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Novel lineage patterns from an automated water sampler to probe marine microbial biodiversity with ships of opportunity

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ABSTRACT

There is a paucity of data on long-term, spatially resolved changes in microbial diversity and biogeography in marine systems, and yet these organisms underpin fundamental ecological processes in the oceans affecting socio-economic values of the marine environment. We report results from a new autonomous Water and Microplankton Sampler (WaMS) that is carried within the Continuous Plankton Recorder (CPR). Whilst the CPR with its larger mesh size (270 μm), is designed to capture larger plankton, the WaMS was designed as an additional device to capture plankton below 50 μm and delicate larger species, often destroyed by net sampling methods. A 454 pyrosequencing and flow cytometric investigation of eukaryotic microbes using the partial 18S rDNA from thirteen WaMS samples collected over three months in the English Channel revealed a wide diversity of organisms. Alveolates, Fungi, and picoplanktonic Chlorophytes were the most common lineages captured despite the small sample volumes (200–250 ml). The survey also identified Cercozoa and MAST heterotrophic Stramenopiles, normally missed in microscopic-based plankton surveys. The most common was the likely parasitic LKM11 Rozellomycota lineage which comprised 43.2% of all reads and are rarely observed in marine pelagic surveys. An additional 9.5% of reads belonged to other parasitic lineages including marine Syndiniales and Ichthyosporea. Sample variation was considerable, indicating that microbial diversity is spatially or temporally patchy. Our study has shown that the WaMS sampling system is autonomous, versatile and robust, and due to its deployment on the established CPR network, is a cost-effective monitoring tool for microbial diversity for the detection of smaller and delicate taxa.

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1. Introduction

Microbial communities are important drivers of marine food webs that are responsible for 50–75% of primary production and play a fundamental role in carbon assimilation and nutrient recycling (Jiao et al., 2010). Photosynthetic Pico- (0.2–2 μm) and nanoplankton (2–20 μm) contribute 50–70% and up to 30–50% of Carbon fixation in Atlantic respectively (Poulton et al., 2006). An increasing number of publications attest to the diversity of marine microbiota, and their roles are varied. Mixotrophy is common and relations with metazoa can be complex – for example, heterotrophic dinoflagellates can predate on zooplankton at several life cycle stages (Jeong, 1999). At the same time, scientists lament the lack of understanding of eukaryotic microbes within the microbial food web and their influence on higher trophic levels (Sherr and Sherr, 2002). A large proportion of picoplankton consist of bacterioplankton, of which it is estimated up to 50 percent are grazed by nano- to pico-sized heterotrophic eukaryotes that can be present at elevated levels ($\leq 10^3$ cells ml^{-1}) (Fuhrman and McManus, 1984; Fenchel, 1986). The diversity and roles of microbial heterotrophs, parasites and detritivores are particularly understudied and deserve more attention if we are to understand how the environment influences fluctuations in microbial community structure and how these changes impact the larger organisms that depend on marine microbes.

One reason for this lack of understanding is due to the limitations in accessing the marine environment for sampling and data

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collection. Compared to terrestrial monitoring, marine monitoring is expensive (Radulovici et al., 2010) and therefore mostly limited to coastal regions, with sampling programs typically deployed from one or few locations. This leaves extensive spatial areas under-sampled, resulting in gaps in our collective understanding. A second reason is the assumption that microbial organisms, particularly nano- and pico-sized microbes, are essentially cosmopolitan and have no biogeographical limits or habitats (Finlay and Fenchel, 2004). Such ideas are being challenged, providing increasing evidence of localisation both in space and time. Examples of this include, the habitat boundaries of the mineralising nanoplankters such as *Emiliania huxleyi*, *Fragilariopsis* spp. and *Tetraparma pelagica*, which are formed along major circumpolar fronts (Hinz et al., 2012), whilst the picoplankter *Ostreococcus* is restricted to distinct depths to take advantage of different light wavelengths (Demir-Hilton et al., 2011).

Marine systems are changing quickly on a global scale due to climate change (Edwards et al., 2006; Beaugrand, 2003; Edwards and Richardson, 2004) and anthropogenic pressures, such as pollution and the introduction of new species. Thus the need to understand the marine microsphere at larger scales is imperative. The diversity and distribution of marine microbes is currently a known knowledge gap which needs to be filled in order to implement marine stewardship such as the Ecosystem Approach to management, as required, for example, in the EU by the Marine Strategy Framework Directive (MSFD) Beaugrand, 2003 and Canada's Department of Fisheries and Oceans Ecosystem Science Framework (2007).

The Continuous Plankton Recorder (CPR) survey, the longest-running marine survey, has been operating since 1931 (Reid et al., 2003) and has proved to be an invaluable tool for identifying and recording plankton. It has extensive coverage across the North Atlantic and North Pacific, and is now being deployed by other sister surveys populating regions such as the Arctic Ocean, Southern Ocean (AusCPR) and South Atlantic (Flavell, 2012). The autonomous nature of this sampling survey has allowed vast sampling distances to be covered. CPRs are deployed using 'ships of opportunity' such as cargo ships and passenger ferries on their regularly scheduled commercial routes, enabling the CPR survey to collect plankton at relatively low cost. Through visual identification the CPR has thus far recorded ~500 plankton taxa (Edwards et al., 2011) and has sampled >6 million nautical miles of ocean, making it the world's most spatially-extensive marine monitoring programme. To take advantage of the automated CPR platform, an automated Water and Microplankton Sampler (WaMS) was developed as an additional device to capture planktonic organisms under 50 μm- especially the pico (<2 μm) and nano (<20 μm) sized fractions (Fig. 1) and be flexible enough to capture other parameters such as nutrients. This device is a pump connected to sampling bags that is deployed inside the CPR's cargo bay and in this instance can take up to 10 sample bags of up to 100–150 ml each. The autonomous software control triggers sample acquisition where sampling volume, location, timing and peristaltic tube flushing can be controlled. Autonomous sampling devices collect a wide range of volumes from 100 ml (e.g. the Remote Access Sampler (MacLane laboratories) and the AQI Water Sampler for Underwater Autonomous Vehicles (Wulff et al., 2010)) up to 120 l (Wommack et al., 2004) from a semi-autonomous Large-Volume Water Sampler, but are generally under 2 l and often constrained by the size of the housing needed for protection or floatation, particularly when deployed on gliders.

To investigate whether the CPR can be augmented to more adequately survey the pico- to microenvironment (plankton less than 50 μM), the WaMS was deployed on board a CPR in the English Channel. This is a marine system where there are long-standing taxonomically monitored stations on the Devon coast at the Western Channel Observatory (L4 and E1), (reviewed by Southward et al. 2004) and on the French coast, at SOMLIT-Astan (Guilloux et al. 2013) and the estuarine Dourduff station (Romari and Vaulot, 2004). Samples collected using the WaMS were tested for marine eukaryotic microdiversity using 454 pyrosequencing to obtain longer amplicons for better taxonomic resolution but at a relatively shallow-scale of detection (8000 sequences per sample) for Next Generation Sequencing (NGS) to conform to a low-cost testing platform often required in long-term marine surveys. We chose the V4 region of the 18S ribosomal DNA marker as it is the most populous marker in public databases for eukaryotes and has been chosen as a primary barcode candidate for protists (Pawlowski et al., 2012), which constitute most of the smaller-sized plankton.

2. Methods

2.1. Sample collection

The WaMS (Cefas Technology Ltd.) was designed to fit into the empty tail plane of a CPR unit. The WaMS consists of a battery-operated peristaltic pump that discharges water via microprocessor-controlled valves at set intervals into VA Parenteral Nutrition Container plastic medical reagent bags (Baxter, IL, USA). A detailed description is shown in Fig. 1. The tubes attached to the bags were tied together to avoid getting lost during deployment. Similarly the bags were attached to the cage and given extra protection within the steel tail plane area of the CPR. The instrument has a seawater switch, a sensor that detects when it is underwater that is then activated to start collecting samples. The WaMS can take up to 10 samples each of 100–150 ml. Between 12–24 h prior to deployment the WaMS was pumped through with 1 l of bleached water (1000 ppm) and then rinsed with 1 l of MilliQ water to clean the tubing. The sampling bags were reused after soaking overnight in bleached water (1000 ppm), then washed in detergent, which was rinsed off thoroughly with MilliQ water on the day of deployment. The washing process was done as near to deployment to avoid bacterial growth.

The CPR fitted with the WaMS was deployed and towed behind the Brittany Ferries ship MV Armorique on monthly crossings from Roscoff, France, to Plymouth, UK, at a depth of 10 m between February and May 2011 (Fig. 2, Table A1). The WaMS was programmed to collect five pairs of seawater samples (ten in total) at 70 min intervals along the route (Table A1) using proprietary software supplied with the instrument. The time period between collection and sample processing (filtration – see below) was between 5.5 h (first sample) and 1.5–2 h (last sample). Samples were kept underwater after collection and were processed within 1–1.5 h. Each sample bag pair was combined together before processing. In all, thirteen samples were collected between February and May 2011. Samples were collected for flow cytometric quantification of preserved samples (2 ml samples in polypropylene cryovials with 1% TEM grade glutaraldehyde (final concentration)) and processed as described below. The remaining water was processed for molecular analysis of biodiversity using cleaned, sterilised apparatus. In February 2011, only two locations were sampled and, due to sample collection faults in March, there was only sufficient water to carry out 18S-diversity analysis at two of the five possible locations.

2.2. Enumeration of phytoplankton and bacteria by flow cytometry

Phytoplankton and bacteria were enumerated using a BD Accuri™ C6 flow cytometer equipped with a 50 mW solid state laser providing blue light at 488 nm. Samples were analysed at a

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