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Abstracts

A national scale "BioBlitz" using citizen science and eDNA metabarcoding for monitoring coastal marine fish

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Monitoring of biodiversity across large spatial extents are challenging. We here combined citizen science with eDNA metabarcoding to map coastal fish biodiversity at a national scale. We engaged 360 citizen scientists to collect filtered sea water samples from 100 sites across Denmark over two seasons, and by sampling the same date within the same hour across all 100 sites, we obtained an overview of fish biodiversity largely unaffected by temporal variation. Comparing our eDNA data with National Fish Atlas data we found a positive correlation between species richness and congruent patterns of community compositions. We retrieved distribution patterns matching known occurrence for both invasive, endangered, and cryptic species, and detected seasonal variation in accordance with known phenology. These findings support the use of eDNA based citizen science to detect patterns in biodiversity.

eDNAbyss: Setting up a standardized chain of protocols to achieve a FAIR pipeline for the DNA-based exploration of the largest biome on Earth

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The emergence of environmental DNA has granted access to hitherto nearly unreachable compartment of diversity. This includes the largest biome on Earth: the deep ocean floor (DOF), whose biodiversity and contribution to large biogeochemical cycles remain largely unknown. The still large gap affecting our knowledge of the DOF covering more than half our planet, is largely due to technical challenges associated with its remote access and to the amount of material needed. Moreover, the exploration of biodiversity across this vast biome, also requires the integration of inventories based on distinct sampling strategies and performed by different experts in distinct areas of the world (i.e. the famous observer effect). The relatively small amount of material required to perform metabarcoding and metagenomics assessments based on eDNA, and the level of standardization they allow offered new promises to advance toward complementary and interoperable biodiversity assessment and improve our understanding of the extent and drivers of deep sea biodiversity. It may also allow unravelling completely unknown lineages, the so-called "dark matter", whose identification requires challenging bioinformatics analysis to separate the wheat from the chaff. In the framework of two projects, Pourquoi Pas les Abysses (Ifremer, 2016-2019), eDNAbyss (France Génomique, 2018-ongoing), we developed a series of standard protocols from sampling to libraries construction and bioinformatics analysis, to assess benthic diversity of the deep sea floor across the Tree of Life. During the development of those protocols, some issues could be solved while others remain awaiting for new methodological developments. This standard pipeline of protocols was applied to a diversity of ecosystems already, allowing to foresee the ability to gather concerted efforts across the international community, to gain a global holistic appraisal of the large reservoir of biodiversity in the deep ocean.

Spatial distribution of sedDNA in small catchments and lakes: Is single core enough?

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Comprehensive use of environmental DNA (eDNA) for biomonitoring present and past biodiversity provides new opportunities in the fields of ecology and biology. The interpretation of eDNA studies is contingent upon our understanding of taphonomical aspects DNA transportation mechanisms, degradation and preservation processes. Recent studies using sedimentary DNA (sedDNA) as a proxy to explain past biodiversity have also addressed the same questions concerning the taphonomy of DNA. Further investigation needed to understand how sedDNA represents the ecosystem over time and how representative a single core is of local biodiversity. We aim to test the homogeneity and representativity of a single core for characterizing the vegetation communities surrounding the lake. For this, we have taken 42 pairs of lake-sediment surface samples (0-2cm) over a small lake, Stabbevatnet located in Northern N Roadmap for implementing environmental DNA (eDNA) and other molecular monitoring methods in Finland – Vision and action plan for 2022–2025 in Norway. The lake is ideal for this study as it has a distinctive pattern of vegetation around the lake varying from Boreal forest and heath to agricultural land. Our objective is to compare two datasets: 1) the sedimentary DNA samples collected using systematic sampling method inside the lake 2) and a systematic vegetation survey within the catchment and surrounding the lake. This is the first study in Northern Norway that uses multiple sediment samples sedimentary DNA in order to elucidate the relationship between sedDNA and catchment vegetation.

Assessing biodiversity in 20 Norwegian ponds in the context of urbanization

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According to the Living Planet Report, ca 60% of animal species populations have declined as a result of human activity in the past four decades. Urbanization induces the loss of natural habitats and opens a gate for invasive species. Ponds are freshwater habitats that exist in both urban and non-urban landscapes, often found to be biodiversity hotspots of many rare and endangered species. As ponds typically are not monitored systematically, knowledge about overall pond biodiversity still remains poor. Here, we will present the results of the diversity of 20 Norway ponds (including bacteria, fungi and invertebrates) based on eDNA and assess the impact of urbanization in Oslo and Trondheim. Pond water was sampled and filtered from urban and rural ponds in Oslo and Trondheim areas. DNA libraries were prepared using PCR-based amplification of the different regions depending on organism identification (16S rRNA for bacteria, LSU rRNA for fungi, COI for invertebrates). Sequencing was performed on the Illumina MiSeq platform rendering 2 x 250 bp paired-end sequences. Ponds were described as urban or rural based on distance from metropolia and human modification level (QGIS) and several factors describing water chemistry were measured when sampling. The level of significance for the difference between the Shannon index in urban vs rural locations is: $p=0.016$ (invertebrates, COI) for Oslo and $p=0.043$ (fungi, LSU) for Trondheim. For both, higher biodiversity was observed in rural areas. At the same time for the Jaccard dissimilarity index we have $p<0,05$, for: bacteria and invertebrates in Oslo and fungi in Oslo and Trondheim. We further used the ANCOM model to determine taxa separating urban and rural areas. These results showed that in Oslo, which is a more urbanised city, the differences in biodiversity between urban and rural ponds are more significant than in Trondheim, especially if we consider beta-diversity for all studied taxa.

Fungi – facilitator or limitator for rhododendron invasion in western Norway

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Rhododendron spp. is a garden plant in Norway with long horticultural traditions. In several parts of Europe some varieties have become highly invasive, but in Norway, the seedling establishment remains relatively limited and quite random despite the high fecundity and distribution of this genus. *Rhododendron spp*. seeds are minute, lacking survival abilities in terms of germination and persistence, and germination success may in fact be dependent upon having the right fungal facilitators for successful seedling establishment and persistence. However, rhododendron varieties mostly used in horticulture, are descending mainly from Asia and America, thus, potentially having a completely different fungal root microbiome compared what naturally existing within native Norwegian soils. In this project, I have assessed this "missing mutualist hypothesis" as an explanatory factor for why rhododendron is not more widespread within western Norway. Root-associated fungal communities have been assessed from Rhododendron spp. seedlings together with adjacent native ericaceous plants including *Calluna vulgaris*, *Vaccinium myrtillus* and *Empetrum nigrum*. The native plants are all key stone species within various nature types in Norway. In parallel, the fungal communities were captured across all the plant root systems using culturing techniques to assess how eDNA approaches and more traditional methods such as culturing, and sanger sequencing fungi compare at capturing the root associated fungal community. Preliminary data from culturing demonstrates that only one fungal species was identified unique to rhododendron, and the native plants shared more fungal species compared to numbers shared with *Rhododendron spp*. This may indicate that fungi are a limiting factor for rhododendron seedling establishment, thus regulating the invasiveness of these varieties. However relatively few fungal species were identified across the culturing data, hence the emerging data from the currently running high-throughput sequencing have the potential power to elucidate these patterns further.

Monitoring terrestrial biodiversity using airborne eDNA

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DNA metabarcoding is a powerful tool for assessing biodiversity. For this, many sample types have been employed of which one has recently gained considerable interest: air. Studies have shown that bulk samples of vira, bacteria, fungi spores and plant pollen can be collected from air and their taxonomic origins determined through metabarcoding. Further, studies have shown that environmental DNA, eDNA, filtered from air can be used to detect insects, plants and vertebrates. Together, these studies highlight metabarcoding of airborne particles as a promising new approach for DNA-based biomonitoring. My group's contribution to this work focuses on terrestrial vertebrates. Terrestrial vertebrates are experiencing declines due to human activities and environmental change. To inform conservation efforts, survey data is needed. However, monitoring can be costly and laborious, and although metabarcoding is a strong tool to assess biodiversity, few sample types effectively capture terrestrial vertebrate diversity. We recently demonstrated eDNA captured from air to be the solution. In Copenhagen Zoo, we collected airborne eDNA in a stable, the rainforest house and outdoors. Through metabarcoding, we detected 49 vertebrate species spanning mammal, bird, amphibian, reptile and fish. These included animals from the zoo, animals used to feed zoo animals and local animals. While this demonstrated airborne eDNA as an untapped source of terrestrial vertebrate biodiversity data there is an obvious need to demonstrate the applicability outside urban zoo environments. My research team is now exploring the use of airborne eDNA for terrestrial vertebrate monitoring in nature. For example, the low quantity of airborne eDNA collected in nature increases the demands for sample collection and laboratory workflows to optimize detections while minimizing false positives. Pending these and other developments and explorations, airborne DNA has the potential to revolutionize the way we survey and characterize terrestrial ecosystems.

Earth BioGenome Project (EBP) - Sequencing Life for the Future of Life

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Understanding and preserving biodiversity is imperative for human survival and prosperity. Each species has a unique "blueprint" encoded by its genome, which contains all the information that the species needs to survive, interact with its environment and reproduce. Sequencing the entire genome of all species on Earth will provide fundamental new insights into biology, biodiversity, conservation and fuel future biotechnology to provide humanity with food, medical treatment, drugs, vaccines, biofuels and biomaterials. The Earth BioGenome Project is a global non-profit initiative that aims to sequence and catalogue the genomes of all of Earth's 1.5 million currently described eukaryotic species over a period of ten years. This catalogue will greatly benefit current and future eDNA monitoring and research projects, which are dependent on good reference databases. These genomes will resolve phylogenetic relationships and result in deeper insights into ecological and evolutionary dynamics in an ecosystem. Moreover, complete genome references allow us to monitor any gene or genomic region of interest in addition to traditional barcodes. EBP-Nor is the Norwegian initiative of EBP, and a partnership of the major universities in Norway (UiO (lead), NMBU, UiB, NTNU, Uni Nord and UiT), the research institute SINTEF, and several non-academic institutions. EBP-Nor is currently underway sequencing species native to Norway, in coordination and collaboration with other national and international efforts. The genomic resources generated by EBP-Nor will contribute to unveil the “dark matter” of biology held in the genomes of millions of known and unknown species worldwide. These genomes could hold the key to unlocking the potential for sustaining planetary ecosystems on which we all depend.

Does it look like a duck? Quack like a duck? Capturing uncertainty in metabarcoding taxonomic assignments

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Biodiversity monitoring using eDNA metabarcoding methods is heavily reliant on accurate identification of species from sequence fragments of limited length. We present a series of bioinformatic controls that provide insight into uncertainty in metabarcoding taxonomic assignments related to non-target amplifications, marker insufficiencies, and database incompleteness. Recommendations include:

1) The use of probabilistic taxonomic assignment tools which allow setting a threshold for correct assignment at a given taxonomic level

2) Controls against a broad database for removal of non-target sequences

3) Implementing internal database controls using BLAST to identify potential marker insufficiencies

4) Implementing external database controls against GenBank to identify potential database contamination and insufficiencies

5) Evaluation of per-taxon coverage of database for the geographic area studied

6) Comparison of taxonomic assignments against local species checklists to identify cases requiring manual verification or further investigation

These bioinformatic controls are further used as criteria for categorizing taxonomic assignments as having high, medium, or low confidence, which can guide and inform further use of metabarcoding occurrence data.

Distribution patterns of eDNA from four salmonid species, including the invasive pink salmon (*Oncorhynchus gorbuscha*), in a river in Northern Norway

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The invasive species pink salmon is increasing in rivers in Northern Norway. It spawns earlier than other salmonids, such as Atlantic salmon (*Salmo salar*), and may displace native fish. Grense Jakobselv, known for recreational fishing, is a 49 km long river, running from lake Vuorjánláđvi to the Varanger Fjord. Anadromous salmonids can travel unhindered to the Jakobselv lake, 39 km inland. In recent odd-numbered years, the migration of pink salmon upstream in the river has been extensive but largely unexplored. To assess how far the pink salmon travels along Grense Jakobselv, and to compare the distribution patterns of eDNA from pink salmon to other salmonid species in the river (Atlantic salmon, sea trout (*Salmo trutta*) and Arctic charr (*Salvelinus alpinus*)), we sampled eDNA at six stations, 10-35 km from the fjord, and quantified eDNA concentrations using qPCR. Sampling was done in mid-August 2021, around the time of pink salmon spawning, and before the mass death which occurs after spawning in this species. The results show differing eDNA distribution patterns of salmonids in Grense Jakobselv. eDNA concentrations of pink salmon, Atlantic salmon and sea trout decreased along the river, on average -30%, -22% and -11% per km, respectively. Pink salmon had the highest eDNA concentrations, except at the station farthest inland. eDNA from all species was detected at all stations, indicating that they travel at least 35 km upstream. During sampling, pink salmon was observed at highest densities closer to the sea, decreasing along the river, and not observed at the last station. By contrast, charr, which has both anadromous and stationary populations in the river, had similar eDNA concentrations across all stations, although lower than the other species. More knowledge on species-specific eDNA shedding, and degradation and dispersion rates is however needed before fish distributions can be inferred from eDNA.

UiO Research and Training program: Coastal Biodiversity Dynamics under anthropogenic pressures

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The main efforts to preserve marine biodiversity and ecosystem services involve legislative approaches to regulate individual and corporate human behavior, setting aside protected areas, and habitat restoration. Establishment of marine preserves and habitat restoration are the key tools in conservation efforts. However, flexible methodological frameworks are needed to utilize and further improve these developments. Here, we propose novel data collection approaches for big marine biodiversity data based on citizen science and the use of novel molecular and modelling tools including machine learning for improved understanding and management of land-ocean interactions. This includes how marine biodiversity and food webs are influenced by the ocean circulation and run-off from land, as well as how biodiversity and ecosystems services are impacted by increased human activities and other environmental perturbations. I will present ideas and first results of our ambitious project including gut analysis of a top predator to reconstruct marine food webs, monitoring of fish biodiversity using citizen science and results from machine learning to model biodiversity.

Pipeline for metagenomic database construction from plant genome skims

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Plant genomes can be large, ranging up to 19 800 Mb (*Picea abies*), but shallow pass shotgun sequencing, or genome skimming, has proven to be a cost-effective method for generating a large amount of genomic information from these organisms. High copy number regions such as nuclear ribosomal and plastid DNA can be assembled from these data while other low sequencing depth regions of the genome can also provide useful information in their unassembled form. Including these low sequencing depth regions in classification databases results in increased sensitivity and detection of taxa when analyzing shotgun sequenced eDNA samples. Memory-efficient data structures like the Ferrangina-Manzini index implemented by Centrifuge (https://ccb.jhu.edu/software/centrifuge/) allows for the creation of a database that is orders of magnitude smaller than the combined raw genome skims. After comparing pros and cons of several of such programs, we provide here, a proof of concept pipeline for the decontamination of full genome skims, their assembly-free incorporation into a metagenomic database, and their application as a taxonomic classifier of shotgun sequenced data. We use the ~2,000 genome skims generated by the PhyloNorway project to demonstrate the scale and usability of this workflow. This pipeline can facilitate the creation of similar databases from other genome skim collections as well as for the maintenance of existing databases as additional species/individuals are sequenced.

Developing methods for routine monitoring of marine zooplankton communities using metabarcoding

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Metabarcoding is a rapidly developing tool in marine zooplankton ecology, although most zooplankton surveys continue to rely on visual identification for monitoring purposes. We attempted to resolve some of the biases associated with both metabarcoding and traditional zooplankton sorting by sequencing a 313 b.p. fragment of the COI gene in 34 "mock" samples from the North Sea which were pre-sorted to species level, with biomass and abundance estimates obtained for each taxonomic group. The samples were preserved either in 97% ethanol or dried for 24 hours in a drying oven at 65C (the routine way of preserving samples for dry weight measurements). The visual identification yielded a total of 59 unique holoplanktonic and 16 meroplanktonic species/taxa. Metabarcoding identified 76 holoplanktonic and 107 meroplanktonic species/taxa, which included all but 2 of the species identified visually as well as numerous species of hard-to-identify crustaceans, hydrozoan jellyfish and larvae of benthic animals. We demonstrate robust correlations of relative sequence abundances to relative biomass for most taxonomic groups, highlighting the quantitative potential of metabarcoding, and suggest conversion factors for different taxa to account for sequencing biases. We also suggest a list of semi-quantitative parameters that can be calculated from a combination of metabarcoding and bulk biomass data and used as "indicators" of marine pelagic ecosystems for routine monitoring purposes. We also discuss the limitations of metabarcoding, and emphasize the continued need for an integrated methodological approach when assessing marine zooplankton communities.

Challenges & unexpected opportunities from constructing a lichen DNA barcode database for Norway

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Lichens, as symbiotic organisms, contain at least one fungal species and one photosynthetic species. In addition to these primary partners, additional fungi, algae, and bacteria are often found within the lichen thallus. This mix of species within lichens creates challenges and opportunities for DNA barcoding. As part of the Norwegian Barcoding of Life, the Oslo Lichen Herbarium has sampled and sent over 8000 lichen specimens for dna extraction and sequencing at the Canadian Centre for DNA Barcoding. The resulting nrITS dna barcodes are published to the Barcode of Life Data System (BOLD). In this process, more than 5800 sequences have been generated, with more than 1324 publically available for lichenized fungi on GenBank to date. These sequences have aided taxonomic investigations, including combinations of species and the discovery of new lineages. We have also found interesting sequence length variation in the nrSSU section of the barcode, caused by type 1 introns. In addition to lichenized fungi, we have recovered sequences from potential algal symbionts and fungal endophytes. These unintended sequences spur additional questions and projects about species found in lichens. We will discuss the results and implications of this ongoing construction of a lichen reference database through the lens of eDNA.

What drives the changes of biotic communities after the global retreat of glaciers?

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Multiple factors can determine biotic colonization of terrains exposed after the retreat of glaciers, including time after glacier retreat, macro-climatic differences across areas of the world, and the rapidly evolving abiotic and abiotic features of these environments. Until now, the complete reconstruction of soil communities was hampered by the complexity of identification of organisms, thus analyses at broad geographical and taxonomic scale have been so far impossible. We used the metabarcoding of environmental DNA extracted from soil to reconstruct the evolution of communities in chronosequences from 48 glacier forelands from four continents; we targeted all the major taxonomic groups (bacteria, fungi, plants, protists and soil animals). Soil animals colonize ice-free areas almost immediately. While both taxonomic and functional diversities quickly increase over time, this is modulated by climate so that colonization starts earlier in forelands with less cold summer temperatures. Colder forelands initially host poor communities, but the colonization rate then accelerates, eventually leveling biodiversity differences between climatic regimes after 150 years. Nevertheless, the rate of colonization is strongly different across taxonomic groups. Micro-organisms already attain high richness immediately after glacier retreat, but then community richness increases at a slow rate. Conversely, for animals and plants the rate of increase accelerates through time. This occurs because of the interplay between time, soil features and biotic components of communities. Environmental DNA allows an all-inclusive study of community ecology, which reveals how complex biotic interactions arise through time, and will help to predict the impacts of climate change on whole ecosystems.

Examples of eDNA-based monitoring in Norwegian management

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eDNA-based methods are increasingly being suggested as both more sensitive and more cost-effective alternatives to many conventional biodiversity monitoring methods. However, implementing the new methods in practical management have proven difficult and such suggestions are often not being included in ongoing monitoring programs. We argue that the expectations of eDNA-based methods often are unrealistically high and when presenting caveats and reservations, the substantial improvement is consequently ignored. Here, we showcase some examples of suggestions that have been implemented in practical management in Norway, including both aquatic and terrestrial ecosystems. We discuss how eDNA-based methods can improve monitoring programs of rare elusive species and threatened ecosystems, even with several caveats and reservations.

Who wins the battle? Interspecific combative interactions between wood-decay fungi

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Dead wood is a dynamic and complex ecological niche in which saprotrophic fungi play a central role in nutrient decomposition and recycling. The interactions and competitive success of saprotrophic fungi, as well as environmental factors can influence the rate of wood decomposition and modulate the resulting carbon release, which constitute the Earth's largest pool of aboveground terrestrial carbon. It is well established that some saprotrophic fungi colonise wood earlier than others, but there is large stochastic variation in the timing of species colonisation and the interactions between species. From a metabarcoding study of the internal transcribed spacer (ITS2) marker in dead spruce logs, we selected 40 saprotrophic fungi based on their order they fruit and their co-occurrences. From axenic cultures, we assessed the growth rate and measured interspecies interactions. We amplified and Sanger sequenced the full ITS to confirm species identification. Growth rate was evaluated by growing three replicates of each of the 40 species on MEA medium for two weeks. Based on gross mycelial contact on malt extract media using dual species interactions, we evaluated the combative abilities of species. The combative capabilities were scored as competitive exclusion (one fungus displaces the other), or deadlock (no displacement), allowing species ranking based on the competitive hierarchy. The interaction responses of fungi were subjected to ANOVA analyses. We correlated competition ability to growth rate, which is highest under more favourable growth conditions, where competition is stronger. Understanding the functional traits underlying the dynamics of wood decomposing fungi is a first step towards untangling the decomposition processes.

Ecology, chemistry and eDNA analyses– effective monitoring tools for status in terrestrial and aquatic ecosystems. Case studies from Sweden

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As eDNA is maturing as a method the use of the tool becomes more reliable and powerful in combination with ecological knowledge and physical and chemical background data. Here we present two aquatic surveys in Sweden using combined methods where species composition in a river system and in a lake system was mapped out with eDNA and in combination with environmental background data. A similar concept was used for fungi, bacteria and metazoan analyses in different forest habitats and clear differences were shown between protected and commercial forests. We discuss new discoveries, strengths and pitfalls with the method The results from the surveys that were used as information for decision making for restoration and protection actions will be presented.

Metagenomics vs. total RNA sequencing: most accurate data-processing tools, microbial identification accuracy, and implications for freshwater assessments

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Metagenomics and total RNA sequencing (total RNA-Seq) have the potential to improve the taxonomic identification of diverse microbial communities, which could allow for the incorporation of microbes into routine freshwater assessments. However, these targeted-PCR-free techniques require more testing and optimization. In this study, we processed metagenomics and total RNA-Seq data from a commercially available microbial mock community using 768 data-processing workflows, identified the most accurate data-processing tools, and compared their microbial identification accuracy at equal and increasing sequencing depths. The accuracy of data-processing tools substantially varied among replicates. Total RNA-Seq was more accurate than metagenomics at equal sequencing depths and even at sequencing depths almost one order of magnitude lower than that of metagenomics. We show that while data-processing tools require further exploration, total RNA-Seq might be a favored alternative to metagenomics for targeted PCR-free taxonomic identifications of microbial communities and might enable a substantial reduction in sequencing costs while maintaining accuracy. This could particularly be an advantage for routine freshwater assessments, which require cost-effective yet accurate methods, and might allow for the incorporation of microbes into freshwater assessments. Further research on environmental samples is required to confirm the advantages of total RNA-Seq over metagenomics in applied settings.

Assessing the performance of benthic metabarcoding for environmental monitoring and baseline biodiversity of the Norwegian Shelf

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Metabarcoding is a powerful tool to characterize benthic biodiversity and understand species distribution. Yet, there are many ways to implement this method, muddying the waters for analysis between studies. Furthermore, a lack of frame of reference from a large body of existing metabarcoding data limits interpretation when compared to morphological taxonomy community data, which restricts its ability to draw ecological conclusions. On the Norwegian continental shelf and slope, the MetaMon and MetaBridge projects conduct metabarcoding sampling alongside regular monitoring based on morphological macrofaunal identification and chemical impact parameters, providing a unique opportunity to compare metabarcoding biodiversity data on a large scale against a complementary dataset of sediment macrofauna both as a general baseline biodiversity tool, and as a parameter of anthropogenic impact. Here, we present some major findings connected to this work, comprising COI macrofauna and 18S eukaryote metabarcoding data from sediment environmental DNA and faunal bulk samples, and morphological taxonomy. We show that metabarcoding can be applied in several ways depending on specific research goals, governing choice of target organism groups, sample type, sampling intensity and degree of integration with morphological taxonomy. Further, we emphasise how metabarcoding compliments traditional taxonomy in biodiversity biomonitoring: Studies combining taxonomic competence and complimentary metabarcoding data represent an especially attractive strategy for poorly known areas such as the deep sea, where they can enable simultaneous gains in both biodiversity and connectivity for baseline research and impact-related monitoring and assessment of extinction risk.

BIOSCAN Europe

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Research programs led by the International Barcode of Life (IBOL) consortium have transformed the use of DNA sequencing for specimen identification and species discovery. The BIOSCAN Europe initiative has been recently launched as part of IBOL, to bring together and support existing European networks, scientists, and projects that work on characterisation and monitoring of biodiversity using DNA. It aims to build an efficient European system of interconnected facilities for rapid DNA identification and monitoring of species. This presentation introduces the BIOSCAN Europe initiative, and its developing programme of work, with a focus on activities being delivered as part of the Horizon Europe funded Biodiversity Genomics Europe consortium.

Optimalization of the ponds eDNA procedures for research prokaryote and eukaryote biodiversity

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Ponds are anthropopressure-sensitive freshwaters serving as biodiversity hotspots, especially for urban areas[1,2]. One of the non-invasive ways to study the pond's biodiversity is eDNA metabarcoding. It especially works for rare endemic species, invasive species early in their settlement, and short lived species. The aim of this study was to optimize the method of water filtering and filter preservation, which will allow for the most in-depth analysis of the biodiversity of the two (urban and rural) ponds located in Poland. The water was sampled all around the ponds from 20 cm and 80 cm depth, then it was filtered through filters with a pore-size 2.0, then 0.45 and finally through 0.22 µm. Filters were preserved in three different preservatives: 96% ethanol, ATL, and RNA-later. We compared the biodiversity data across depths, filter porosities and conservatives. At the same time, macrozoobenthos samples were taken NGS libraries were prepared using PCR-based amplification of the different minibarcodes for bacteria (16S rRNA), fungi (LSU), vertebrates (16S rRNA), invertebrates (COI) and all eukaryotes (18S rRNA). Results on genetic and species diversity across depths and filter porosities differ at the alpha-diversity level depending on the analyzed taxonomic group. The greatest biodiversity was detected on 2.0 µm filters, but on each filter pore size we detected some unique taxa, undetected on the other filters. Therefore, while aiming at total biodiversity study, all three pore size filters should be used (specifically for bacteria). However, for the studies focusing on the vertebrates, invertebrates and fungi 2.0 µm filter seems to catch the significant majority of studied taxa. We did not note any differences in the efficiency of conservation of genetic biodiversity between preservatives. Not all invertebrates identified in macrozoobenthos samples were detected with NGS and *vice versa*, so it is recommended to add a metabarcoding of bulk samples as already suggested.

Genome-wide analysis of eDNA

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Within the last decade, sequencing costs, high-performance computer clusters and genome assembly databases have improved by orders of magnitude. It is now financially feasible to sequence nearly all of the DNA within an environmental sample and together with the rapidly expanding genome databases (e.g. the European genome atlas, with a prominent role for Sweden, aims to assemble genomes for 200.000 higher organisms within the next decade), leverage the information contained within eDNA. To do so, improved algorithms and pipelines, capable of performing genome-wide analysis from metagenomic samples are needed. The aim of my Ph.D. research is to bridge the current gap between the ever-increasing amount of eDNA data and the lack of tools to analyze these to their full extent. I will briefly present a summary of my ongoing bioinformatic developments that allow for genome-wide inferences from ancient sediment and modern eDNA samples.

Barcoding of freshwater taxa for improved assessment of biodiversity (FRESHBAR)

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FRESHBAR (2019-2022) is a Swedish project aiming at contributing to the further development of libraries of key freshwater organism groups with a focus on freshwater benthic diatoms and invertebrates. FRESHBAR is funded by and performed in close cooperation to the Swedish Agency for Marine and Water Management, with the aim to integrate and establish DNA-barcoding techniques with ongoing national monitoring of lakes and streams in Sweden. Here, we will present the an overview on the first results, including the new generated barcodes and the related taxonomical identification work for more than 300 DNA barcodes of Swedish freshwater diatoms to be added to the diatom reference library Diat.barcode, and to be vouchered in the collection of the BGBM. We will especially focus on our attempts for taxonomical quality control, and discuss its challenges. We will also present the first overview on the results on the invertebrate work.

Long-term management history affects seasonal diet composition of semi-domesticated reindeer

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Semi-domesticated reindeer exert a major top-down control on plant biomass and biodiversity dynamics within the Fennoscandian tundra ecosystem. Reindeer herding practices are complex and have been implemented for centuries by indigenous Sámi people. The interplay between historically established seasonal migration patterns and the management of reindeer stocking densities has resulted in considerable differences in pasture productivity, likely altering the nutritional base for reindeer. But surprisingly, how pasture productivity levels impact reindeer feeding preferences has never been investigated before. Comparing two herding districts with contrasting reindeer densities, we assessed the effect of pasture productivity on the composition and diversity of the reindeer annual diet in Eastern Finnmark. Using a DNA metabarcoding approach, we quantified variations in the relative proportion of plant, lichen and mushroom taxa in the reindeer diet, during their annual migration between winter and summer pastures. We found that seasonality explained the largest part of the variation in diet composition. Contrary to our expectations, diet diversity did not differ between districts, except during winter where reindeer from the high-density district displayed higher diversity in their plant diet. But the intake of lichen did differ, with reindeer from high density areas incorporating a significantly smaller proportion of lichen in their winter diet. However, we also show that contrary to the common understanding, lichen represents a prevalent food resource in all seasons, and its proportion in the reindeer diet remains high regardless of potential differences in availability. Our results suggest that the impact of stocking densities on reindeer feeding preferences is complex and strongly dependent on the season. In higher densities areas reindeer do have a lesser intake of lichen during winter but also a more diversified plant diet. This adds to the evidence that persisting herbivory might induce a vegetation shift towards more diversified and productive tundra plant communities.

The effect of *Silene acaulis* on soil bacterial communities across elevational and latitudinal gradients in Scandinavia

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Microbes are ubiquitous, extremely diverse, and contribute to important ecosystem functions. Soil bacterial communities, in particular, play an important role in carbon and nutrient cycling, through decomposition and carbon sequestration. In addition, soil bacteria establish mutualistic relationships with plants, animals and other microorganism. However, soil microbial communities may change in diversity and composition in response to changes in climate, which may disrupt the interactions between soil microbes and other organisms, such as plants. The impact of climate change in arctic and alpine environments is expected to be disproportionately large, thus it is important to understand how soil microbial communities in these environments respond to changes in temperature, precipitation, and plant communities. The alpine cushion plant *Silene acaulis,* is a dominant, foundational species known for its facilitative effects in stressful environments. There have been several studies investigating the facilitative effects of cushion plants on other plants. However, less is known about the relationship between cushion plants and their soil microbiome. Here, we investigated the effect of *S. acaulis* on its soil bacterial community. We also investigated the effect of latitude and elevation on the soil bacterial communities in the soil under *S. acaulis,* as proxies for climate change. We found that *S. acaulis* had a significant effect on the soil bacterial community composition. There was a significant effect from latitude on the bacterial composition. However, it appears that site specific factors, such as pH, are more influential along the latitudinal gradient. *S. acaulis* may have a converging effect on the soil bacterial community composition along environmental gradients, and as such, the direct effects of climate change may be weak. Further research is needed to better understand the relative importance of the different factors driving soil bacterial communities in alpine environments.

Hidden diversity and host specificity of *Pertusaria*-residing *Tremella* fungi in Norway

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Hidden from plain view, the diversity of lichen-inhabiting *Tremella* fungi may be underestimated. *Tremella* occasionally form fruiting bodies on lichens and the more common yeast stage is asymptomatic on lichen thalli. Lichenicolous *Tremella* in the filamentous stage (symptomatic; forming fruiting bodies) is known to be host specific, while the host specificity and ecology of the yeast stage (asymptomatic) is poorly studied. Prior to this study, only one species of *Tremella* was known to reside symptomatically in the Pertusariaceae, *namely T. pertusariae.* However, while DNA barcoding species of the Pertusariaceae, contaminant ITS sequences of *Tremella* were obtained from asymptomatic specimens. Through this project, I have (1) studied the diversity of asymptomatic *Pertusaria*-residing *Tremella* fungi in Norway and (2) investigated their host specificity to *Pertusaria*. I used high-throughput amplicon sequencing targeting the ITS2, using both general fungal- and *Tremella* specific primers in two different PCR setups, on samples collected from various locations in Norway. I assessed the diversity with phylogenetic analyses, and I visualized host specificity using circos plots. Most of the tremellalean species diversity obtained from the various *Pertusaria* hosts was found using the general fungal primers rather than the *Tremella*-specific primers. My phylogenetic results show that several tremellalean amplicon sequence variants (ASVs) group with a variety of tremellalean species, also outside of the genus *Tremella*, which is observed for the first time in *Pertusaria*. Most of the obtained ASVs do, however, group with the *T. pertusariae* reference. These ASVs appear as several rather distinct genetic lineages, suggesting *T. pertusariae* represents a species complex. My study clearly shows that the tremellalean yeast stage is more common than its filamentous stage in species of Pertusaria. The tremellalean yeast stage also does not show any signs of host specificity, as opposed to various filamentous *Tremella* species.

Integrating Molecular Biodiversity – ELIXIR3 Norway

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The term biodiversity is according to the World Health Organization (WHO) all life on earth in its genetic and cultural diversity. Many ecosystems in countries around the globe are losing biodiversity and its ecosystem service at an alarming rate due to human activities. In an effort to stagger this momentum several worldwide initiatives have engaged in the conservation effort. In this respect, the Global Biodiversity Information Facility (GBIF), Earth BioGenome Project (EBP) and International Barcode of Life are committed in the endeavor to connect, preserve and cultivate sequenced genetic knowledge of existing species. Molecular data generated from sequencing projects has the benefit of providing additional layers of information and contributing with elevated capacity towards monitoring biodiversity and correcting taxonomy. However, efficient progress on a large scale, as expected through the mentioned projects, require collaboration and alignment towards common bioinformatic strategies. As such, data management and standardization of metadata is fundamental in connecting resources and research infrastructures so data of great value can be created. In this landscape, ELIXIR is a European coordinator of life sciences that caters expertise in interoperability, cloud data storage, databases, compute, tools and training – all of which serve to connect infrastructures. The ELIXIR focus-group on biodiversity, initiated in 2019, is on the advance to get status as an ELIXIR Community – emphasizing the desire to drive the development of biodiversity services across ELIXIR resource platforms. Focus on biodiversity is also at the national level with ELIXIR3 Norway organizing a dedicated work package (WP6). The work package aims to coordinate networking between national research infrastructures and provide services in data management and data-mobilization. The Norwegian EBP-branch, EBP-Nor, is an example where the national ELIXIR node has an active service contribution towards data mobilization.

Multiplexing markers: Increasing the amount of data that can be obtained from a single amplification reaction

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Metabarcoding has proven to be a powerful tool for the recovery of ecological data from a variety of environmental samples. Over the years, a multitude of different metabarcoding primer pairs have been developed that are either universal in nature and can amplify a wide range of organisms, or that are aimed at more specific taxonomic groups. These pairs, however, may have certain limitations. Universal primers often lack species level identifications for a large number of taxa, while specific primers on the other hand can provide improved identifications, but for narrow taxonomic ranges. Any application that requires high resolution taxonomic data for a large group of organisms, such as ecosystem reconstructions, thus has to rely on multiple metabarcoding analyses using different specific primers, which increases both time and costs and can cause complications when DNA extracts are limited. In a multiplex PCR reaction multiple primers can be combined into a single amplification reaction, where each primer included can target a different loci or taxonomic group. This greatly increases the amount of data that can be generated from a single amplification and with the right markers yield improved taxonomic identifications compared to a single metabarcode marker. Here we present our experiences with the multiplex PCR method for improved identifications of a single taxon from fragmented sedimentary ancient DNA, including the process of marker development and subsequent testing and improving our methodology.

Tracking of threatened aquatic invertebrates using eDNA – a Swedish showcase

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Aquatic environments harbour around 370,000 species worldwide and the number of undescribed invertebrate species is still unknown. Many aquatic species are threatened by the consequences of a warmer climate, invasive species and pollution. Even if several directives exist in Europe to protect those habitats (e.g., Habitats Directive, Marine Framework Directive), the conservation status for many aquatic invertebrate species remains unknown and their red list status is *Data Deficient* (DD). Our Swedish FORMAS project focuses on marine crustaceans and freshwater mollusks and aims to track threatened species using different approaches: classical sampling, DNA barcoding and metabarcoding of sediment and plankton samples. The project was initiated with a gap analysis of red listed aquatic species of marine crustaceans and freshwater mollusks in Northern Europe to obtain an overview of existing occurrence records and barcode data. The analysis for 12 Northern-Atlantic European countries showed large data gaps for these aquatic invertebrates. We will also present preliminary data tracking i) freshwater pea clams (*Pisidium* spp.) from 15 Swedish lakes and rivers and ii) marine crustaceans from 14 sampling locations on the Swedish west coast, in the Gullmar fjord and Kosterhavet National Park, using classical morphological and molecular methods. Sorted material was identified using morphological characters and COI barcoding. Sediment and plankton samples from the same locations were analyzed with metabarcoding using group specific and general metazoan primers. In this talk, we will present our results from morphological identification and barcoding as well as preliminary data from molecular sediment analyses from 30 Swedish aquatic habitats. We will discuss the possibilities for using molecular methods in the tracking of threatened invertebrates, investigate the pros and cons of each method and how these might be best used in future to speed up the assessment of conservation status for aquatic organisms.

Environmental DNA-based tracking of species community in Norwegian freshwater systems

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Ecosystems worldwide are changing at unprecedented rates, driven primarily by increasing anthropogenic activities such as habitat degradation and climate change. Freshwater habitats are now among the most endangered habitats as these systems are exposed to the negative impact caused by species introduction/biological invasions and their associated pathogens, leading to biodiversity loss. In Norway and various other European countries, the recent discovery of newly invasive amphipods (sometimes vectors of parasites affecting native species) has highlighted their rapid and silent spread. Here, we conducted a baseline survey in Southern Norway using environmental DNA to assess the health and status of Norwegian aquatic systems and to investigate the potential presence of any newly invasive species. A total of 30 sites were investigated, including Enningdalselva, Haldenvassdraget, Glomma, Drammenselva and Numedalslågen rivers. We now present an updated list of species occurring in freshwater systems in southern Norway and discuss the implications of the presence of these species.

Biodiversity assessment of benthic fauna in an Arctic glacier-influenced fjord based on morphological and eDNA metabarcoding survey

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The ecosystems of the Arctic fjords characterize high dynamism, where organisms are exposed to various natural and human-induced stressors that shape their taxonomic structure and functioning. The activity of glaciers, meltwater discharge, strong sedimentation along with advection of sea water masses from shelves form a variety of environmental gradients that influence marine biota. In this study we compare the effects of glacially mediated disturbance on biodiversity of benthic fauna assessed using morphological and eDNA metabarcoding based methods. Materials were collected at 6 stations along the environmental gradient of decreasing impact of a glacier in Hornsund (Svalbard fjord) and included samples for macrofauna and samples for meiofauna (nematodes) and sediment eDNA. Three genetic markers were used for the PCR amplification of eDNA targeting: metazoans - mitochondrial COI, meiofauna - nuclear 18S VIV2 and Foraminifera – nuclear 18S 37f, and were sequenced using the Illumina MiSeq platform. Bioinformatic analyses of the sequence data were performed using the SLIM pipeline and ASVs were determined with dada2. The data assessed for each group of organisms and each eDNA marker showed a decrease in taxonomical richness along the fjord towards the glacier. The pattern of this decrease was particularly similar between the morphologically analysed macrofauna and eDNA assessed with COI marker (sequences assigned to Metazoa). The analysis of beta diversity showed similar nMDS ordination patterns among samples in the case of morphological macrofauna data and eDNA data obtained with the use of the V1V2 (only sequences assigned to Metazoa and regarded as benthic taxa) and the 37f markers, contrary to the data derived with the COI marker, where no pattern could be observed. Our results show that eDNA metabarcoding-based methods may be successfully used for impact assessments of Arctic benthic fauna and may provide results comparable to traditional taxonomical surveys of benthic fauna.

Any gaps to fill? The presence of the flora and fauna of Northern Norway in the database

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The Norwegian barcode of life (NorBOL) has successfully barcoded more than 22 000 species from all parts of mainland Norway, Jan Mayen and the Arctic Archipelago of Svalbard. Most species reach their northern distribution limit somewhere along the south-north gradient, which results in a lower diversity in the north. However, there are also arctic species found in Svalbard and/or northern Norway, which are absent in the southern part of the country. When working with eDNA it is important to know if the species present in the ecosystem also is present in the reference library, and studies have pointed out that regional representation is important for correct identification. Here we review the DNA barcode coverage of northern ecosystems and point to which gaps should be filled for optimal use in biodiversity assessments and monitoring.

Refining enumeration of planktonic sea lice larvae detected by ddPCR; calibration by serial spiking

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Lepeophtheirus salmonis and *Caligus elongatus* cause significant economic and environmental damage to both the Norwegian salmonid aquaculture industry and the country's wild salmonid populations. Understanding the dispersal and infectivity of their larvae remains a challenge to researchers and regulators due to a limited availability of louse monitoring methods suitable for broad-scale use. Rapid accurate quantification of lice larvae from within plankton samples remains the greatest bottleneck to generating high resolution coastwide louse density and dispersal data from their earliest life stages, which are currently only produced by models. However, in recent years molecular methods have shown considerable promise due to their heightened sensitivity, increased accuracy, and decreasing costs. To further refine digital droplet PCR for use as an absolute quantification method, we spiked samples at five densities (1-30 individuals) for two planktonic stages (Nauplii II, Copepodid) of both louse species. Results generated by two species-specific amplification runs of COI fragments extracted from the 60 samples showed a clear linear relationship between the quantity of positive droplets detected and the number of spiked louse individuals for both life stages. A lower number of positive detections per louse was observed in the copepodid than the nauplii II stages of *C. elongatus* but no difference was detected between the stages of *L. salmonis.* Furthermore, by testing a 10x dilution of extracted DNA on secondary runs of all samples, it was confirmed that no inhibition occurred within the PCR reaction due to saturation in any of the groups. These results showcase another step towards establishing higher accuracy estimates of louse larval abundance using a calibrated and highly precise molecular quantification method.

Nordic Seas metabarcoding and eDNA taphonomy

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Recent development of DNA metabarcoding led to spectacular accumulation of metabarcoding data, especially for microbial and meiofaunal biomes. However, despite rapidly increasing number of DNA metabarcodes, their taxonomic assignment is still very limited. The large proportion of metabarcodes remains unassigned even at higher taxonomic level, impeding their ecological interpretation and sometimes making it difficult to distinguish between planktonic and benthic taxa. To overcome this issue, we propose to establish a reference database of barcodes obtained from morphospecies known to be present in the Nordic Seas and metabarcodes obtained in this and other eDNA studies from the same area. We will target selected taxonomic groups that are of particular interest to this study, e.g. foraminifera, diatoms, and copepods. Moreover, we will assign metabarcodes to plankton and benthic community, based on their occurrence and relative frequency in water column and sediment DNA datasets. We expect that this will help to better understand the taphonomic processes involved in a transfer of DNA from water column to the sediment, and to determine whether all planktonic taxa are equally recorded in sedimentary DNA.

Roadmap for implementing environmental DNA (eDNA) and other molecular monitoring methods in Finland – Vision and action plan for 2022–2025

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Technological development in molecular methodology has been extremely fast in the past two decades, and groundbreaking approaches have been introduced. This presents a huge opportunity to improve the coverage, accuracy and cost-efficiency of monitoring, enabling a more complete picture of biodiversity and the state of the environment. As the new European Biodiversity Strategy for 2030 and other international policies to halt biodiversity loss and the degradation of habitats are translated into concrete measures, the quality of the monitoring data will play a crucial role in determining their success or failure. We present a Roadmap commissioned by the Finnish Ministry of the Environment, in which we assess the state-of the-art in molecular monitoring methods in Finland within the international context, identify challenges and development areas that remain to be addressed and propose an action plan for promoting the coordinated implementation of molecular methods in national monitoring programs. Our analysis is based on recent scientific literature, survey results, direct enquiries and interviews. Participation of the national community of experts from different sectors was enabled and invited at several stages of the roadmap preparation. We estimate that extensive, routine implementation of a wide range of molecular monitoring methods is conceivable in Finland before 2030. As the primary development areas for reaching this goal, we identify (i) international coordination and standard development, (ii) networking across sectors, (iii) education, (iv) infrastructure, (v) reference sequence libraries and the mapping of whole genomes, and (vi) modelling and analysis tool development. For concrete actions in 2022–2025, we propose (1) a cross-governmental funding instrument, (2) a permanent working group responsible for national and international coordination, (3) a national network and (4) an online platform to enhance interaction and knowledge transfer, as well as (5) a national data management system with collectively agreed data and metadata formats and standards.

Fungus–arthropod networks in boreal forests: insights from metabarcoding

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Fruiting bodies (mushrooms) produced by forest fungi constitute an important resource for a multitude of organisms, including humans. Food webs consisting of mushrooms and their associated arthropods are species-rich and ecologically diverse, but also extremely difficult to study using traditional methods based on rearing and morphological identification. These practical difficulties have considerably hampered research on the ecology and evolution of fungus–arthropod networks. To overcome these problems, we developed methods for inferring mushroom-associated arthropod communities based on metabarcoding of DNA extracted from entire fungal fruiting bodies. By analyzing the metabarcoding-based community data using Hierarchical Modelling of Species Communities (HMSC) and other multivariate approaches, we tested how arthropod community composition within single mushrooms is determined by fungal identity and phylogeny, the state of decay of the fruiting body, and latitude. While we found fungus-associated arthropods to be relatively generalized in their use of available mushrooms, the multivariate analyses also revealed a clear imprint of fungal identity and phylogeny on the networks structures. Arthropod species richness was higher in older mushrooms, possibly as a result of accumulation of insect remains throughout the short life span of each individual fruiting body. Interestingly, arthropod species richness in forest mushrooms did not vary with latitude, *i.e.*, communities were as diverse in northernmost Finland as they were in southern Estonia. However, species composition changed across the analyzed latitudinal gradient, resulting in latitudinal turnover in community structures. Evidently, metabarcoding-based inference of mushroom-associated arthropod communities is a powerful approach for targeting general ecological and evolutionary questions concerning the factors that determine trophic associations and community structures in species-rich food webs.

eDNA in environmental monitoring and biodiversity assessment - remaining issues

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Environmental DNA is a powerful, non-invasive and cost-effective tool to monitor aquatic biodiversity. However, eDNA surveys sometimes produce false negative results (the species is present but not detected by eDNA) and false positives results (the species is absent but reported by eDNA). Detection of an endangered or highly invasive species may have high societal consequences, and current confidence level of eDNA results in many cases is not sufficient for management purposes. Our recent research project aimed to remedy some of these problems, focusing on the following questions: 1. How do eDNA results compare with traditional biodiversity surveys? 2. How long does eDNA stay in the environment after the source has disappeared? 3. Can eDNA detect all groups of aquatic organisms, or some may be missed? Within the project we have developed dPCR assays for several endangered (e.g. freshwater pearl mussel, thick-shelled river mussel) and invasive species (e.g. round goby, pumpkinseed fish). Overall eDNA methods for the target species had higher sensitivity than traditional surveys. Further, in eDNA decay rate in the fish tank experiments the signal disappeared after a few days, confirming other studies and suggesting that repeated sampling after two weeks is one way to rule out false positives. Estimating total biodiversity based on eDNA metabarcoding was however more complex, especially in the open marine systems. Sampling in the Danish stone reefs, Swedish shallow bays and public marine aquaria with open flow showed that some organisms are missed in eDNA surveys, while others become dominating, likely due to variation in eDNA release rate between organisms and primer selectivity. Further, eDNA can spread very long distances, resulting in false positives. The project resulted in several practical protocols for authorities and educational activities, aiming to increase standards, reliability and transparency of eDNA methods.

Environmental DNA as part of an observational toolkit for marine biodiversity monitoring in a global change context: The ABOVe-DNA project

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Maintaining biodiversity is critical to sustain healthy ecosystems, and the loss of biodiversity has ecological, economic, and societal consequences. Climate change and anthropogenic stressors such as pollution threaten the maintenance of biodiversity all over the globe. The fast-progressing effects of human disturbances necessitate the establishment of ecosystem monitoring programs to map the diversity and distribution of species. Comprehensive biodiversity assessments of vulnerable marine ecosystems are imperative for understanding the impact of anthropogenic stressors to inform policymakers on the developing state of ocean systems. Researchers at SINTEF Ocean AS have long experience in research related to environmental fate and effects of marine pollutants (and their degradation products) such as oil, chemicals, and plastics. The research also involves the pollutants effect on both eukaryotic organisms and procaryotic communities. As part of the national infrastructure Ocean Lab, SINTEF Ocean AS have access to two floating observational buoys, equipped with a broad array of sensors, performing continuous measurements of ocean environmental data. These buoys practically function as floating laboratories, and the infrastructure also guarantees easy access for sampling of eDNA. Combining the biological and chemical data gathered, modelling of marine conditions and the available research expertise and tools, will allow establishment of an observational framework for a comprehensive marine environmental monitoring. The ABOVe-DNA project aims to develop an operational toolkit to observe and describe changes in the marine organismal community structure, for both procaryotes and eukaryotes by using eDNA analyses. The outcomes will be used for assessing the impacts of various single or combined natural and anthropogenic stressors, including habitat artificialization (e.g., in- and off-shore artificial structures instalment), economic important species exploitation (e.g., fishing and aquaculture activities), pollution and changes in climatic conditions (e.g., temperature increase, sediment loads).

Quantification using DNA metabarcoding: the case of airborne pollen

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Airborne pollen monitoring is of global socio-economic importance as it provides information on presence and prevalence of allergenic pollen in ambient air. Traditionally, monitoring has been done using microscopic determination of pollen grains which is a time-consuming procedure that requires high expertise. Therefore, novel techniques are constantly being developed to automate this process. Among these, DNA metabarcoding has the highest potential of increasing taxonomic resolution, but uncertainty exists whether the results can be used to quantify pollen. In this study, DNA metabarcoding is performed on airborne pollen collected at two monitoring stations in the Netherlands over two consecutive years and using both a nuclear ribosomal marker (nrITS2) and a chloroplast plant marker (*trn*L). First, it is shown that the use of nrITS2 highly increases taxonomic resolution as compared to *trn*L, also revealing the significant contributions of invasive alien and cultivated species to an intensified and extended hay fever season. Second, this study shows highly positive correlations between DNA read abundances and relative abundances of manual pollen counts, although the slope of this correlation is shown to be species-dependent. These results will be placed in a wider context related to quantifying pollen using molecular methods.

Environmental DNA of aquatic macrophytes: potential for reconstructing past and present vegetation and environment

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Sedimentary DNA is increasingly used to reconstruct past biodiversity from various environments, including freshwater systems. We reviewed studies which used shotgun sequencing, targeted capture, and various metabarcoding markers, to explore the methods used to detect freshwater vegetation diversity and change. We used a case study to infer past freshwater habitats from sedimentary DNA records of macrophyte taxa, based on their abiotic niches. We found that, with sufficient DNA barcode libraries, using metabarcoding (n = 40) to target the P6 loop region of the chloroplast *trn*L marker is the best compromise between taxonomic resolution and diversity of past macrophyte communities. Fewer studies used shotgun sequencing (n = 7) or targeted capture (n = 6), and the method choice ultimately depends on the aimed taxonomic breadth. Across all markers, metabarcoding identifies down to species level on average 92.5 % of all aquatic taxa detected, and 85 % of all vascular taxa. From our case study, we reconstructed several environmental parameters such as annual thermal range, water pH, nutrient availability, or light conditions. We showed that sedaDNA data can be used to identify past environmental conditions over time, at both regional and local scales. Metabarcoding of sedimentary DNA is thus powerful to measure present freshwater macrophyte diversity, and to reconstruct past communities. When near-exhaustive, short barcode databases and traits databases are available, like for northern Fennoscandia, metabarcoding can support wider ecological reconstructions, not limited to aquatic plant taxonomic inventories but also past environmental changes ranging from water-quality to climate.

Ancient DNA of bryophytes: current status and future potential

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Bryophytes are important components of the ecosystems globally as they influence nutrient, carbon, and water cycling as well as acidification. Due to cool climate and short growing seasons, northern ecosystems are rich in bryophytes. The broad geographic ranges and narrow habitat tolerances of most of the bryophytes make them a suitable taxonomic group to study the impact of disturbance and climate change on the northern ecosystems resilience and change. However, palaeoecological research of bryophytes is largely limited due to scattered occurrences as macrofossils. To that end, sedimentary ancient DNA (*seda*DNA) may provide high resolution taxonomic data for bryophytes. Using *sed*aDNA data from ten Fennoscandian lakes covering the time from deglaciation until present, we explored temporal diversity of the bryophytes. Out of the 77 taxa detected, nearly 42% and 14% were identified to species and genus level respectively. The taxonomic richness of bryophytes continuously increased throughout the Holocene in most of the lakes except a short core showing negative trend for last ca. 2500 calibrated years before present (cal BP). Of the two lakes showing non-linear trend, richness decreased after ca. 7000 and 2500 cal BP. Two major groups of the bryophytes i.e. bryophyta and marchantiophyta reached the northern region around the same time after deglaciation. However, there were some differences in the dispersal ability between different orders of major taxonomic groups of bryophytes. We highlight that bryophytes have been, so far, reported as the by-catch of *seda*DNA projects targeting vascular plants and reference library of bryophytes is incomplete. Thus, there is a need to develop bryophyte specific markers and expand the reference library to fully harness the power of *seda*DNA in bryophyte research.

DNA as a tool for dealing with arthropod diversity

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Arthropods dominate the animal kingdom. They do so in terms of abundance, diversity, and they share innovativeness in terms of life cycles and styles. Each of these features make them a nightmare for anyone wanting to keep track of their state and of the general state of the environment. Now DNA-based tools offer great promise for dealing with arthropods – but also new questions on how best to approach them, and new challenges calling for innovative solutions. In this talk, I will consciously avoid addressing the many biases associated with DNA recovery, sequencing technologies, bioinformatics pipelines and biases in species detectability – hoping that other talks will address these fundamental challenges. Instead, I will focus on the ecological features of insect communities, and the specific features that make it hard – or less hard – to characterize them by DNA-based tools. These features relate to e.g. the extreme rarity of most insects, the wild turnover of species between samples, and the Herculean efforts needed to populate reference libraries for any hyperdiverse group of organisms. Each of them are interesting in themselves, and each urges solutions in downstream ecological analyses.

Applying long-read sequencing to environmental DNA to map Eukaryote diversity in soil

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Soil-dwelling microorganisms are an understudied collective of highly ecologically important communities, among them a host of microeukaryote taxa. In recent decades, the main approach to soil biodiversity has been metabarcoding from environmental DNA in soil samples, however taxonomic informativeness and diversity estimates from metabarcoding may be limited by region selection. To further advance the depth of knowledge on soil communities, long read sequencing has emerged as an approach to gain potentially highly informative diversity data to be utilised for both ecology and phylogeny purposes. As part of a mycology project investigating the fungal group Archaeorhizomycetes, soil samples were collected from all over Norway in 2020. The sample sites and their accompanying metadata represent geographical and ecological extremes of the country and as such, offer an opportunity to assess diversity of soil microorganisms and potential drivers of this diversity. DNA extracted from litter and soil in 90 of these locations (n=180) has been made available for this new project, with the primary aim of conducting explorative mapping of microeukaryote diversity using long-read sequencing (PacBio HiFi), including parts of the SSU, the whole of the ITS and parts of the LSU region. In addition, the resulting diversity data will be used to investigate trends in organismal and phylogenetic diversity patterns relative to recorded environmental variables and gradients and in this way assess which factors might drive microeukaryote diversity. Lastly, diversity estimates from the long-read sequence data will be compared to estimates from selected shorter regions (ex. SSU) within the dataset to assess limitations to their respective informativeness - and the effect of such limitations on subsequent analyses on diversity drivers and phylogeny. Findings may add to the body of knowledge on soil biodiversity and diversity drivers as well as inform future approaches to diversity assessment through sequencing.

The applicability of eDNA-based assessment of biodiversity in Austrian protected areas

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In the face of global change and biodiversity decline, a need for effective biodiversity monitoring methods is growing rapidly. Thus, novel methods for assessment of biodiversity across different ecosystems and especially in protected areas are gaining importance. In particular, the detection of species from environmental DNA (eDNA) is gaining traction in biodiversity assessments and conservation practices due to time- and cost-effectiveness, and user-friendly implementation. The eDNA-based biodiversity detection methods have especially large potential to be implemented regularly in biodiversity assessments and conservation actions of protected areas due to their non-invasive nature and capacity for comprehensive species detection and identification. Here, we present examples of biodiversity assessment in protected areas based on eDNA metabarcoding approaches conducted in the frame of the project Biodiversity Monitoring Technologies (BioMONITec). We tested practical aspects of eDNA metabarcoding for detection of fish, macroinvertebrates, and fungi in a floodplain forest and in an alpine bog situated in a biosphere park. The main aim of the analysis was to provide protocols that can be easily and efficiently used by protected area managers for biodiversity assessment. In particular, we tested the efficiency of different sampling approaches (syringe vs. pump water sampling), compared performance of three eDNA extraction methods, compared sample processing methods in the field and in the laboratory, and cost-, and outcome-effectiveness of tested approaches. We determined biodiversity indices of assessed habitats and compared them to samples acquired by traditional sampling approaches (e.g. bulk samples). The methodological and scientific outcomes of the project will be communicated to the Monitoring Global Guideline (MoniGloG) and communicated to the protected area managers and stakeholders who will apply these methods to their monitoring practices in biosphere reserves and national parks.

eDNA capacities for aquatic biodiversity assessment at the Swedish University of Agricultural Sciences (SLU)

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The Department of Aquatic Sciences and Assessment (Institutionen för vatten och miljö, IVM) at SLU has been a flag ship of aquatic monitoring in Sweden for more than 60 years. Biological assessment of Swedish inland waters to this day happens primarily by traditional methods. However, recent and planned infrastructural and personal expansions provide crucial capacities for the integration of eDNA approaches into the work of the department. From nucleic acid extraction to bioinformatics and statistical data analyses we have the potential to evaluate the biodiversity of microorganisms up to vertebrates and produce indispensably valuable data about the status of Swedish inland waters.

Utilization of eDNA methods in water status and biodiversity assessment (eDNA-monitor)

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Implementation of EU directives sets requirements for the description and monitoring of the abundance, composition, species, and functional diversity of the biological quality elements of water ecosystems. Traditional methods for monitoring and status assessment require good species identification skills, are time-consuming and expensive. DNA- and RNA-based technologies have potential in complementing, renewing, and enhancing monitoring, thus helping to reveal the true diversity of aquatic organisms in a cost-effective manner. In this project, the application possibilities of the DNA and RNA of environmental samples are investigated in the assessment of the status of water bodies and monitoring of biodiversity. For evaluation, 16S rRNA, 18S rRNA or CO1 (mitochondrial cytochrome c oxidace 1) genes will be isolated, amplified, and the obtained sequencing results are compared with reference sequence library databases. Currently, the use of molecular methods is limited by the fact that a large part of the reference sequences of microscopic aquatic organisms (phytoplankton, benthic diatoms, protozoa) are missing from the reference databases. On the other hand, benthic invertebrate sequences are better represented. In the project, deficiencies in the reference libraries will be mapped and missing reference sequences will be supplemented. We will also test alternative ways to utilize genetic information [OTU (Operational Taxonomic Unit) and ASV (Amplicon Sequence Variants)]. The project utilizes both new and previously collected samples from lakes, rivers, and the Baltic Sea. In addition, the project utilizes data stored in the Hertta database of the Finnish Environment Institute SYKE, produced using traditional methods. The results will be examined in relation to water quality and the ecological status of water bodies. The goal is to validate and create guidelines for DNA-and RNA-based, more cost-effective monitoring of biological quality elements. The project is funded by the Ministry of the Environment of Finland.