



Marine Microbial Biodiversity, Bioinformatics & Biotechnology



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Summary

With 191 participating marine stations the Ocean Sampling Day performed on June 21st 2014 became a landmark for the Micro B3 project. Originally planned as a single event in 2014, it has proven to be a unique opportunity for orchestrated and integrated scientific work across the Atlantic and beyond. The addition of the Citizen Science project “MyOSD”, which was not part of the initial planning (DoW) of OSD, is a big step forward with respect to raising awareness for marine science and especially the importance of marine microbes for ocean health by “hands-on” experiences.

In agreement with the Micro B3 consortium and the Scientific Advisory Board Micro B3 has used the momentum and repeated OSD and the citizen science project MyOSD on June 21st 2015. The marine stations and citizen scientists were in favour of this repetition, especially since the resulting standardized data will be compared and first trends will become visible.

JacobsUni has taken the lead, as a new task within WP 1, and was in charge of organising OSD and MyOSD in 2015.

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Ocean Sampling Day 2015

Organising OSD 2015 followed the proven concept of OSD 2014 as described in D2.3, D5.3 and D2.6 and took into account the feedback of the OSD 2014 participants (D9.8). A paper describing the assembly and operation of OSD 2014 is available at Kopf *et al.*, 2015 “The ocean sampling day consortium”. GigaScience 4:27

(<http://www.gigasciencejournal.com/content/4/1/27>)

Summary statistics for OSD 2015

Stations: 131

Samples: 137 (131 surface and 6 from several depth, provided by OSD2 Roscoff, OSD15 Villefranche, OSD51 Panama, OSD100 GOS and OSD 152)

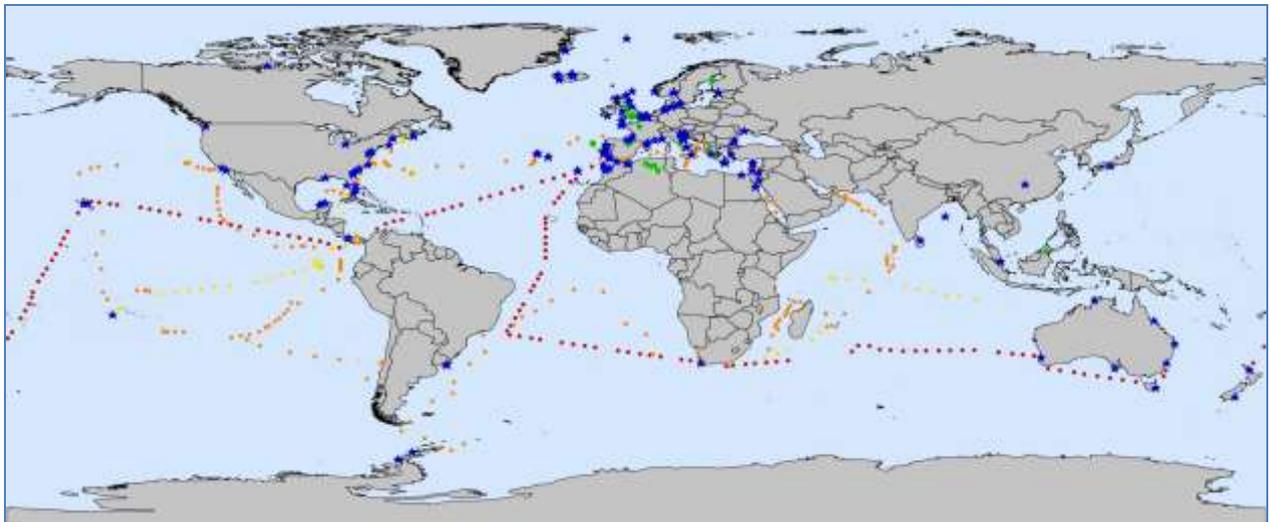


Figure 1 World map illustrating the current status of global marine sequencing projects. OSD: blue stars, RSD: green dots, Tara Oceans: orange dots, Malaspina cruise: red dots, Global Ocean Sampling (GOS): yellow dots.

DNA of all samples has been extracted at the Biologische Anstalt Helgoland as part of the Alfred Wegener Institute for Polar and Marine research and shipped to the sequencing company LGC Genomics in September 2015. Sequencing is currently ongoing.

Change of primers for OSD and MyOSD 2015 amplicon sequencing

Recent advancements in science clearly document that the primers used for OSD 2014 were suboptimal for an important group of bacteria (SAR 11) in the marine environment. This has been documented by Jed Fuhrman in two recent papers (Fuhrman *et al.*, 2015 and Parada *et al.*, 2015). To keep pace with the new knowledge, it was necessary to run extensive primer tests, re-sequence parts of the OSD 2014 samples and go for deeper sequencing of OSD and MyOSD 2015 samples. The results and strategy have been extensively discussed with the Scientific Advisory Board of Micro B3. Furthermore, it has been presented to the Micro B3

partners in the General Assembly of the Final Micro B3 conference in Brussels on 05/11/2015 (D9.10). The SAB and GA have acknowledged the procedure.

The citizen science project MyOSD 2015

Citizen science (CS) involves the participation of the lay community in scientific research activities and often centers on the collection of data. Over the last few decades, CS has grown in popularity and it is now regularly featured in conservation science. Besides data collection, CS is also a means to enhance the scientific literacy of the lay community and influence their attitude towards environmental issues.

Already in 2014, OSD was not only popular among scientists, but also the general public was interested and contributed to OSD via the CS project called MyOSD. Although, various CS projects exist there are only two widely known projects, the American Gut and uBiome, where volunteers can sample for microbes. However, both of these projects focus solely on the human microbiome, and to our knowledge there is no CS project working with microbial diversity of marine systems.

In 2014, MyOSD participants were asked to measure environmental parameters such as salinity or water temperature, which have crucial impact on microbial community structures. In 2015, OSD and MyOSD went into the second round, and MyOSD took a new turn. Given that some MyOSD participants from the previous event in 2014 had requested to also be able to sample for marine microorganisms, we started to develop a new strategy to include their wish in the MyOSD project.

This strategy had to address two main challenges, which previously prevented microbial sampling by citizen scientists in 2014.

The first challenge concerned the sample storage and transportation. Following the OSD 2014 protocol, the Sterivex filters—which are used to collect the microbes out of the water— were supposed to be frozen directly after the filtration either at -80°C or in liquid Nitrogen and stored at -80°C for long term preservation. Afterwards they had to be shipped on dry ice to Bremen, Germany, in order to keep them from defrosting. These preservation steps are crucial as bacteria species react differently to changing environmental conditions and interruption of the cool chain might result in a shift in the original community composition. Since liquid Nitrogen or -80°C freezers are entities not used in common life, it is very unlikely that non-professional scientists will have access to them. Therefore, fulfilling these requirements is almost impossible for citizen scientists. Additionally, liquid Nitrogen is a dangerous chemical, which should be handled with care and by experienced people. As an alternative to the aforementioned freezing steps, we decided to use the chemical RNAlater. RNAlater is a quaternary ammonium salt-based solution, which is suitable to preserve RNA or DNA over a longer period of time at room temperature.

With RNAlater a sample can be stored at room temperature for up to one week, in a standard $+4^{\circ}\text{C}$ fridge for one month, and at -20°C or -80°C indefinitely. These properties make RNAlater a feasible alternative preservation method for both, MyOSD and OSD

participants. It not only allows a straightforward sample storage but also relatively cheap and uncomplicated sample shipment.

The second challenge was the necessity of holding legal permits for international biomaterial transfer. The Micro B3 Consortium developed OSD in compliance with the Nagoya Protocol on Access and Benefit Sharing as well as with the Convention on Biological Diversity. It is therefore concerned about the ownership of and the permits required to send biomaterial samples across borders. All of the OSD samples had to be shipped to Bremen, Germany for centralized DNA extraction and sequencing routine to secure comparability and reduce bias to a minimum. Therefore, OSD participants—depending on the country of the sampling location—need to hold permits for the legal transfer of biomaterial to Germany. Since it is very unlikely that non-professional citizen scientists are able and willing to obtain such permits, the idea of MyOSD hubs was born to overcome this issue.

A call for interest in becoming a hub was sent to all the OSD participants in January 2015, 31 of them replied positively (Figure 2). MyOSD hubs are research institutes, preferentially already involved in OSD, which hold the legal permits to ship biomaterial out of the country of origin. The MyOSD coordination team equips these hubs with MyOSD sampling kits which were then distributed to citizen scientists in the country of the respective hub. After completion of the sampling procedure, the citizen scientists returned the Sterivex filters and a water sample for salinity determination back to the MyOSD hub, which then shipped all samples to the MyOSD coordination team in Bremen, Germany. Besides the advantage to solve the legal aspect, MyOSD hubs provided a great opportunity for institutes to get in contact with the broad public and support educational outreach. In addition, they gave MyOSD participants the possibility to get help and information from a contact person in their native language.

Having these two main issues solved, a special MyOSD sampling kit was developed (Figure 3) and a video tutorial was published online on MicroB3 website and on YouTube (<https://www.youtube.com/watch?v=Mtmp4Ltr4xo>). The MyOSD coordination team developed also a MyOSD handbook, translated in several languages. The kit keeps the sample procedure straightforward and simple, while still producing adequate scientific samples. Hence, the MyOSD citizen science project expanded the OSD 16S rRNA data set of 2015 even further resulting in an even higher resolution of the marine microbial community snapshot OSD is aiming for. However, it also gives citizen scientists the possibility to investigate the prokaryotic life in the waters of their interest and the chance to learn more about the importance of marine microbial life with hands-on experience.

Moreover, the MyOSD coordination team developed a dissemination strategy for the 2015 event: the MyOSD webpages were redesigned and improved (<https://microb3.eu/myosd>) and the initiative was presented and reported to the general public at several events:

- Open Talk at Stazione Zoologica Anton Dohrn (Naples, Italy, 2014)
- European Marine Science Educators Association Conference - EMSEA 14 (Göteborg, Sweden, 2014)

- Maritime Woche (Bremen, Germany, 2015)
- College of Exploration - NOAA (online <https://vimeo.com/127170429> May 2015)
- Science Slam (Bremen, Göttingen, Flensburg and Osnabruck, Germany, 2015)
- Beneath the Waves Film Festival (Hamburg, Germany, 2015)
- Lange Nacht der Wissenschaft (Berlin, Germany, 2015)



Figure 2 The 31 MyOSD hubs displayed on a world map. Visualization was done with Google maps and the tool MapAList.

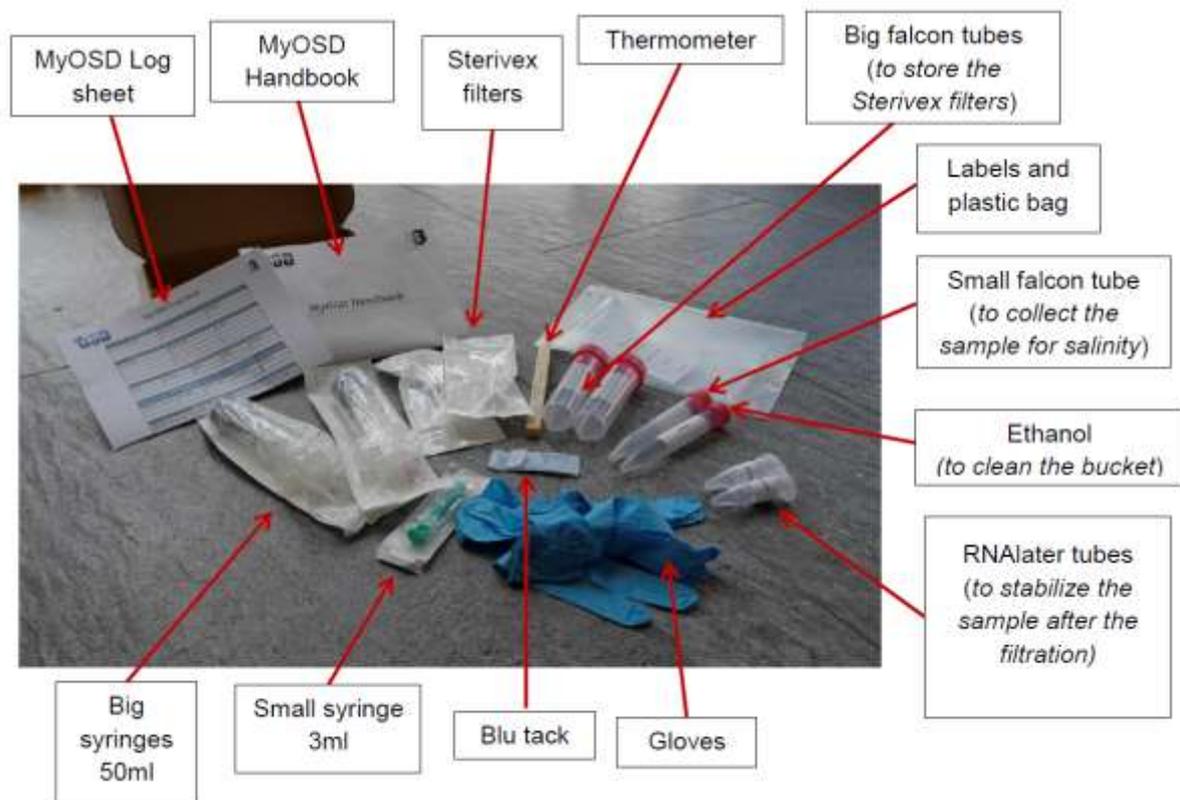


Figure 3: The MyOSD 2015 sampling kit.

In total 250 sampling kits have been distributed via the OSD hubs. 192 kits have been returned and 319 DNA extractions have been performed. DNA has been shipped to LGC Genomics for sequencing the 16S ribosomal RNA in September 2015. The MyOSD sequence and contextual data, just like the OSD data, will be made publicly available to everyone via the International Nucleotide Sequence Database Collaboration (INSDC) umbrella study PRJEB512926 and at PANGAEA.

The 16S ribosomal RNA sequences will be taxonomically classified by the SILVAngs pipeline. However, we assume that the vast majority of MyOSD participants are neither experts in microbiology nor in sequence data analysis. Within the MyOSD Germany 2016 project, funded by the Federal Ministry of Education and Research, we will develop a strategy to present these data in an understandable and educational way.

References

Fuhrman JA, Cram JA, Needham DM (2015) Marine microbial community dynamics and their ecological interpretation. *Nat Rev Micro* 13:133-146

Parada AE, Needham DM, Fuhrman JA (2015) Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ. Microbiol.*