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Crystal ball – 2009

In this feature, leading researchers in the field of environmental microbiology speculate on the technical and conceptual developments that will drive innovative research and open new vistas over the next few years.

How to use a crystal ball in environmental microbiology: developing new ways to explore complex datasets

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A crystal ball is an instrument which – when used properly – helps to gain information on the past, present or future by other means than common human senses and the standard technologies supporting them, with the purpose to use this information to generate knowledge and to aid decision making. Hence, it could be a very useful tool in environmental microbiology, which deals with an enormous number and diversity of unknown populations, processes and habitats, many of which escape human scales, and almost all of which are not traceable in the past – if we would only know how to use the crystal ball. Esoteric handbooks say that: (i) one must have a specific question to ask, (ii) one must be able to completely concentrate on this question and be very patient until the fog goes away and some information-containing images develop, then (iii) one must accept what one sees and not be blocked by preconceived opinions, finally (iv) the crystal ball is a sensitive tool that must be well maintained, cultivated and surrounded by positive energies. So, doesn't this sound like the good advices we get from our statistics experts and data base managers in how to deal with large and complex data sets?

Let us face it, the big challenges in environmental microbiology – such as estimating total microbial diversity and its temporal and spatial patterns in a given habitat, studying evolution and succession of populations, recording and predicting the effects of global change, deciphering biological interactions, understanding the links between genomes and metabolomes in organisms – can only be solved by supernatural clairvoyance, or by a painstaking effort to improve observation, data recording, data accessibility and availability. In other words, the urgent questions in environmental microbiology are known, the methods and technologies are largely available, but the culture and art of retrieving and dealing with large multidisciplinary data sets have remained underdeveloped in our field, and are still largely missing from the education of new generations of scientists.

The coming years will be associated with dramatic changes in the way we hitherto have approached microbial diversity and functions in natural environments. The drivers of these profound changes are already noticeable. First, major environmental issues (think global warming, ocean acidification, species extinction, altered land use) create a number of real-life, large-scale natural experiments with uncertain outcome, and increase the pressure to provide more predictive knowledge. Second, neighbouring disciplines such as oceanography and the geosciences have already prepared for new scales of global earth observation to which environmental microbiology could be hooked in many advantageous ways. Third, powerful sequencing tools are now offering a more detailed snapshot of the extensive diversity in natural environments. Fourth, the current revolution in single-cell imaging is just starting to open new dimensions in our understanding of what microbial life is about. Not only can we now look at who is there, but also at what and when they do it on an individual basis. Microbial ecology is showing the signs of a profound mutation of concepts, approaches and methods because microbial diversity and functions can now be questioned routinely over a huge range of spatial, temporal scales and environmental gradients, just as macrobial studies.

From those simultaneously occurring scientific revolutions that describe the infinitely small and immensely big patterns of microbial life, exciting progress will logically come from the merging of those fields, so that butterfly effects at the single-cell level can be detected in their complex and ecosystem-sustaining context. Although successful results have been obtained by the description of specific cells to ecosystems, it will be also needed to place the pieces of the big puzzle back together: the understanding of the ever-increasing -omics databases will not be fully comprehended if we impose our compartmentalized vision of the world to the objects of our research: Terrestrial and marine realms, sediment, water column and atmosphere are all interconnected. Moreover, macro- and microorganisms are also connected in ways we have not understood. More studies are needed that investigate biological interactions and compare diversity patterns across taxa, not only to determine whether the same scaling rules apply, but also to identify common structuring factors, and the taxonomic levels at which

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Culture-based studies provide control, specificity and relative ease (in comparison with natural systems) in characterizing the physiology and activity of the cultured organisms. But cultures presented with environmentally realistic energy fluxes may grow so slowly that the time required to generate sufficient biomass for study with traditional methods could be unappealingly long. Nonetheless, the development of new culture methodologies that control and limit energy fluxes, and maintain rather than grow biomass, will be crucial to advancement in this arena. The development of analytical and imaging techniques that can perform characterizations on smaller scales (with much less biomass, or even on single cells) will aid significantly in this regard.

Access to highly energy-limited natural populations will expand substantially in the years ahead. Methodological and technological advances that are tailored to such environments – improved detection limits and accuracy for enumerating low cell numbers and for quantifying concentrations, fluxes and turnover of substrates at extremely low levels; techniques for assessing metabolic status *in situ*; and methods for linking genetic (metabolic) identity with biogeochemical function at low activity levels – will dramatically enhance our understanding of life at the energetic fringe.

Thus, energy will emerge, in the years ahead, as a path toward understanding the 'silent majority' of microbes that occupy the current frontiers of environmental microbiology.

References

Manske, A.K., Glaeser, J., Kuypers, M.M.M., and Overmann, J. (2005) Physiology and phylogeny of green sulfur bacteria forming a monospecific phototrophic assemblage at a depth of 100 meters in the Black Sea. *Appl Environ Microbiol* **71:** 8049–8060.

Morita, R.Y. (2000) Is H_2 the universal energy source for long-term survival? *Microb Ecol* **38:** 307–320.

Price, B.F., and Sowers, T. (2004) Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. *Proc Natl Acad Sci USA* **101:** 4631–4636.

Valentine, D.L. (2007) Adaptations to energy stress dictate the ecology and evolution of the *Archaea*. *Nat Rev Microbiol* **5:** 316–323.

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Microbial observatories in the sea

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All marine habitats support diverse and dynamic assemblages of microorganisms, including bacteria, archaea, eukarya and their viral and metazoan predators. These complex communities interact through a variety of genetic, metabolic and ecological processes to sustain life in the sea. Recent discoveries of novel microbes, new metabolic pathways and their intimate interrelations have challenged pre-existing paradigms and have led to a renewed commitment to a comprehensive study of the biology and ecology of marine microorganisms from genomes to biomes (e.g. http://cmore.soest.hawaii.edu).

Our general understanding of the structure, function and regulation of microbial communities in the sea has changed radically during the past three decades but is still fragmentary. Currently, we are woefully ignorant of the time–space domains of microbial processes in the sea, in large part because of the extreme magnitudes of scale (seconds to millennia for the temporal domain and micrometre to ocean basin for the spatial domain). Because most marine ecosystems are remote and out of direct human sight, they are sparsely observed and grossly under-sampled; indeed, the scales that matter for microorganisms may be far removed from the scales that our own senses can perceive. Furthermore, anthropogenic forcing of the Earth since the dawn of the industrial revolution has created a 'no analogue' new world and we may be altering the ocean habitat in ways that we do not understand. The changing ocean may lead to different, perhaps novel, marine habitats that can select for new microbial assemblages. The lack of comprehensive temporal observation and facilities for long-term field experimentation currently limit and will ultimately restrict our rate of progress in the field of marine microbial ecology, in my opinion. There is no substitute for at-sea observation and experimentation, and no short cuts either. This situation needs to change, and it will improve in the near future according to my crystal ball!

Much of our extant knowledge concerning the role of microorganisms in the sea is based on either laboratory study of selected isolates or the results obtained during oceanographic research expeditions. The latter are, at best, single freeze frames of what are probably full-length motion pictures. Consequently, this expeditionary mode of data collection provides an incomplete account of the dynamic and stochastic nature of microbial life in the sea. Marine microbial community structure and the ecosystem services that they support are complex, time-varying, non-steady-state features of a changing planet, and must be studied as such. This demands a financial commitment for the establishment of microbial observatories for long-term observation of oceanic phenomena, an intellectual investment in the establishment of transdisciplinary research teams and the development of novel instrumentation for continuous, remote observations of marine microbes. Above all, there must be sustained 'access to the sea', not in the international political sense of Hugo Grotius' classic treatise *Mare Liberum* (1609), but in a modern ecological research sense.

Long-term ecological studies are common in terrestrial and limnological research. They are ideally suited for investigation of subtle habitat changes, irregularly spaced stochastic events and complex interdependent phenomena. In 1988, two open ocean observatories were established to track the temporal changes in a variety of microbial processes in response to physical and chemical dynamics of the selected habitats. The two study sites, one in the North Atlantic Ocean near Bermuda (Bermuda Atlantic Time-series Study, BATS) and the other in the North Pacific Ocean near Hawaii (Hawaii Ocean Time-series, HOT), were initially focused on the ocean's carbon cycle from solar energy-based carbon dioxide reduction to particulate matter export and deep-sea carbon sequestration. At the time of their creation, there were no marine analogues of the atmospheric pCO_2 record (the so-called Keeling Curve) and no microbial-based ecosystem models for accurate predictions of carbon fluxes in the sea. To put our ignorance into proper context, *Prochlorococcus* – a tiny marine photoautotroph now recognized as the most abundant plant on Earth – had just been discovered, and the 'marine microbial genomics revolution' had not yet begun (the first full genome sequence of a marine microorganism was not available until 1996).

The design of the sampling and core measurement programme for HOT and BATS was based on the best information at that time; an approximately monthly frequency, ship-based programme lasting for at least one decade was believed to be a minimum investment for establishing the seasonal climatologies and variability spectra for most key physical, biogeochemical and microbial processes. Now, 20 years later, the data from these ocean time-series programmes continue to reveal previously unrecognized phenomena including, but not limited to, novel ecological processes and unpredicted climate-driven biogeochemical connections. These microbial ocean time-series programmes have attracted the interest of collaborators who have benefited from the existing core measurement programmes and from shared and leveraged access to the sea. The new knowledge gained is being used to improve existing ecosystem-based models, and to develop new ones.

At the beginning of the HOT programme, we had no idea that our efforts would facilitate the discovery of novel microorganisms, unexpected metabolic pathways and new ecological insights. We also had no idea that a monthly sampling frequency would be deemed inadequate for all key microbially mediated biogeochemical processes that we had endeavoured to understand. For example, it is now known that stochastic physical processes with time scales of less than 1 month can trigger a pulsed delivery of nutrients, resulting in changes in microbial community structure and a cascade of independent ecological processes. Higher temporal frequency microbe detection to track these rapid perturbations will require the development of new sensors and sampling procedures since it is impossible to sustain continuous human presence at this remote field location. Significant microbial and biogeochemical changes have also been observed on decadal time scales, possibly triggered by large-scale changes in the coupling of the ocean to the atmosphere (e.g. El Niño) or by secular changes in habitat due to greenhouse gas-induced climate warming. Ultimately, these long-term changes are the result of processes taking place over a broad range of scales eventually propagating into the habitat that impacts microbial processes. Understanding how marine microbial assemblages in different oceanic habitats may evolve over time in response to climate change will require not only a characterization of the microbes' response to physical and chemical changes but also the development of how interactions among microbes contribute to the metabolic plasticity and resilience of the biome as a whole. Prior to the start of the HOT programme, the North Pacific subtropical gyre – Earth's largest contiguous biome – was thought to be relatively stable in time and coherent in space, a habitat supporting a climax-type microbial community that was well tuned to its environment and resistant to change. We now recognize a fundamentally different biome, one that is dynamic, variable and poised for ecological opportunity. The design of future ocean time-series programmes and field experiments can benefit from lessons learned in HOT and related time-series programmes.

In summary, marine microbial communities are genetically gifted, metabolically talented and functionally literate. All microbial and biogeochemical processes in the sea reside in a temporal domain, and each process has a characteristic time scale of variability. A major achievement of the HOT programme is an improved understanding of the time-varying changes in microbial community structure and fluxes of energy and carbon at a key, open ocean site. If the ultimate goal of ecological research is to understand how organisms regulate metabolism and growth, coexist in complex and diverse assemblages, and respond to various environmental forces, then the future research prospectus needs to be comprehensive, ranging from extensive field observations, to well-controlled laboratory studies using model microorganisms, to whole ecosystem experiments. The design and conduct of meaningful field experiments demands an *a priori* understanding of habitat dynamics and metabolic controls, which ultimately requires extensive at-sea observations in advance of formulating the specific research question. The emergent HOT data sets are serving as the basis for the generation of new ideas, improved models, controlled experiments and other forms of hypothesis testing. Future ecological research will focus on the interrelationships among climate,

habitat, microbes and their collective metabolic function. Most important to our success will be continued, indeed enhanced, access to the sea for sustained observation and experimentation. By the next year of the dog, I predict that at least a dozen new ocean time-series programmes will have emerged with a collective focus on the key roles that microbes play in sustaining planetary habitability. These comprehensive, transdisciplinary field programmes will fully integrate physical and chemical oceanography with theoretical ecology and modelling into the wonderful world of marine microbes. It should be a very exciting decade of discovery.

This article is dedicated to the past, present and future scientists, students and technical staff of the Hawaii Ocean Time-series programme on their 20th anniversary (1988–2008) of successful field operation (http://hahana.soest.hawaii.edu).

A visit to the paediatrician in the not-so-distant future

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'Open wide and say "Aahh" Timmy', Dr Lemon instructs the child, then swiftly takes a swab sample from his throat. 'That wasn't so bad, was it? Now, I want you to do one more thing and I promise we'll be done: step into the restroom and please pee into this little jar.'

As the child leaves the room, Dr Lemon turns her attention to Timmy's mom who has been sitting quietly in the corner of the room. 'I am delighted to see Timmy mostly on well visits now. . . . I'll send the throat sample away for microbial community profiling and the urine for a metabolite screen. We'll know by tomorrow if the beneficial microbe cream you've been using on his nostrils is still keeping those nasty Staph, Strep and H-flu at bay . . . From the looks of him, I suspect those wonderful good germs are well-established and continuing to do their work. We will only call you if we detect any imbalances in his microbiota. . . .'

Dr Lemon has the experience to know that just a couple of decades ago the way she would have approached Timmy would have been dramatically different. He could easily have been one of those typical cases of children with recurrent ear and throat infections. At the first symptoms of otitis media she would have prescribed antibiotics, in great part to assuage parental anxiety. Chances are she would have seen Timmy come back many times and eventually amoxicillin would have proven ineffective as evolution played out its drama and resistant microbes took over the child's upper respiratory tract. Now, Dr Lemon knows full well that powerful antibiotics can be held in reserve for use in life-threatening systemic infections. The wonderful small molecule natural products made by beneficial microbes that routinely colonize the upper respiratory tract can be delivered 'naturally' using the topical probiotic cream she prescribed to Timmy. Early following of Timmy's microbiota by culture-independent methods had provided her with the clues that his particular microbiota put him at risk for colonization by *Staphylococcus aureus* and *Haemophilus influenza*. Prophylactic treatment with a consortium of microbes known to produce a cocktail of bioactive secondary metabolites almost always rectified the imbalances in microbiota such as she had detected in Timmy . . . Ever since the use of diverse probiotic approaches became widespread among physicians, the incidence of difficult-to-treat antibiotic-resistant infections had steadily decreased.

If the advances being made today through the study of the ecology of human microbiota do not begin to impact the way physicians treat their patients, we – the community of microbial scientists – will have failed. I strongly believe we will not fail. Recent advances in massive parallel DNA sequencing are already making microbial community profiling rapid and relatively inexpensive. The latest mass spectroscopic approaches allow rapid metabolite profiling. These facts combined mean that we now can begin to move from a human microbial ecology that has greatly focused on determining 'who is there' in a relatively small number of individuals to a human microbial chemical ecology that can ask 'who is there and what are they doing' on very large numbers of individuals in longitudinal studies worldwide. The information thus obtained will give rise to models where the patterns of microbial community composition dynamics and activities may be used to predict clinical outcomes. Couple this to a growing acceptance of the beneficial role of microbes, along with the perennial concern that the 'scorched earth approach' inherent in antibiotic therapy spells ecological disaster for the human microbiota, and you have the stage set for the development of probiotic prophylactic approaches to be applied in the clinical setting.

Experimental community -omics

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With microlitre-sized niches and generation times measured in hours rather than years, microbial communities are eminently manipulable. Microbial responses to system-wide perturbations can be measured on gratifyingly short time frames. Indeed, it was while reading about microbial reactions to manipulated watersheds at the Hubbard Brook Experimental Forest that I was inexorably pulled away from my fledgling career in plant ecology about 25 years ago. And while I was drawn into a world of