidase (HRP)- labelled probe MY1574 (a, c), whilst the blue colour is wheat germ agglutinin binding of chitin cell walls (b, d) and the red colour constitutes the positive signal of the PRYM02 probe after TSA-FISH

Figure 4. Epifluorescence micrographs of (a) a Prymnesiophyceae (PRYM02 probe, red) and (b) a Chrysophyceae (CHRYSO1037 probe, red) in association with chitin structures (stained with wheat germ agglutinin, in blue/white).

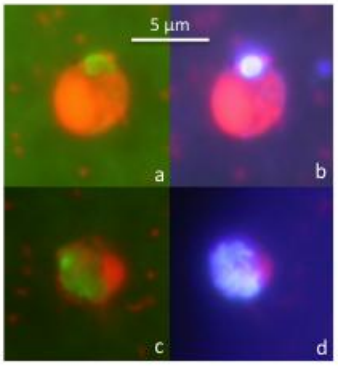
Supplementary Table 1. The location, abundance and composition of PPEs at specific stations along AMT19.



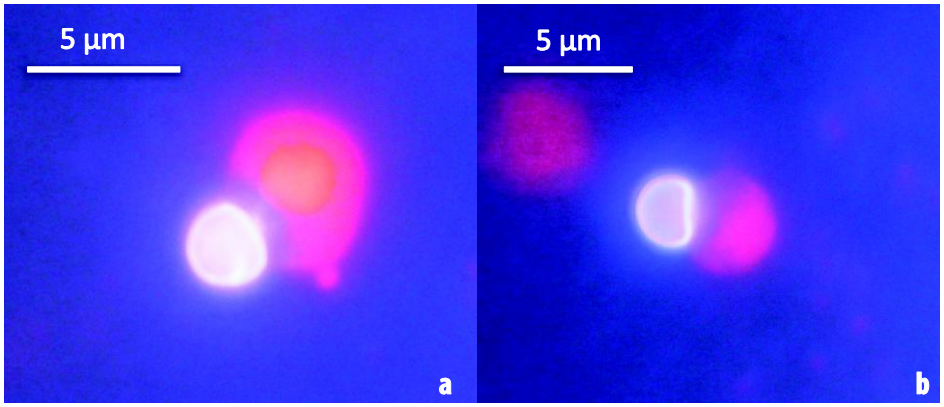
EMI4_12339_F1



EMI4_12339_F2



EMI4_12339_F3



EMI4_12339_F4

Table 1. The percentage contribution of Syndiniales and Fungi, targeted by the ALVO1, MY1574 and Chyt1061, probes respectively, to the total eukaryotic community (<5 µm).

			% total euk		
			Syndiniales	Fungi	
	Station	Depth (m)	ALV01 probe	MY1574 probe	Chyt1061 probe
SG	JC039053	5	1	12	1.2
SG	JC039053	25	0	8.2	1.9
SG	JC039055	5	0	3.3	0.8
SG	JC039056	5	0	11	5
SG	JC03967	5	0	2.1	0
SG	JC03967	25	5	8.2	2.1
SG	JC03969	5	2	5.1	2
SG	JC03970	88	0	8	2.1
ST	JC03971	5	7	1.5	0
ST	JC03972	5	26	9	3.6
ST	JC03974	10	0	14	9.3
ST	JC039	5	3	2	0

Table 2. The percentage association between PPEs and fungi along AMT 19

			Plast-L			
			% association with Prymnesiophyceae (PRYM02)	% association with Chrysophyceae (CHRYSO1037)	% association with Prymnesiophyceae (PRYM02)	% association with Chrysophyceae (CHRYSO1037)
	station	Depth (m)	General fungi probe (MY1574)		Chytridiales probe (Chyt1061)	
SG	JC039053	5	7	5	5	1
SG	JC039053	25	5	2	0	0
SG	JC039055	5	9	0	3	0
SG	JC039056	5	0	0	5	2
SG	JC03967	5	0	0	0	0
SG	JC03967	25	0	1	0	0
SG	JC03969	5	6	0	3	0
SG	JC03970	88	0	0	0	0
ST	JC03971	5	4	6	3	3
ST	JC03972	5	0	0	0	0
ST	JC03974	10	12	4	2	0
ST	JC039	5	2	0	0	0
	Mean		4	1.5	2	0.5