Environmental DNA for biodiversity monitoring and conservation

According to recent research, the loss of biodiversity could have an ecological impact as significant as climate change. Important processes such as water purification, natural flood protection, pollination, soil fertilization and CO2 capture are services provided free of charge by biodiversity.

The workshop will showcase new developments in implementing environmental DNA-related tools for biodiversity monitoring in freshwater and marine environments. Effective biodiversity policy and impactful conservation/ restoration actions are hampered by the lack of rapid and cost-effective tools for species identification and monitoring. Rapid development of high-throughput sequencing technologies is changing the approach to biodiversity research and conservation at different levels. DNA metabarcoding provides a unique holistic tool to study complex aquatic ecosystems and is gradually implemented in diverse practical contexts from detecting particular species to monitor the whole communities and assess their sensitivity to environmental changes. We propose a future-oriented, focused workshop to present latest advances in eDNA technology and its implementation in routine biomonitoring and bioassessment.

Environmental DNA for modern ecology

Pierre Taberlet, CNRS Grenoble, France, The Arctic University of Norway

From biomonitoring to conservation: the multifaceted roles of genomic approaches in ecology

Alexandra Anh-Thu Weber, Department of Aquatic Ecology, Eawag, Dübendorf, Switzerland
The first part of the presentation is a basic introduction to environmental DNA, with definitions and timing of emergence. After giving the simplified experimental protocol, the different topics that can be addressed with environmental DNA will be sequentially presented. These are several research topics. (i) Assessment of soil biodiversity in an agricultural context, using different metabarcoding markers. (ii) Biodiversity assessment from freshwater samples: brief presentation of the potential. (iii) Diet analysis applied to domestic and wild animals. (iv) Assessment of past ecosystems: using lake sediments to trace changes in plant and animal communities. The last part of the presentation will focus on the experimental design and on the limitations of environmental DNA. A DNA metabarcoding experiment seems easy to implement according to the simplicity of its different steps, namely (i) sampling and extracting environmental DNA, (ii) amplifying a metabarcode, (iii) sequencing on a next-generation sequencer, and (iv) sequence analysis based on published bioinformatic pipelines. Unfortunately, this is not the case, and metabarcoding suffers from many difficulties due to several categories of experimental artifacts. By knowing all the potential problems, it is possible to design an experimental protocol that will limit their impact and secure the final results.
**Pierre Taberlet**, Centre National de la Recherche Scientifique “Laboratoire d’Ecologie Alpine”.

Pierre Taberlet studied biology and geology at the University Joseph Fourier of Grenoble (France) from 1972 to 1976. He was a biology teacher in a high school from 1978 to 1989. He obtained a PhD in 1992 from the University Joseph Fourier, and the Habilitation in 1993. He joined the Centre National de la Recherche Scientifique in 1994. He has worked in the “Laboratoire d’Ecologie Alpine” (Grenoble), first as junior scientist (1994-1998), and then as senior scientist (1999-2019). He was the head of this laboratory from 1999 to 2010. For the past 20 years, his research team has been working in the field of molecular ecology, with emphasis on the phylogeography of wild animals and plants, on conservation genetics (development of non-invasive sampling), and on domestication of goats and sheep. More recently, he is involved in biodiversity assessment using DNA metabarcoding, i.e. using environmental samples (soil, water) and new sequencing technologies. He is author or co-author of 300 scientific publications in peer-reviewed journals and is a highly cited researcher (hcr.clarivate.com). He is currently senior editor of Molecular Ecology, associate editor of Science Advances. He received the Molecular Ecology Prize in 2007, and coordinated three large collaborative European projects between 2004 and 2014.

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**From biomonitoring to conservation: the multifaceted roles of genomic approaches in ecology**

Alexandra Anh-Thu Weber, *Department of Aquatic Ecology, Eawag, Dübendorf, Switzerland*

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**Next-generation sequencing applications in ecology**

Biodiversity assessment
- Community level
  - Discovery
  - Description
  - Metagenomics
  - Metabarcoding

Conservation / Invasion biology
- Species level
  - Species-specific monitoring
  - Reference database
  - Barcoding
  - Reduced-representation genome sequencing
  - Whole-genome sequencing

Barcoding
- The invasive Quagga mussel
- The native duck mussels

Conservation
- Demographic history
- Connectivity
- Species delimitation
- Adaptation
- Genetic diversity

Next generation sequencing (NGS) has revolutionized many fields of ecological research over the last decades. However, it is not always trivial to choose the optimal sequencing target and effort for each specific biological question. In this talk, I will present the main applications of...
NGS for: i) biodiversity assessment and monitoring (metabarcoding; metagenomics); and ii) conservation (barcoding; reduced-representation genome sequencing; whole-genome resequencing; reference genome generation). I will give an overview of the various biological questions different datasets can answer, and the requirements in terms of: i) source of genetic material (eDNA; eRNA; tissue samples); ii) the sequencing effort; iii) bioinformatic infrastructure and expertise for data analysis. Then, I will present two case studies on the use of NGS data for biomonitoring and conservation in aquatic ecosystems: i) the invasive Quagga mussel in Switzerland: from early detection to real-time biomonitoring using eDNA; ii) the native duck mussel in Switzerland: conservation genomics of an ecosystem engineer with potential cryptic speciation.

**Dr Alexandra Anh-Thu Weber** is a group leader at Department of Aquatic Ecology, Eawag, Dübendorf, Switzerland. She obtained PhD degree PhD in Oceanography from IMBE, Aix-Marseille University, Marseille in 2015, and subsequently conducted research in Switzerland, Australia and France. Her scientific interests focus on adaptive evolution, speciation, conservation, genomics, ecological genetics. She author of over 20 publications on biodiversity in aquatic ecosystems. Currently she is working on native and invasive freshwater bivalve species in Switzerland but she has worked in the past on a variety of study systems such as fishes, echinoderms and protists.

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**Translating Illumina®-derived massive sequence data into biologically meaningful information for biomonitoring via SML**

**Verena Rubel & Thorsten Stoeck**, Department of Ecology, University of Kaiserslautern, Kaiserslautern, Germany

![Pipeline diagram](image)

*Fig.: Pipeline from Illumina®-derived ASVs (Amplicon Sequence Variants) to a SML-based (Supervised Machine Learning-based) monitoring system via bioindicator DB (database) construction.*

As Illumina® sequencing is becoming cheaper and faster than ever, traditional biomonitoring methods requiring expert-based taxonomic assignments can be replaced with more efficient
molecular methods. Here I present a case study of biomonitoring focusing on disturbed coastal marine ecosystems. Such environments are under the influence of mariculture, e.g. salmon aquaculture. One of the key pollutants introduced is organic carbon, which accumulates on the sea floor due to superfluous feed and fish feces. In the future, bacterial 16S eDNA metabarcoding pipelines can replace the labor-intensive, traditional monitoring procedures. The main building blocks of this novel method are Illumina sequencing and a subsequent Supervised Machine Learning (SML) approach. I will present the pipeline which uses Illumina®-derived sequences to infer Amplicon Sequence Variants (ASVs) in a first step. Based on such ASVs, SML is used in a second step to create a taxonomy-free database of bioindicator ASVs. This pipeline enables the construction of a molecular tool to conduct ecological quality assessments of samples with previously unknown ecological conditions. Only with a cautiously standardized pipeline, a robust indicator database can be constructed, enabling salmon farm biomonitoring in a broad geospatial context.

Dr Verena Rubel (née Dully) is a Post-Doc researcher in the Lab of Prof. Dr. Thorsten Stoeck at the Department of Ecology at the University of Kaiserslautern, Germany. Since her master thesis, she focuses on marine coastal monitoring under salmon aquaculture impact and how to predict its influence on the environment. She recently obtained her PhD focusing on the standardization of the eDNA metabarcoding pipeline for the development of SML-based biomonitoring approaches. At the moment she is working on further pipeline optimization steps and the inclusion of metagenomic and metatranscriptomic data for sequencing-based biomonitoring systems.

Diatom’s Molecular Index – latest advances in genomic biomonitoring

Kristina Cermakova, ID-Gene ecodiagnostics, Switzerland
Based on sequencing of environmental DNA (eDNA) targeting the diatom community, ID-Gene has developed a Diatom Molecular Index as a tool to determine the ecological status of rivers and streams. The diatom DNA is specifically amplified from total eDNA extracted from epilithic biofilm samples and sequenced using the high-throughput sequencing technology. Thousands of diatom DNA sequences are obtained per sample. The analysis of these samples using specifically tailored computer algorithms allows to predict with high accuracy a **Diatom Molecular Index**, as well as to provide a list of referenced diatom species present in the sample.

Current biodiversity assessment and monitoring are largely based on morphological identification of bioindicator taxa. However, in the case of diatoms, the microscopic identification of tiny and highly variable diatom frustules is time consuming and requires very good taxonomic expertise, which is not always available. These limiting factors contrast with the need of a fast routine assessment for the management of water quality. To overcome these issues, we propose the **ID-Gene™ Diatom Molecular Index** to assess water quality directly from diatom high-throughput DNA sequence data. This method allows processing a large number of samples over a short period of time.

**Kristina Cermakova** joined ID-Gene in 2017. She graduated from University of Geneva with master’s in biology and a Certificate in Industrial Life Sciences. During her studies she has been granted the Excellence Master Fellowship for outstanding students. She is successful co-applicant in several Swiss and European grants. At ID-Gene she acquired strong expertise in metabarcoding. Kristina’s solid scientific background and analytical skills has effectively contributed to development of novel biomonitoring tools.

**Tools for measuring biodiversity changes**

**André Eggen**, Agrigenomics, Illumina, France