2.9. *Microbial Observatories as a tool to detect and describe changes in marine (microbial) diversity and ecosystem functioning: lessons learnt from the Blanes Bay Microbial Observatory*

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Unlike larger marine organisms, microbes cannot be easily identified based on morphology alone. This is particularly true for the smallest ones, archaea and bacteria, but also holds true for most protistan eukaryotes where crypticity is a widespread characteristic. However, prokaryotes comprise most of the Earth's living biomass, are the most abundant living particles in the sea, are the only relevant dissolved organic matter (DOM) transforms in the sea and comprise the largest living surface in the ocean. Furthermore, they are the largest "unknown" pool of genomic and metabolic (i.e. functional) diversity. As an example, a recent report (Yooseph *et al*. 2007, PloS Biol. 5: e16 doi:10.1371/journal.pbio.0050016) analysing metagenomic data from the ocean's surface has identified >1000 new bacterial protein families, indicating that there may easily be many unknown functions yet to be discovered in the ocean and that can be assigned to microbes.

Microbes are also extremely diverse. Surveys of bacterial richness can show hundreds of different organisms per sample depending on the methodology used. But this is based on methodologies which might show only the "tip of the iceberg" of bacterial diversity. Newer, more recent methodologies, adopting a massively parallel tag sequencing strategy, have shown that bacterial communities of deep water masses of the North Atlantic and diffuse flow hydrothermal vents are one to two orders of magnitude more diverse than previously reported for any microbial environment. A relatively small number of different populations dominate all samples, but thousands of low-abundance populations account for most of the observed phylogenetic diversity. This ''rare biosphere'' is very ancient and may represent a nearly inexhaustible source of genomic innovation. The areas of biodiversity that involve small microbes that drive the bulk of ecosystem processes can be analyzed by these newest molecular tools and this makes possible the integration of microbial diversity into studies of ecosystem processes.

In terms of quantifying diversity, however, the problem has to be solved of what to do with the reported microbial microdiversity that is present in the samples when analysed using molecular methods: these are extremely similar sequences, that might represent neutral diversity but, simultaneously, sources of variability for subsequent adaptation. The fact that day after day there are new molecular approaches being developed, indicates the relevance of creating i) well organized repositories of microbial genetic material, accessible to scientists with ideas on how to analyze the material, and ii) ecologically-referenced, with as much ancillary ecological and biogeochemical data collected as possible. While the first metagenomic study of the ocean's microbes was done without even referring to the temperature and chlorophyll data of the sample (something that decreased the relevance of the findings), current programmes should focus equally on the ancillary biogeochemical and food web data.

Studies of this type in well-standardized sites are the basis of what has come to be called "Microbial Observatories" (MO). The long-term goal of the MOs is to study and understand microbial diversity over time and across environmental gradients. The guiding themes are i) discovery of large numbers of, as yet, undescribed microorganisms and microbial consortia from diverse habitats, and ii) characterization of novel biochemical, metabolic, physiological, genomic, and other properties and processes of newly described or poorly understood microbes and microbial assemblages and communities. The American National Science Foundation (NSF) has launched a series of calls for MOs across the USA, encouraging proposals that would "increase knowledge of the biochemical, genetic, physiological and ecological properties and processes that enable diverse microbes to occupy and interact in natural and disturbed habitats"

(Kane 2004, Microb. Ecol. 48: 447-448). Some projects aim to study and characterize entire communities, including viral, bacterial, archaeal, and eukaryotic microbial members. Others consist of targeted studies of assemblages or physiologically and/or phylogenetically defined microbial groups that are of great interest due to genomic, metabolic, ecological, and/or evolutionary aspects of their biology.

Since 1998 we have been running a Microbial Observatory in the Northwestern Mediterranean (the Blanes Bay Microbial Observatory, http://www.icm.csic.es/bio/projects/icmicrobis/bbmo), a coastal site that reflects well the typical Mediterranean seasonality. The suite of techniques currently in use at the Blanes Bay MO include a special focus on single-cell analysis techniques that can be used to relate the function of a given organism with its phylogenetic characteristics, thus linking biodiversity and ecosystem functioning. This is a long-term strategy that seems particularly adequate to determine the effects of global change in microbially-driven ecosystem function. As an example, we have established how anthropogenic and climate forcing can alter the response of the microbial communities to the addition of nutrients, and how bacterial carbon processing is altered by small seawater temperature rises. We believe that the combination of experimental work in tandem with long-term observational studies is the best strategy to predict the effects of global change on the ocean biogeochemistry.

Finally, the establishment of MOs allows going back and forward in time, because we are living in times in which molecular methodologies are developing rapidly. Analyses currently not possible may become so with the development of new or improved (and more affordable) methodologies in the future. Then we can go back to the stored DNA and do the analysis. Just imagine how good it could have been to have DNA collected from the world's oceans from Darwin's times: we would be able to test whether the oligotrophic ocean back then was dominated by SAR11 and Prochlorococcus as is now, and whether these organisms have had genetic changes with the time passed. Our stored DNA is equivalent to the herbaria that can be searched again by interested scientists.

Microbial observatories require team effort. No a single scientist can pretend to run a MO studying microbial diversity and ecological functioning. We are fortunate to work in an environment that allows collective work and promotes interaction. This is also beneficial because it allows the research to be funded for longer times than the typical three-year projects.

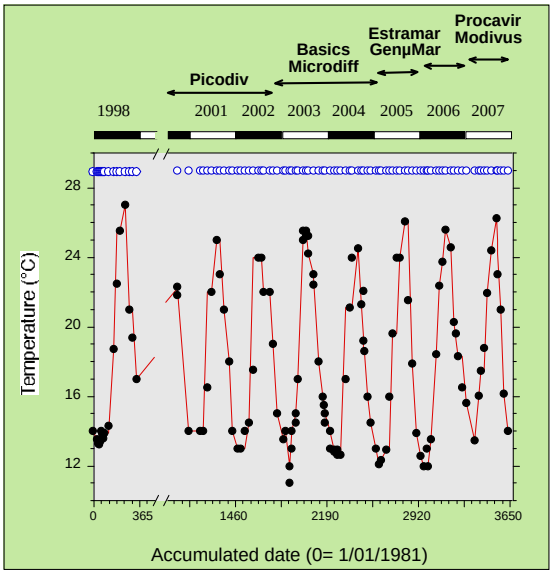

Figure 2.9.1 Temperature profile recorded at the Blanes Bay Microbial Observatory sampled from 1998 to 2007. The upper names are the EU or Spanish projects supporting sampling. The blue dots are those samples in which DNA was collected and is maintained in the DNA repository.

variability and thresholds and assess the requirements to achieve sustainable management of the resources of European Seas. Stakeholders, end-users and policy-makers should be made fully aware of the services that marine ecosystems provide as well as of the inherent ecosystem variability that should be fully acknowledged and reflected in the policy design process. The scientific and technological approaches adopted in SESAME are oriented towards that approach focusing on the study, assessment and prediction of the environmental changes in the Mediterranean and Black Sea ecosystems, in response to natural changes and anthropogenic forcing (regime shifts). The project is also investigating how these changes affect the ability of these seas to provide goods and services with fundamental societal importance, such as tourism, fisheries, mitigation of climate through carbon sequestration and ecosystem stability through conservation of biodiversity.

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- There is an urgent need to realise that long-term monitoring cannot be funded with 3-yr or 5-yr projects. Funding to support monitoring programmes need not be prohibitive but must be provided for long periods.
- Analysis of microbial diversity requires expensive technologies, akin to those used in biomedical research. Projects must be funded sufficiently so that sampling is done on a regular basis, and stocks are maintained.
- There is a need for an efficient way of organising nucleic acid stocks, and for accessing them.
- Ancillary biogechemical data should be collected as part of studies to assess and monitor microbial biodiversity. The more complete the information, the more we will be able to understand microbial biodiversity and how this affects ecosystem function.
- We need at least one microbial observatory from each of the European coastal and deep oceans and we also need deep-sea microbial observatories. There should be some form of agreement to work in cooperation to collect, treat and maintain the DNA using standard methodologies.

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