Living on the edge: biofilms developing in oscillating environmental conditions

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Living on the edge: biofilms developing in oscillating environmental conditions

Sergey Dobretsov, Raed M. M. Abed, Thirumahal Muthukrishnan, Priyanka Sathe, Laila Al-Naamani*, Bastien Y. Quested and Sergey Piontkovski

ABSTRACT

For the first time, the densities and diversity of microorganisms developed on ocean gliders were investigated using flow cytometry and Illumina MiSeq sequencing of 16S and 18S rRNA genes. Ocean gliders are autonomous buoyancy-driven underwater vehicles, equipped with sensors continuously recording physical, chemical, and biological parameters. Microbial biofilms were investigated on unprotected parts of the glider and surfaces coated with base, biocidal and chitosan paints. Biofilms on the glider were exposed to periodical oscillations of salinity, oxygen, temperature, pressure, depth and light, due to periodic ascending and descending of the vehicle. Among the unprotected surfaces, the highest microbial abundance was observed on the bottom of the glider’s body, while the lowest density was recorded on the glider’s nose. Antifouling paints had the lowest densities of microorganisms. Multidimensional analysis showed that the microbial communities formed on unprotected parts of the glider were significantly different from those on biocidal paint and in seawater.

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KEYWORDS

Biofilm; antifouling; chitosan; next generation sequencing; ocean glider; Indian Ocean

Introduction

Ocean gliders are a relatively recent tool in oceanography, which allow autonomous collection of long-term oceanographic data over long distances (Figure 1A). Ocean gliders are autonomous buoyancy-driven autonomous underwater vehicles (AUV), equipped with sensors continuously recording physical, chemical and biological parameters. Compared to other AUV, the lithium sulfuryl chloride battery enables ocean gliders to be operational for up to 10 months and the battery powers different sensors that can be directly controlled by an operator. The examples of sensors typically installed on ocean gliders include temperature, salinity, oxygen and chlorophyll sensors (Eriksen et al. 2001). During the collection of data, ocean gliders can dive from the surface to the sea bottom through changes in their buoyancy (Figure 1B). At such times, the glider and, in particular, its sensors may be vulnerable to transient biofouling. The intensity of biofouling on ocean gliders depends on the environment (Lobe et al. 2010). Generally, more organisms accumulate on artificial substrata exposed in tropical waters than in temporal ones (Moline & Wendt 2011).

Marine biofouling is the undesirable growth of organisms on submerged surfaces (Wahl 1989). Any clean artificial substrata will be colonized by bacteria, diatoms and other microscopic unicellular eukaryotes within hours after submersion (Salta et al. 2013). At this stage, a well-developed biofilm will be formed composed of multiple species of prokaryotic and eukaryotic organisms with dominance of bacteria and diatoms (Dobretsov 2010).

Biofouling has huge economic impacts on maritime industries. Worldwide, countries spend billions of dollars in order to manage and prevent this problem (Callow & Callow 2011). Biofouling may significantly increase vehicle drag of the ocean glider, interfere with the stability of the scientific sensors and limit good data collection (Davis et al. 2003; Medeot et al. 2011). In order to prevent biofouling, maritime industries use biocides or other toxic compounds applied as antifouling (AF) paints (Yebra et al. 2004; Lobe et al. 2010).

Biocidal paints kill marine organisms and cause
undesirable environmental impacts, hence new low toxic and non-toxic AF paints are urgently needed.

Chitosan has been proposed as a promising non-toxic AF agent (Pelletier et al. 2009) due to its antimicrobial properties (Kim & Rajapakse 2005). Chitosan is a naturally occurring linear polysaccharide composed of D-glucosamine and N-acetyl-D-glucosamine obtained by deacetylation of crustacean waste (Xiao 2012). Previous laboratory and mesocosm experiments showed that chitosan led to a reduction in the densities of diatoms and bacteria on experimental paints (Al-Naamani et al. 2017). Another study demonstrated that chitosan-based paints reduced growth of bacteria for four days and inhibited densities of photosynthetic organisms for 14 days in northern estuarine waters (Pelletier et al. 2009). The AF properties of chitosan have not been studied in long-term field marine experiments in tropical waters.
The Sea of Oman, previously known as the Gulf of Oman, is situated between the shallow (<50 m), high salinity waters of the Persian (Arabian) Gulf and the deeper (>1,000 m) Arabian Sea, and hence possesses a unique hydrological regime (Al-Hashmi et al. 2010; Banse 1997; Vic et al. 2015; Queste et al. 2018). One of the most intensive coastal upwelling phenomena in the world characterizes Oman east coastal waters (Reynolds 1993; Al-Hashmi et al. 2010). The circulation is driven by reversing summer and winter monsoons, thus impacting the depth of high salinity Arabian Gulf outflow water and exchanging at the eastern boundary with the Arabian Sea (Vic et al. 2015). The unique circulation and high production in the Sea of Oman create an oxygen minimum zone where there is almost no oxygen in the water column (<2 μmol kg⁻¹, Banse & Piontkovski 2006; Piontkovski et al. 2017; Queste et al. 2018). The Sea of Oman provides a unique opportunity to investigate the formation of microbial biofilms on ocean gliders at gradients of salinity, temperature, oxygen and pressure.

In this study, the formation of microbial biofilms on coated and uncoated parts of an ocean glider during its deployment in the Sea of Oman was investigated. Biofilms developed on the glider over three months and were exposed to continuous variations in salinity, oxygen, light, temperature and pressure. The main objectives of this study were to investigate: (1) the composition of the prokaryotic and eukaryotic communities formed on painted and unpainted parts of the glider, and (2) the AF effect of commercial and non-toxic experimental paints.

### Material and methods

#### The ocean glider’s deployment

A Kongsberg ocean glider was deployed 5 km off the coast of Muscat, Oman, at 23°41.66’N, 58°40.7’W (Figure 1C) on 4 March 2015 and was retrieved on 3 June 2015 (at 23°43.01’N, 58°39.7’W). The intention was to collect data throughout the end of the North-East monsoon and the onset of the spring inter-monsoon period. The ocean glider consisted of an aluminium pressure hull surrounded by a flooded fibreglass fairing. The body of the instrument was not coated with any specific AF agents. Yellow coloured fibreglass maximizes durability and visibility of the glider at the sea. A total of 712 dives with 1,424 vertical profiles of environmental parameters covering over 2,080 km and repeating the survey transects 24 times out over a period of 91 days were carried out. The ocean glider was equipped with a Seabird free-flushing CT sail (Bellevue, WA, USA), an Aanderaa 4330 F oxygen optode (Bergen, Norway), a Biospherical QSP-2150 PAR sensor (San Diego, CA, USA) (spectral region: 400–700 nm), a Wetlabs Triplet ECO sensor measuring chlorophyll a (Kenilworth, UK) (based on fluorescence intensity) and backscatter at 470 and 700 nm (Piontkovski et al. 2017). Satellite communication was used for retrieval of the data in near real time after every dive at a speed of about 25 cm s⁻¹.

### Coatings

In total, five different coatings were tested (Table 1). These include two types of biocidal AF paints (International Micron Extra YBA 920 (AkzoNobel, International Paint Ltd, Felling, Tyne and Wear UK) and Hempel Olympic 86950 (Hempel, Kongens Lyngby, Denmark), referred to as “paint”, PIn and PHe), one experimental non-biocidal chitosan paint (“paint”, PCh) and two primer base coatings (Intershield 300 and Hempel primer, “base”, Bln and BHe). The base coatings did not contain any biocides and served as controls for the AF paints. Chitosan paint was prepared according to Al-Naamani et al.

#### Table 1. Samples taken from the ocean glider and the characteristics of the paints used in this study.

<table>
<thead>
<tr>
<th>Code</th>
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<th>Location</th>
<th>Paint</th>
<th>Type of paint</th>
<th>Active ingredient</th>
</tr>
</thead>
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<tr>
<td>PHeB</td>
<td>Paint</td>
<td>Glider bottom</td>
<td>Hempel Olympic 86950</td>
<td>Biocidal</td>
<td>Cuprous oxide and zineb</td>
</tr>
<tr>
<td>PinT</td>
<td>Paint</td>
<td>Glider top</td>
<td>International micron extra YBA920</td>
<td>Biocidal</td>
<td>Cuprous oxide and dichlofluorid</td>
</tr>
<tr>
<td>PinB</td>
<td>Paint</td>
<td>Glider bottom</td>
<td>International micron extra YBA920</td>
<td>Biocidal</td>
<td>Cuprous oxide and dichlofluorid</td>
</tr>
<tr>
<td>PChT</td>
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<td>Experimental chitosan</td>
<td>Non-biocidal</td>
<td>Chitosan</td>
</tr>
<tr>
<td>BlnT</td>
<td>Base</td>
<td>Glider top</td>
<td>Primer Intershield 300</td>
<td>Non-biocidal</td>
<td>No</td>
</tr>
<tr>
<td>BlnB</td>
<td>Base</td>
<td>Glider bottom</td>
<td>Primer Intershield 300</td>
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<td>Hempel Primer 26050</td>
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<td>Hempel Olympic 86950</td>
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</tr>
<tr>
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<td>No</td>
<td>No</td>
</tr>
<tr>
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<td>No</td>
<td>No</td>
</tr>
<tr>
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<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>UTB</td>
<td>Unprotected</td>
<td>Glider’s tail wing bottom</td>
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<td>No</td>
<td>No</td>
</tr>
<tr>
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<td>Unprotected</td>
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<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>UN</td>
<td>Unprotected</td>
<td>Glider’s nose</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>
was estimated using flow cytometry (FC). The abundance of prokaryotes in each sample was characterized according to Piontkovski et al. (2017). Briefly, chitosan paints were made using 1.5% chitosan (Sigma Aldrich, Gillingham, UK) solution in 1% acetic acid (Sigma Aldrich). The solution was mixed for 10 min and then sonicated for 15 min. The fibreglass surface of the glider was not protected with any specific AF agents ( "unprotected", U). Before application of paints, the surface of the ocean glider was cleaned with ethanol (96%, Sigma-Aldrich, Gillingham, UK). All paints were applied in replicated strips (10 × 70 cm) at the top and bottom of the ocean glider using brushes. The paints were air dried at room temperature for 24 h before the glider was deployed into the sea.

**Sampling**

On 3 June 2015, the ocean glider was gently lifted to the surface of the Research Vessel Al Jamiya, Muscat, Oman. Biofilms from the painted area (~700 cm²) (paint and base, Figure 1D) were scraped off using sterilized microscope slides and collected into individual sterile tubes. Remaining biofilms were washed with sterilized seawater and collected into the same tube. Replicated samples of undisturbed biofilms were scraped off as described above from unprotected parts of the ocean glider covering an area of ~500 cm²: top (UGT) and bottom (UGB) of the body, and the top of the wings (UWT) (Figure 1D, Table 1). Biofilms from the bottom parts of the wings were disturbed during withdrawal of the ocean glider and, thus, were not sampled. The smaller areas (~100 cm²) were also sampled as described above from the glider’s nose (UN), the top tail wing (UTT) and the bottom tail wing (UTB) (Figure 1D). Additionally, one-litre seawater samples (seawater) were collected on June 2015 from the area of the ocean glider retrieval with Niskin bottles (volume 5 l) from the 15 m (SW1), 25 m (SW2), 35 m (SW3) and 50 m (SW4) depths. Biofilms and water samples were immediately brought on ice to the Sultan Qaboos University laboratories and processed (see below).

**Sample analysis**

**Abundance of microbes**

Abundances of phytoplankton eukaryotes in water were determined by direct counts in the Niskin bottle samples using a Zeiss inverted microscope (Germany, 50× and 100× magnification). The taxonomic composition of the phytoplankton eukaryotic community was characterized according to Piontkovski et al. (2017). The abundance of prokaryotes in each sample was estimated using flow cytometry (FC). FC measurements were performed using BD FACSAriaTM III (BD Biosciences, Franklin Lakes, NJ, USA). Before the analysis, each sample was filtered through a 40 μm nylon cell strainer Falcon™ (Thermo Fisher Scientific, Waltham, MA, USA) to exclude large cells, cell clumps and detritus particles. Samples were stained with SYBR green I stain (Molecular Probes, Invitrogen, Carlsbad, CA, USA, excitation/emission wavelengths: 497 nm/520 nm; dilution 1:10,000) and incubated for 10 min in the dark. Each sample was divided into three independent fractions. Thus, three independent FC readings were recorded for each sample. The average number of cells ml⁻¹ for each sample was calculated. The density of microorganisms on the surface of the ocean glider was calculated taking into account the size of sampled area and the amount of liquid used to wash it. The densities of prokaryotes in the water column at different sampling depths and on different parts of the glider were compared using factorial analysis of variance (ANOVA) using Statistica 11 (Statsoft, Tulsa, OK, USA). The normality of the data was verified using the Shapiro-Wilk’s W test. Post hoc Tukey’s HSD test was used to test significance of differences between microbial abundances. In all cases, a p-value <0.05 was considered statistically significant.

**DNA extraction and MiSeq analyses**

The scraped samples from painted and unprotected surfaces of the glider were frozen and kept at ~80°C until the analysis of microbial community composition using next-generation sequencing. Prior to DNA extraction, water samples were filtrated through 0.2 μm Whatman (GE Healthcare, Chicago, IL, USA) filter. DNA from each sample from the glider and water column was extracted using a Power Biofilm (Qiagen, Venlo, Netherlands) kit following the manufacturer’s instructions. Purified DNAs were analysed at the Molecular Research (MRDNA) company (Shallowater, TX, USA). Illumina MiSeq was used to sequence the 16S and 18S rRNA genes. Bacterial V3-V4 regions of 16S rRNA genes were sequenced using the primers 515 F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806 R (5′-GGACTACHVGGGTWTCTAA-3′). Eukaryotic 18S rRNA genes were sequenced using the primers Euk7F (5′-AACCTGTTGATCTCCTGCCAGT-3′) and Euk570R (5′-GCTATTGAGCCTGGAATTAC-3′).

Sequence data were processed using the MRDNA analysis pipeline. In summary, sequences were joined, barcodes were deleted. Then, sequences <150 bp and sequences with ambiguous base calls were removed.
Sequences were de-noised. OTUs generated and chimeras were removed. Operational taxonomic units (OTUs) were defined by clustering at 3% divergence and 97% similarity. Final OTUs were taxonomically classified using BLASTn against a curated database derived from RDPII and NCBI (http://rdp.cme.msu.edu, http://ncbi.nlm.nih.gov).

Rarefaction curves and diversity indices (OTU richness, Chao-1 and ACE) were calculated using Mothur software (Schloss et al. 2009). Statistical analysis of sequencing data was carried out using the PAST program (Paleontological Statistics, version 1.47, http://folk.uio.no/ohammer/past) and the R v.2.15.0 statistical platform using the Vegan package. The table containing samples by OTUs was used to calculate pairwise similarities among samples based on Bray-Curtis dissimilarity index (Clarke 1993). A multivariate analysis of all samples was performed using multidimensional scaling (MDS) based on Bray-Curtis dissimilarities as between biofouling communities developed on paint, base, unprotected and present in seawater. Ordination of the Bray-Curtis dissimilarities was performed using non-metric MDS, with 100 random restarts, taking into account the presence/absence, as well as the relative abundance of OTUs in all samples. The MDS results were plotted in two dimensions. Analysis of similarities (ANOSIM) with Bonferroni corrected p-values was carried out to test for significant differences between the defined sample groupings. ANOSIM produces a sample statistic R, which represents the degree of separation between test groups (Clarke 1993). Similarity percentage (SIMPER) analysis was performed using the PRIMER® software to compare microbial communities from seawater, coated with paint and base, as well as unprotected parts of the glider. OTU partitioning was used to find out the number of OTUs that are specific for each dataset in the MDS analysis and the number of shared OTUs between different datasets. This was done on OTU datasets using Microsoft Excel and a custom R script.

Figure 2. Total microbial abundance of microbial cells in seawater samples, on unprotected ocean glider surface and coated with paints and base coatings. Data are the means ± standard deviation (SD). For the sample abbreviations, see Table 1.
Results

Environmental parameters

During the study, the ocean glider dived from the surface to a depth of 1,015 m. Vertical profiles of physical, chemical and biological characteristics recorded by the glider showed high variability during the study period (Supplemental material Figure S1). This variation was attributed to the movement of the ocean glider and of mesoscale eddies, as well as seasonal changes.

The average seawater temperature during the period of investigation varied from 27.8° to 12.2 °C (Figure S1A). As expected, the highest temperatures were recorded at the surface, while the lowest ones were measured at depth >560 m. The averaged vertical temperature profile implied that the surface mixed layer extended to ~25 m and followed by the seasonal thermocline (Figure S1A). In turn, the thermocline layer was underlined by the Arabian Gulf waters, with the core at 250 m. This water mass, located between 150 and 350 m, flowed eastward.

The vertical profile of salinity differed from that of temperature (Figure S1B). The highest salinity of 36.9 ppt was recorded at the depth of ~260 m, co-occurring with the Gulf outflow, while the lowest salinity was found at depth. The average salinity varied from 35.7 to 36.9 ppt during this study.

The photosynthetically active radiation (PAR) varied from 0 to 2,400 μE m⁻² s⁻¹ during this study. A characteristic feature in the vertical distribution of the fluorescence intensity was the fluorescence peak observed at ~30–40 m deep, persisting throughout the deployment period (Figure S1C). This peak was formed by the phytoplankton community dominated by the dinoflagellate Noctiluca scintillans (see Abundance of microbes).

The vertical distribution of the dissolved oxygen concentration showed a decline from saturated surface water (~220 μmol kg⁻¹) to near anoxic conditions in the oxygen minimum zone (< 2 μmol kg⁻¹; below 400 m). This pattern was interrupted by the outflow of Gulf water, injecting high salinity oxygenated water (~140 μmol kg⁻¹) between 150 and 350 m (Figure S1D).

Abundance of microbes

Taxonomic analysis of Niskin seawater samples collected within the layer of fluorescence peak (see Figure S1C) showed the presence of the dinoflagellate N. scintillans. This species made up about 90% of the total phytoplankton abundance (data not shown). As for the upper mixed layer, the processed samples showed that Noctiluca abundance was equal to ~120,000 cell l⁻¹ at the beginning of the experiment whilst at the end of the study, Noctiluca abundance became ~20,000 cell l⁻¹.

The highest densities of microorganisms were recorded at a depth of 35 m, which correlated well with the presence of N. scintillans (Figure 2). The densities of microorganisms at the other depths were 14-fold to 23-fold lower. The densities of microorganisms on the ocean glider were significantly (ANOVA p < 0.05) different (Figure 2). Among unprotected surfaces, the highest density of prokaryotes was observed on the ocean glider bottom (UGB) followed by the glider’s wing (UWT) and the glider’s top (UGT). The lowest density (HSD p < 0.05) was recorded on the glider nose (UN). Among paints, the lowest density (HSD p < 0.05) of microorganisms was found on PChT. The densities of microbes on the top and bottom surfaces were different (ANOVA p < 0.05; Figure 2). There were significant differences (HSD p < 0.05) in the densities of microbes on the different base coatings tested; three-fold higher densities were observed on BIn in comparison to that on BHe. The density on BIn was 1.5–2-fold lower (HSD p < 0.05) than on the unprotected glider (UG) surfaces. The densities of microbes on AF paint PlnB were 1.3-fold lower than on UGB (Figure 2). There was a significant difference between the densities of microorganisms in biofilms collected from the top and the bottom of the unprotected parts of the ocean gliders (HSD p < 0.05). Generally, the density of microbes was lower on the top of the glider than on the bottom (Figure 2). Similarly, the density of microbes on PlnT was nine-fold lower (HSD p < 0.05) than the density on the same paint located at the bottom of the glider (PlnB).

Microbial diversity

A total of 1,259,486 and 584,473 of 16S and 18S rDNA sequences, respectively, were obtained by Illumina MiSeq sequencing (Table 2, Figure S2). The lowest number of OTUs was observed on the ocean glider wing (UWT) and the highest number on base BHeT (Table 2). A similar pattern was observed for Chao-1 and ACE indices. For eukaryotic communities, the highest number of sequences was found on the bottom of the ocean glider top tail (UTT), while the lowest number on the bottom of the tail (UTB). The highest number of OTUs and the highest diversity Chao-1 and ACE indices were observed on the
AF paint PInB and the lowest at the bottom of the glider’s top tail (UTT) (Table 2).

The bacterial communities that developed on paints, base and uncoated surfaces had 1,158 OTUs in common (Figure 3). The eukaryotic communities shared 355 OTUs (Figure 3). The highest number of unique bacterial OTUs was observed for paints, while the lowest one was found in biofilms on base. For eukaryotic communities, biofilms which developed on unprotected parts of the glider had 138 unique OTUs, while the numbers of OTUs on base and AF paints were lower (Figure 3).

Multidimensional analysis (MDS) showed that bacterial communities formed on the ocean glider and present in seawater were different (Figure 4), which was supported by ANOSIM analysis (r = 0.94, p < 0.006). While bacterial communities formed on unprotected parts of the glider (U) shared some similarity with the base (BInT and BInB) and the experimental chitosan paint (PCh), they were different from the copper-based paints (PIn and PHe) (Figure 4). A similar tendency was observed for eukaryotic communities. MDS analysis showed that eukaryotic communities in the seawater and on the ocean glider were significantly different (ANOSIM r = 0.62, p > 0.05). Eukaryotic communities formed on unprotected and base surfaces shared some similarities, as well as communities from the base and paints.

SIMPER analysis demonstrated that bacteria belonging to genera *Dasania*, *Pantoea* and *Vibrio* contributed from 14% to 22% of dissimilarity between communities developed on the ocean glider and existing in seawater (Table 3). Additionally, bacterial communities formed on unprotected and base surfaces differed by the presence of the genus *Exiguobacterium*. Eukaryotes belonging to the Bacillariophyceae and Hydrozoa accounted

---

**Table 2.** Amplicon library size and diversity estimators for bacterial and eukaryotic communities of the samples using MiSeq.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>No. of sequences</th>
<th>No. of OTUs</th>
<th>Chao-1</th>
<th>ACE</th>
<th>No. of sequences</th>
<th>No. of OTUs</th>
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<td>286</td>
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<td>975</td>
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</tr>
</tbody>
</table>

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**Figure 3.** Venn diagram showing the number of shared and unique OTUs in bacterial and eukaryotic communities developed on paints, base coatings and the glider’s unprotected surface.
for >21% each between communities developed on the glider and existing in the seawater (Table 4). Additionally, Holozoa (Ichthyosporea) and different fungi (Agaricomycetes and Dothideomycetes) contributed for >10% each for dissimilarities between eukaryotic communities.

Bacteria belonging to the classes Gamma- and Alphaproteobacteria dominated biofilms on the ocean glider during the experiment (Figure 5A). These were mainly represented by the genera *Vibrio*, *Pseudomonas*, *Alteromonas*, *Marinobacter*, *Dasania*, *Teredinibacter* and *Cycloclasticus* (Gamma-proteobacteria), and *Pseudoruegeria*, *Parvibaculum*, *Sphingomonas*, *Hyphomonas*, *Erythrobacter* and *Tateyamaria* (Alpha-proteobacteria). The class Bacilli (mainly genus *Exiguobacterium*) was abundant on all unprotected parts of the ocean glider. Sequences belonging to chloroplasts of diatoms (Bacillariophyceae and Fragillariophyceae) were detected. There were clear differences between the compositions of bacterial communities on AF paints, base and unprotected parts of the ocean glider. Sequences belonging to chloroplasts of diatoms (Bacillariophyceae and Fragillariophyceae) were detected. There were clear differences between the compositions of bacterial communities on AF paints, base and unprotected parts of the ocean glider (Figure 5A). Copper-based AF paints had a high (51–66%) relative abundance of Gammaproteobacteria. Among AF paints, the chitosan paints (PChT and PChB) were characterized by the lower relative abundance of Gammaproteobacteria (14–33%) and the higher relative abundance of Betaproteobacteria (28–29%). Bacteria belonging to the genera *Dasania*, *Erythrobacter* and *Cycloclasticus* were the most common on paints containing copper. In contrast, *Ralstonia* sp. was dominant on the chitosan experimental paint (PCh) and base. Among the base coatings, the high relative abundance of Beta-proteobacteria was observed on BHeT and BHeB, while the high abundance of Bacilli was observed on BlnT and BlnB. The bacterial genera *Pantoea* and *Exiguobacterium* were dominant on base and unprotected parts of the ocean glider. Different unprotected parts of the ocean glider had distinct communities. For example, in biofilms on the wing (UWT) Gammaproteobacteria (85%) dominated, while a lower relative abundance of Alphaproteobacteria (7%) and Bacilli (7%) was recorded. On the other hand, there were no Bacilli in the biofilms on the glider’s nose (UN).

Eukaryotic communities on the ocean glider were highly diverse and represented by different groups of fungi, microalgae, nematodes, arthropods and hydrozoans (Figure 5B). Sequences of some macrofouling genera, such as *Megabalanus*, *Hydractinia*, *Actinostola* and *Dicoryne* were found. Additionally, sequences belonging to some planktonic species, such as *Nectopyrampus* sp. (Siphonoporae), were detected. Hydrozoa had a high relative abundance on the base coatings (BlnB 58%; BHeT 79%), the glider’s nose (UN 79%) and the AF paint (PInB 53%). High fungal diversity (represented by five different classes) in marine biofilms was detected (Figure 5B). The fungal class Agaricomycetes was highly abundant (relative abundance 72%) on paint PHeT, while the class Dothideomycetes (relative abundance 46%) and Eurotiomycetes (relative abundance 14%) were highly abundant on unprotected parts (UTB). The relative abundance of the fundal class Sordariomycetes on the antifouling paint PHeB was
Table 4. The contribution of particular eukaryotic taxa towards the total dissimilarity (Contrb., in %) between the bacterial communities using similarity percentage (SIMPER) analysis. Groups with contribution ≥2% are shown.

<table>
<thead>
<tr>
<th>Paint vs control</th>
<th>Paint vs base</th>
<th>Paint vs seawater</th>
<th>Control vs base</th>
<th>Control vs seawater</th>
<th>Base vs seawater</th>
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<tbody>
<tr>
<td>Taxon</td>
<td>Contrb. %</td>
<td>Taxon</td>
<td>Contrb. %</td>
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<td>3.754</td>
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Table 3. The contribution of particular bacterial genera towards the total dissimilarity (Contrb., in %) between the bacterial communities using similarity percentage (SIMPER) analysis. Groups with contribution ≥2% are shown.

<table>
<thead>
<tr>
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</tr>
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<tbody>
<tr>
<td>Taxon</td>
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<td>Taxon</td>
<td>Contrb. %</td>
<td>Taxon</td>
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<td>3.08</td>
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<td>4.10</td>
</tr>
</tbody>
</table>

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7%. The highest relative abundance (89%) of the class Ichtyosporea (Mesomycetozoa, Holozoa) on PlnT was recorded (Figure 5B). Diatoms (Bacillariophyceae), mainly Amphora and Cylindrotheca species, dominated biofilms on the ocean glider’s unprotected surfaces (UWT 82% and UTT 94%), the chitosan paint (PChB 71%; PChT 59%) and base (BlnT 73%). More than half of the sequences (54%) obtained from the unprotected bottom of the ocean glider (UGB) belonged to the Chlorophyta (Pycnococcus sp.).

**Discussion**

This is the first study to investigate microbial fouling on an ocean glider by next-generation sequencing. Previous studies reported the presence of macrofouling organisms on ocean gliders (Nicholson et al. 2008; Moline & Wendt 2011), but neglected microbial biofilms, which may also affect the performance of the glider and its sensors (Davis et al. 2003; Cetinić et al. 2009).

During the study period, vertical profiles of temperature, oxygen, chlorophyll and salinity showed high variability. The study area is highly influenced by three hydrodynamic processes: (1) the outflow from the Arabian Gulf, (2) the inflow from the northern Arabian Sea, and (3) the mesoscale (cyclonic and anti-cyclonic) eddies persisting in the region (across the Gulf) and connecting the northern-banked inflow and the southern-banked outflow (Al-Hashmi et al. 2010; Vic et al. 2015). Thus, it is assumed that variations in the physical and biological parameters in the study region could be attributed to the spatial shifts in the location of the mesoscale eddy affecting regional circulation over the shelf in the Muscat region. Additionally, seasonal changes (ie increase of temperature from March to June) have affected vertical profiles of the physical and biological parameters (Piontkovski et al. 2017).

Biofilms on the surface of the ocean glider were exposed to continuous fluctuations of oxygen (0–287 μmol kg⁻¹), temperature (12.2–32.3 °C), salinity (35.4–38.0 ppt), depth (0–1,015 m), pressure (0.04–102 Bar) and light intensity (0–2,400 μE m⁻² s⁻¹) for > three months. It was expected that microbes on the glider would differ from those in the water column. Indeed, as shown by the MDS plots, the composition of bacterial and eukaryotic organisms on the ocean glider and in the water column was different. Previously it was reported that attached and particle-bound bacteria are more abundant and more metabolically active than unattached bacteria (Kirchman & Mitchell 1982; Dang & Lovell 2016). The densities of microorganisms on
the glider’s surface varied from 8,820 to 228,000 cell mm$^{-2}$. Among unprotected surfaces the lowest densities of microbes were found on the nose of the ocean glider (UN). This is probably due to the higher dynamic pressure and velocities on that part of the glider (Isa et al. 2014; Chen et al. 2013). Additionally, the densities of microorganisms on the top of the ocean glider were generally lower than on the bottom. The orientation of different parts of the ocean glider could affect the densities of microbes (Bellou et al. 2012).

Areas of the ocean glider coated with AF paints had lower densities of microorganisms than unprotected parts of the glider. This is not surprising, as AF paints contain chemical compounds that kill or prevent growth of microfouling organisms (Cassé & Swain 2006; Molino et al. 2009; Briand et al. 2012; Briand et al. 2017). The lowest densities of microorganisms were observed on the AF paints PHeT and PHeB that contained the biocides cuprous oxide and zineb. In previous studies, both biocides were recognized as effective AF agents (Hunter & Evans 1991). Among 11 commercial AF paints tested during a one-year study in Oman coastal waters, the lowest microbial biomass was recoded on the paint containing these biocides (Muthukrishnan et al. 2014).

Compared to other studies of marine biofilms on artificial surfaces utilizing the Illumina MiSeq technique, the diversity of communities formed on the ocean glider was similar to that found in Australian (Tan et al. 2015) and Swedish (Oberbeckmann et al. 2016) coastal waters. Bacteria belonging to the classes Gamma- and Alphaproteobacteria, mostly Vibrio, Pseudomonas, Teredinibacter, Cycloclasticus, Pseudorangea, Parvibaculum, Sphingomonas, Erythrobacter and Tateyamaria, dominated the biofilms. Similarly, previous investigations demonstrated the dominance of Alpha- and Gammaproteobacteria in marine biofilms on various artificial substrata (Dobretsov et al. 2013; Tan et al. 2015; Sathe et al. 2016; Flach et al. 2017; Hunsucker et al. 2018). Differences between bacterial communities developed on the ocean glider were due to the genera Dasania, Pantoea, Exiguobacterium and Vibrio as indicated by SIMPER analysis. Dasania spp. are obligately aerobic bacteria (Lee et al. 2007), which were previously found associated with the deep sea tubeworm (Forget & Juniper 2013). Exiguobacterium profundum was previously isolated from a deep sea hydrothermal vent (Crapart et al. 2007). This might suggest possible adaptations of these bacteria to high pressure and low oxygen conditions. Archaea dominate deep sea waters (DeLong 1992; Jensen et al. 2012) but this group was not detected in the present study. While the universal 16S RNA 515F/806R primers used in this research have been used to study both archaea and bacteria (Bates et al. 2011; Walters et al. 2011), it is possible that the absence of archaea was due to poor amplification of this group of microorganisms (Eloe-Fadrosh et al. 2016).

Eukaryotic communities on the ocean glider were predominantly represented by fungi, hydrozoans and arthropods. While only biofilms were observed on the glider, sequences of macrofouling organisms, such as the barnacle Megabalanus sp. and the hydrozoan Hydractinia sp., might indicate recruitment of these species on the ocean glider. Barnacles are reported as the main fouling species on ocean gliders (Lobe et al. 2010). Additionally, sequences of photosynthetic species belonging to the classes Bacillariophyta, Dinophycae, Chlorophyta and Mediophyceae were recorded. This might indicate that some photosynthetic species on the ocean glider, such as Amphora sp. and Cylindrotheca sp., can survive for some time without light. It has been shown that the benthic diatoms Amphora coffeaeformis and Cylindrotheca closterium can survive in the dark, under anoxic conditions for 6–28 weeks (Kamp et al. 2011). These researchers found that these diatoms accumulated nitrate and used it for respiration in the absence of oxygen and light.

The composition of microbial communities developed on paints, base coatings and unprotected parts of the glider was different. This could be explained by different chemical (chemical composition) and physical (wettability) properties of the unprotected and coated surfaces. For example, biologically and physically inert substrata, such as glass, fouled more rapidly and had more diverse communities than active substrata, similar to copper-nickel alloys (Marszalek et al. 1979). The 454 pyrosequencing of 16S genes revealed the presence of different microbial communities on different AF paints (Muthukrishnan et al. 2014; Briand et al. 2017). The copper AF paint resulted in significant changes in both bacterial and eukaryotic communities in New Zealand waters (von Ammon et al. 2018). Similar results were obtained in experiments with plastic panels painted or not painted with AF paints in Swedish waters (Flach et al. 2017). Bacteria belonging to Cryomorphaceae and Alcanivoraceae were exclusively present on polyethylene terephthalate, but not on glass surfaces in another study in the North Sea (Oberbeckmann et al. 2016). In the present study, bacteria belonging to the genera Dasania, Erythrobacter and Cycloclasticus were common on AF paints containing cuprous oxide. While the genus Dasania was previously detected on AF paints, the genus Erythrobacter was observed in biofilms on
AF paints containing cuprous oxide (Muthukrishnan et al. 2014). *Cycloclasticus* was one of the two most abundant genera in biofilms on AF paints exposed to fouling in Swedish coastal waters (Flach et al. 2017). This could suggest that bacteria belonging to *Dasania*, *Erythrobacter* and *Cycloclasticus* are commonly associated with AF paints.

Bacterial and eukaryotic communities on the chitosan paint were different from those on other AF paints. Differences in AF mechanisms can explain differences between the community composition of chitosan and copper-based paints. Copper-based paints kill microorganisms due to the displacement of essential metals in proteins (Thurman et al. 1989). Copper ions may alter enzyme and nucleic acids structure and function, facilitate their hydrolysis and have an adverse effect on oxidative phosphorylation and osmotic balance (Borkow & Gabbay 2005). On the other hand, chitosan inhibits biofouling due to its cationic nature and interactions with positively charged microbial cell membranes (Alishahi & Aider 2012).

The current study was conducted using only one ocean glider. While replicated samples were collected, these cannot be treated as true replicates. It is difficult in practice to replicate naval structures. Several similar studies of biofilms on ships’ hulls also did not have true replicates (Hunsucker et al. 2014; Inbakandan et al. 2010; Zargiel et al. 2011). Gliders are expensive autonomous vehicles and cannot be easily replicated, thus, conclusions of the present study need to be treated with caution.

In conclusion, for the first time the presence of diverse microbial biofilms formed on the surface of an ocean glider exposed to oscillating environmental conditions was demonstrated using next-generation sequencing techniques. The densities and compositions of the microbial communities on different parts of the glider were different, which could be explained by differences in hydrodynamic conditions on different parts of the glider. Additionally, the chemical composition of surfaces coated with base and AF paints shaped the composition of microbial communities on the surface of the ocean glider. This is the first attempt to investigate microfouling on ocean gliders and much work is required in the future to confirm the findings. The differential AF performance of paints suggested that proper AF solutions for long endurance of autonomous underwater vehicles need to be developed.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**ORCID**

Sergey Dobretsov

http://orcid.org/0000-0002-1769-6388

Bastien Y. Queste

http://orcid.org/0000-0002-3786-2275

**References**


