Within the project, we are working on the optimization of both bulk and environmental DNA (eDNA) extraction methods for invertebrates, as well as metabarcoding techniques to capture the diversity of life, using aquatic beetles as one of the main taxon groups under study. Since aquatic beetles (and macroinvertebrates in general) and their DNA behave differently from more commonly studied taxa such as fish and amphibians, the usual methods for detection may be of limited use. An analysis pipeline will be developed together with partner organizations in the field of freshwater ecology and quality monitoring, to enable more uniform and reliable monitoring of species present in freshwater bodies. The results may be applied to 1 the assessment of water quality based on DNA circumventing morphological analysis, 2 the early detection of exotic species or potentially harmful invasive species, and 3 the detection of (potential) vectors of human or livestock diseases in the tropics.
At the very least, the cumbersome morphological sorting and identifying of macroinvertebrate samples can be replaced by molecular tools, eliminating a significant source of error which has been proven to exist in various audit studies. The ideal situation would work towards eliminating the need of specimen collection in its entirety, although much work needs to be done before any such method will become standard practice. By adapting strategies to better fit the needs of macroinvertebrate detection and identification, we can open up the field of molecular freshwater monitoring to much more integrative approaches of defining communities and assessing freshwater health. We will present our recent findings on the topic of macroinvertebrate identification and detection, and discuss the implications on the applicability of molecular tools for the study of macroinvertebrate communities.

25-O Proteomic evidence of methanotrophy in methane-enriched hypolimnetic lake water.

Nina Ullrich [1] - Peter Casper [1] - Andreas Otto [2] - Mark O. Gessner [3]

Leibniz-institute of Freshwater Ecology and Inland Fisheries (IGB), Stechlin, Germany [1] - Institute of Microbiology, Ernst-moritz-arndt University, Greifswald, Germany [2] - Leibniz-institute of Freshwater Ecology and Inland Fisheries (IGB), Berlin Institute of Technology (TU Berlin), Berlin, Germany, [3]

Methane is one of the most potent greenhouse gases. Formed primarily in anoxic sediments, methane can be released into the overlying water of surface waters and ultimately to the atmosphere. However, emissions are mitigated by effective methane oxidation when oxygen is present, mostly at oxic-anoxic interfaces. The objective of the present study was to adopt a proteomic approach to identify effects of methane concentration in oxygenated lake water on patterns of enzymes involved in methanotrophy. We collected bacterial communities in natural lake water from the oxygenated hypolimnion of a deep temperate lake, allocated the water samples to six flasks that were subsequently aerated and either enriched with methane or left untreated to serve as controls. After one week, proteins were extracted from the water of each replicate flask, digested with trypsin, the resulting peptides partially separated by gel electrophoresis and then analysed by LC-MS/MS connected to an EASY-nLC II system. Methane was effectively oxidized when methane concentrations were high, reducing oxygen levels from 12 to 3 mg/L, well below those in the controls (7 mg/L). The key enzyme of methane oxidation, methane monooxygenase (MMO), was identified in both enriched and control flasks, whereas enzymes involved in metabolic pathways leading to carbon assimilation related to methane oxidation (RuMP pathway and serine cycle) were restricted to the enriched microcosms. All enzymes had best sequence matches with type I methanotrophs. Therefore, the detection of enzymes required for the serine cycle was unexpected, since this pathway is considered characteristic of type II methanotrophs. However, we did not detect the truly specific enzymes of the serine cycle, hydroxypyruvate reductase (HPR) and serine hydroxymethyltransferase (STHM). This might imply that the four identified enzymes involved in the serine cycle assumed metabolic roles independent of methane metabolism (e.g. in carbohydrate metabolism). Overall, our proteomic analysis provides convincing evidence that a suite of genes required for methanotrophy are quickly expressed when the presence of both methane and oxygen creates conditions characteristic of oxyclines in lakes.

25-O A lake's microbiome: from zooplankton to sediments.

Ester Eckert [1] - Stefano Amalfitano [2] - Andrea Di Cesare [1] - Gianluca Corno [1] - Diego Fontaneto [1]

Institute of Ecosystem Study ISE CNR, MEG, Verbania, Italy [1] - IRSA CNR, Rome, Italy [2]

This study determines and compares the microbiomes of various microenvironments representing different spatial niches within a lake ecosystem. We sampled three coastal stations of Lake Maggiore and sequenced the 16S rRNA gene of the bacterial communities associated with daphnia, copepods, and those living in water samples at 10 and 40m depth,

in the upper sediment layer, and in epilithic biofilms at the shore. We hypothesised that the animal associated microbiomes were less diverse (thus more specialised) when compared to the other habitats, due to the specificity of a microenvironment associated to an animal.
The total microbial community comprised little less than 3500 operational taxonomic units (OTUs). Most of these OTUs (i.e., 1978) were found uniquely in the sediment, which was clearly the habitat with the highest diversity, followed by epilithic biofilms, 40m-deep waters, 10m-deep waters, and copepods and daphnids at last. Beta-diversity analysis showed that both daphnia and copepod harboured a similar microbiome, since they clustered into the same. This animal cluster then grouped with the water samples (10 and 40m), and the so-formed animal-water group clustered with the stone microbiome. The most distant group contained the sediment microbes. Comparing the different niches, each habitat-pair shared between 107 and 254 OTUs, thus a similar amount of OTUs was shared between all couples. Considering non-rare OTUs (>10 reads in dataset, around 700 OTUs), 55% were found in only one habitat, with an average of 29 reads per OTU, whereas 1.75% were found in all 6 habitats, which however had an average of 1206 reads per OTU, and were thus much more abundant.
In order to determine if each OTU could be considered a habitat specialist or generalist, a niche breadth index was calculated by considering how frequent an OTU is and how evenly its abundances are distributed. We found that very few OTUs could be considered generalists and that these OTUs were not particularly abundant. Despite the largest part of OTUs were clearly specialists, also theses OTUs had rather low abundances. All habitats, except for the sediment, were dominated by OTUs that could not be defined specialist nor generalist and that were found in similar abundances in other habitats, too.
In conclusion, the animal-associated bacteria community was similar, and slightly less diverse then in the surrounding available microenvironments. However, the animal microbiome was not more habitat-specialised then the water or stone microbiomes and OTUs on animals are shared with any other of the habitats.

25-P Distribution of serotonin and the clock protein period in the brain of *Daphnia pulex*.

Piotr Bernatowicz [1] - Marta Polańska [2] - Piotr Bębas [2] - Bohdan Paterczyk [3]

Dapartment of Paleobiology and Evolution, Faculty of Biology, University of Warsaw, University, Warsaw, Poland [1] - Department of Animal Physiology, Faculty of Biology, University of Warsaw, University, Warsaw, Poland [2] - Laboratory of Electron And Confocal Microscopy, Faculty of Biology, University of Warsaw, Warsaw, Poland [3]

Recently described biological clock of *Daphnia pulex* consists of molecular oscillator and its input and output pathways. The *D. pulex* oscillator is composed of genes and proteins that seem to be coupled in a form of feedback loops. Period protein (PER) plays the key role in this mechanism.
Serotonin is a biologically active monoamine. It has been described as neurotransmitter involved in the regulation of many physiological functions in crustaceans including these associated with behavioral rhythms.
The aim of our study was to map a connection between groups of clock containing neurons and serotonergic neurons (which constitute at least one of the clock outputs pathways) in *Daphnia* brains.
To reach this goal we characterize PER and serotonin distribution in the brains of *D. pulex* using immunochistochemical method with anti-PER and anti-5HT antibodies followed by tissues analysis in the confocal microscope.
We found PER protein expression only in two neurons of *D. pulex* protocerebrum, while serotonin was identified in six neurons, both in proto- and deutocerebrum. We found no co-localization of PER and serotonin, which were detected always in different populations of neurons. However, nerve endings of both PER and serotonin neurons are localized very close to each other, supporting hypothesis of the synaptic connection between them.
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25-P Phylogenetic evidence for a new species of *Barbus* in the Danube River basin.

László Antal [1] - Brigitta László [2] - Petr Kotlík [3] - Attila Mozsár [4] - István Czeglédi [4] - Miklós Oldal [5] - Gábor Kemenesi [5] - Ferenc Jakab [5] - Sándor Alex Nagy [1]

Department of Hydrobiology, University of Debrecen, Debrecen, Hungary [1] - Department of Medical Microbiology, University of Debrecen, Debrecen, Hungary [2] - Laboratory of Molecular Ecology, Institute of Animal Physiology And Genetics, The Czech Academy of Sciences, Libechov, Czech Republic [3] - Balaton Limnological Institute, MTA Centre for Ecological Research, Tihany, Hungary [4] - Virological Research Group, Szentágothai Research Center, University of Pécs, Pécs, Hungary [5]