# Meeting Report: ALTER-Net Workshop about the "Application of Molecular Techniques to Study Biodiversity, Structure and Function of Planktonic Communities in Lakes" at Blanes, Spain, February 15-16, 2007

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Biodiversity is important for its contribution to the sustainable function of different ecosystems and for supplying goods and services essential for human survival. To

understand this role and the way biodiversity might be threatened through various pressures, such as land use change, pollution, climate change and invasive species, we must first assess its richness and how it changes throughout time and varies under different conditions. One important way of doing this are Long-Term Ecological Research (LTER) programmes that provide the necessary data series to find temporal changes and therefore allow us to determine their possible causes. The EU Network of Excellence ALTER-Net (A Long-Term Biodiversity, Ecosystem and Awareness Research Network; http://www.alter-net.info/) has been established to achieve sustainable integration of European research capacities in this field, combining currently 24 partners from 17 countries. A related Network of Excellence, MARBEF (http://www.marbef.org/), is focussing on marine biodiversity, while ALTER-Net is addressing biodiversity research in terrestrial and freshwater ecosystems.

The ALTER-Net workshop organised by Lluis Camarero and held at the Centre for Advanced Studies of Blanes (CEAB-CSIC) on the 15/16 February 2007 had the topic to discuss "Applications of molecular and genetic techniques to assess and monitor the impact of environmental drivers on the biodiversity, structure and function of planktonic communities in lakes". Scientists from seven European countries and representing different aspects of freshwater biodiversity research gave examples of their work that included the application of various molecular techniques. The talks covered a broad range of organisms (from prokaryotes (*Bacteria* and *Archaea*), through phyto- and zooplankton, to fish), lake ecosystems (boreal to tropical) and scientific fields (ecology, evolutionary biology, physiology) and were followed by a day of discussion about the use and usability of molecular methods in the assessment of biodiversity of freshwater systems.

Biodiversity studies associated with LTER programmes in freshwater or marine environments usually face two basic limitations. First of all, only a fraction of the recent biodiversity was catalogued by taxonomists and described as species. For instance the majority (>90-99% of taxa) of the recent prokaryotic biodiversity is currently represented by undescribed species. Secondly, almost all prokaryotes and many of the smaller eukaryotic microorganisms, e.g., many flagellated protists smaller than 10 µm, lack features enabling their identification by morphological criteria. On the other hand, biodiversity assessments are traditionally performed by morphological identification of organisms, which consequently results in a lack of insights in the biodiversity of smaller protists and almost all prokaryotes. Notably, however, these two categories undoubtedly represent in all ecosystems on earth the numerically dominating and metabolically most active organisms. Thus, traditional approaches for assessment of biodiversity in a particular ecosystem are missing a substantial part of the biodiversity present.

Molecular methods offer possibilities to overcome those limitations, either on their own or in combination with traditional approaches. Various techniques can be used to assess the overall biodiversity of an ecosystem, identify new and unknown organisms, reveal and trace spatial and temporal distribution of species and even link biodiversity and physiological response of organisms to environmental changes directly. Therefore, these methods allow new directions of research that would not have been possible with classical methods alone. In addition, molecular methods can be used to improve existing procedures, make them more time- or cost-effective, and develop "next-generation monitoring tools". They may allow processing numerous samples automatically and identifying species or groups of protists or specific lineages of bacteria with fine taxonomical resolution.

It should not be given the impression though that molecular methods are the solution to all problems, but they are promising for innovative lines of investigation to be included in on-going LTER and offer chances for new and exciting research with much deeper insight into biodiversity of ecosystems.

It is clear that in biodiversity research it is necessary to define the taxonomic level or phylogenetic resolution (in the case of prokaryotes) at which the analysis should be done. Biodiversity can be defined at the level of functional groups, higher groups, e.g. phytoplankton and zooplankton, or prokaryotes and eukaryotes, classes, genera, species, or even on the intraspecific level of strains. Often this taxonomic level then determines the molecular tools one can apply.

Among those tools, PCR fingerprinting techniques, like DGGE (Denaturing Gradient Gel Electrophoresis), are powerful methods for the analysis of whole microbial communities. They are able to distinguish taxa with only small differences in DNA sequences, enable the identification of predominant members of communities, and allow the monitoring of compositional changes in community structure in a fast and efficient way. DGGE fingerprints usually show 3-35 bands, of which, in the best case, each band represents a single abundant taxon. However, rare species are usually below the detection limit of this method. In contrast, genomic libraries much better represent the whole community, including rare species, but are more laborious and expensive to establish and analyse. **E. Casamayor (**Council for Scientific Research, Spain) spoke about his research on eukaryotic picoplankton and bacteria, and changes in their community structure along environmental gradients, e.g., salinity, oxygen or trophic status gradients using such DGGE fingerprints. PCR primers for the small subunit of ribosomal RNA genes were used to amplify prokaryotic and eukaryotic sequences from samples and their DGGE fingerprints gave a picture of the microbial

diversity. The occurrence of bands (taxa) could then be related to environmental conditions to understand changes in the communities. In addition, the application of DGGE primers for functional genes allowed him to link key metabolic pathways in nutrient cycling to occurring microbial populations.

While DGGE is a powerful technique to qualitatively analyse a microbial community, it can not be used for quantitative assessment of the occurring taxa. Also, the method is labour- and time-intensive and therefore difficult to integrate into routine monitoring programmes. A technique without those drawbacks for monitoring programmes – but of course with others – is flow cytometry, for which **J. Gasol** (Council for Scientific Research, Spain) presented examples of ecosystem research from the marine environment. Gasol showed results from the analysis of HNA & LNA bacteria (high / low content of nucleic acids per cell), cyanobacteria and picoeukaryotic phytoplankton in different water bodies and under different environmental conditions using this technique. Flow cytometry is not *per se* a molecular technique in the sense that it deals with nucleic acids or proteins, but is nevertheless closely linked and may be supplemented and greatly improved through the application of molecular probes in the future. Basic flow cytometry methods detect the characteristics of single cells or other particles while passing a laser beam. The device measures the light that passes the particle or is scattered by it. Furthermore, fluorescence signals emitted by cells or particles due to the presence of autofluorescent substances (e.g. pigments, like chlorophyll) or due to staining with fluorescent dyes (e.g., nucleic acid stains) is measured. Two- or three-dimensional plots of the measured signals allow to group cells in different clusters that share the same characteristics and a tentative assignment to various higher groups (e.g., cyanobacteria, pico- and nano-eukaryotes), which can also be quantified. While flow

cytometry is usually a fast and simple method that could be easily included into aquatic LTER programmes, it is nevertheless limited in its taxonomic resolution. Theoretically, this can be overcome by the application of molecular probes (see below) or various fluorescent dyes, but many of these techniques are still under development and proved difficult in analysing field samples, especially their bacterioplanktonic component.

**T. Buchaca** (National Environmental Research Institute (NERI), Denmark) showed the use of the computer program CHEMTAX for the identification and quantification of phytoplankton groups in freshwater based on their pigment ratios. It makes use of the fact that certain phytoplankton classes have unique marker pigments or at least specific pigment ratios. While this method has a higher taxonomic resolution than flow cytometry, i.e., it allows to identify and quantify phytoplankton at the class-level or below, it also requires the analysis of pigments through HPLC (High Performance Liquid Chromatography) which is more laborious, and is based on a couple of requirements and assumptions regarding species composition and pigment ratios in the analysed water body. Buchaca therefore clearly stated the need for larger datasets, calibration and comparison with other identification methods before a CHEMTAX analysis can be routinely used in freshwater LTER.

As indicated before, the majority of planktonic bacteria and many eukaryotic plankter represent taxonomically undescribed taxa lacking morphological traits suitable for discrimination. Therefore, knowledge of temporal and special diversity patterns of those organisms is very scarce, but one solution for getting insights into their dynamics is the application of molecular probes. Such probes usually target taxon-specific regions of ribosomal sequences and, under the right hybridization conditions, are able to specifically detect the taxon of interest in a mixed sample. They can be

developed for a broad range of taxa that covers all levels of biodiversity from higher groups down to strains, and applied to samples in various ways of which Fluorescence *In Situ* Hybridization (FISH) followed by epifluorescence microscopy is the most common. **M. Hahn** (Austrian Academy of Sciences) developed and applied various phylogenetic probes, which were specific for genus- and species-like groups of freshwater bacteria, to analyse their seasonal dynamics and spatial distribution. Hahn and his co-workers could show that the investigated bacterial populations behaved similarly to eukaryotic populations of phyto- and zooplankton with pronounced and recurrent seasonal dynamics and consistent vertical and horizontal distribution within a habitat, a result that would not have been possible to obtain without using molecular methods. Furthermore, Hahn and colleagues demonstrated by using specific phylogenetic probes complete niche separation in closely related bacterial taxa indistinguishable by morphologic traits.

More applications of molecular and activity probes were given by **K. Horňák** (Biology Centre AS CR, Hydrobiological Institute) in the examination of trophic interactions between heterotrophic nanoflagellates, bacteria and viruses in a freshwater reservoir. In addition to DGGE, he and his co-workers used molecular probes to enumerate bacterial groups and determine the bacterial community composition. A modified FISH assay also allowed them to identify bacterial prey directly in the food vacuoles of nanoflagellate protists and the combination of FISH with microautoradiography (MAR-FISH) made it possible to determine semi-quantitatively the physiological activity of those bacteria under changing environmental conditions. The combination of these methods created a powerful way of understanding the interactions between flagellates, bacteria and viruses at the single-cell level in a couple of microcosm experiments. A set of manipulation

experiments with microbial plankton communities allowed to identify a genus-like cluster of Betaproteobacteria with a stable and relatively high proportion in the community, the largest growth potential of all studied bacterial subgroups and the key role in bacterial production processes in the freshwater reservoir. Changes in the relative proportions and activity of the members of this lineage in the community have been recently suggested as possible indicator of marked changes in the structure and function of natural bacterioplanton that could reflect sudden (biotic and non-biotic) changes in an environment. **D. Diaz de Quintano** (University of Barcelona, Spain) introduced the method of CARD-FISH (Catalyzed Reporter Deposition Fluorescence *In Situ* Hybridization) and the ELF (Enzyme Labelled Fluorescence) technique to the audience. While standard FISH protocols are often sufficient for analysing unicellular organisms, it can sometimes happen that the strength of the fluorescence signal is not high enough for a secure detection, i.e., when there are not enough ribosomal targets for the probe to bind to. This is often the case with small cells, like bacteria or picoeukaryotes, dormant cells or cells in a low-activity physiological state. CARD-FISH uses a horseradish-peroxidase that is bound to the molecular probe to catalyse fluorochrome-labelled tyramide, which amplifies the fluorescent signal 10 to 100 times in comparison to a standard probe. The technique is more elaborate though than normal FISH and requires additional steps and sometimes target-specific optimization. While CARD-FISH is another tool to identify organisms, ELF is a method that gives the opportunity to analyse physiological processes of those organisms, a crucial next step in biodiversity research. **J. Vrba** (Czech Academy of Sciences) showed in his talk such physiological analyses using the ELF technique for detecting the activity of extracellular phosphatases at a single-cell level. The production of this enzyme is usually accompanied by high-affinity uptake of $P_i$ and is

a species-specific or cell-specific feature; i.e., it is not a general response to starvation in either phytoplankton or bacterioplankton. With phosphorus deficiency being frequently the limiting factor in plankton growth and the major source of selective pressure on single microbial populations in a variety of aquatic environments, the ELF technique gives detailed information about the physiological status of natural planktonic microbes.

After biodiversity research at the levels of higher groups down to the species level, intra-specific biodiversity was the topic of **R. Groben**'s (Centre for Ecology & Hydrology, U.K.) talk in which he gave an overview about the development and application of microsatellite and AFLP (Amplified Fragment Length Polymorphism) markers in planktonic species. While "classical" monitoring programmes can maybe analyse biodiversity down to the species level, investigating the hidden intra-specific diversity requires molecular tools. Identification and characterization at strain-level might be important though when it comes to the analysis of toxic versus non-toxic strains of the same species or when strains have varying susceptibilities towards environmental conditions (e.g., viruses, temperature changes or nutrient depletion) that might determine their ecological success. After an introduction into the two marker types and their advantages and disadvantages, Groben gave an overview of research that has been done on molecular markers in marine and freshwater plankton. This showed that the application of these useful techniques to protists is still in its infancy in aquatic sciences and most work is published in terms of methods development. Nevertheless, more and more papers are currently coming out that deal directly with real ecological questions. **J. Mergeay** (KU Leuven, Belgium) illustrated the possibilities of molecular markers further in his talk about the water flea *Daphnia* as a model organism for biodiversity research. *Daphnia* species are key components

in the food webs of most standing waters, have a remarkable ability to cope with environmental changes and, after two centuries of intensive research, is one of the ecologically best known freshwater organisms. Still, molecular methods can add to this pool of knowledge and allow further studies of population genetics, phylogeny and ecology of this genus. For example, molecular markers have allowed the detection of many cryptic species, which has led to a paradigm shift in recent years, from low global species diversity and cosmopolitanism to high species diversity and local endemism or provincialism. In addition, Mergeay showed that neutral molecular markers can provide information on the origin of biogeographic patterns ("phylogeography"), but also on rates of dispersal, colonization and gene flow. Simultaneously, genetic analyses of ecologically relevant and heritable traits (ERT) allow the detection of direct selective pressures, like anthropogenic stress. Moreover, the combination of neutral markers and ERT allows one to estimate the relative importance of neutral effects like drift compared to natural selection. Finally, the application of genetic markers (neutral and/or ERT) to historical archives like lake sediments allows a detailed reconstruction of the response of lake biota to different ecological and evolutionary processes ("paleogenetics").

Further examples of freshwater long-term monitoring sites and the research associated with them were given by **U. Münster** (Tampere University of Technology, Finland) and **M. Ventura** (National Environmental Research Institute, Denmark). Münster used a variety of conventional (bacterial counts & cultivation, metabolic analyses) and molecular methods (DGGE, FISH, sequencing of genomic libraries) to analyse the composition of planktonic and sediment-dwelling prokaryotic communities in Finnish boreal lakes. He and his co-workers link microbial community structure with biocatalytic and metabolic function in order to better understand its value and role in

ecosystem function, stability and resilience. Their research especially focused on the ecological role of *Archaea*, which are not restricted to extreme environments, as was thought in earlier days, but can be found in a wide range of environments. The data presented by Münster demonstrated a large archael diversity in Finnish lakes, including the discovery of novel groups.

While Münster concentrated on boreal lakes, Ventura gave examples of research from the various sites NERI investigates, which range from Greenland to Greece, and also includes lakes outside of Europe. The comparison of biodiversity in the lakes of this huge transect provides a way of elucidating long-term responses to climate changes by analogy observations, i.e., studies of how structure, biodiversity and dynamics change along existing climate gradients (e.g. along gradients of longitude, latitude and altitude) and use this knowledge to estimate the nature of ecosystems and biodiversity at a given time under predicted future climate conditions. It also allows interesting insights into ecosystem structures, trophic interactions and species richness in contrasting lakes. For example, cold northern lakes showed strong top-down effects on zooplankton that diminished with increases in nutrient state or at higher temperatures. In a second comparison, using a subset of different European lakes, bottom-up forces, such as nutrient concentration, were the most important predictors of zooplankton biomass.

One of the aims of ALTER-Net, the building of networks and exchange of information among scientists working on biodiversity of long-term monitoring sites, was clearly achieved during this workshop. The talks and discussions were interesting and fruitful and showed the broad range of topics and research projects within this field. Despite the fact that the speakers presented many exciting research results that

were associated with various biodiversity research projects, the focus of the workshop was definitely methodological and gave many examples of the molecular tools that can be used in analysing the biodiversity of prokaryotes, protists or higher organisms and their links to ecosystem function. Under this aspect, also general questions were discussed, for example, at which level biodiversity is "meaningful" in structuring an ecosystem and regulating its function. Is it necessary to know the diversity at the species or intra-specific level, or is it enough to identify and analyse functional groups to understand how an ecosystem works? Also, is this crucial "ecosystem-shaping" level different for the different groups of organisms (bacteria, phytoplankton and zooplankton)? There are clearly many questions still open in this area and molecular methods can provide valuable tools in answering them and helping us to understand the real importance of biodiversity.

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