Biodiversity Genomics & Environmental DNA: Applications and Advances for Biomonitoring

Robert Hanner

Biodiversity Institute of Ontario, Ontario, Canada

This talk will review the development of biodiversity genomics and its applications, with a special emphasis on fishes. Examples involving alpha taxonomy, conservation, ecology and food security will be presented. We will then examine the outgrowth of the science for applied biomonitoring, using varied technology platforms to detect environmental DNA. Pathways to increase standards and competency of eDNA surveys and related issues in biodiversity informatics will also be discussed.

From samplers to ecogenomic sensors: Use of automated methods and instruments for aquatic eDNA assessment

Einar Eg Nielsen, Brian Klitgaard Hansen and Lars Magnus Wulf Jacobsen.

DTU Aqua, Silkeborg, Denmark.

Recently, environmental DNA analysis has gained significant momentum as a fast and cost-effective method for biomonitoring. This has led to much research on practical detection and possible quantification of aquatic macro-organisms in freshwater and more recently in marine environments. Still, aquatic eDNA surveys require manual collection of water, filtering and transport of samples for subsequent analysis in a dedicated laboratory, often resulting in a significant time lag between sampling and results. Likewise, standardized temporal eDNA based marine monitoring in remote and offshore areas is severely hampered due to the large boat-costs associated with continuous sampling. Despite the apparent need, there has been little focus on completely autonomous e-DNA analysis this far. Here we provide an overview of the technical advances in terms of automation for eDNA analysis, with focus on so-called “eco-genomic sensors”, allowing real-time autonomous genetic analysis as well as collection of samples. We provide insights from practical experience from working with one of these instruments, the 2nd generation Environmental Sample Processor (ESP). The ESP is essentially a DNA lab in a can for subsurface deployment including collection, filtration, extraction and DNA analysis of water samples including two-way communication securing remote control of the instrument and real-time availability of genetic data. We discuss advantages and challenges of using ecogenomic sensors for eDNA based monitoring and point to potential future developments within the field, which are likely to increase the application of fully automated eDNA analysis in the field.

Application of eDNA surveys to fisheries: Recent advances and future directions

Ian Salter

Faroe Marine Research Institute, Tórshavn, Faroe Islands

The application of environmental DNA surveys to aquatic environments offers great promise for ecosystem monitoring. Due to the socio-economic importance and political significance of managing capture fisheries there has been recent emphasis on the application of eDNA to marine fish surveys. Early experimental studies in closed body systems highlighted the potential for eDNA approaches in describing species inventories and biomass/abundance of model organisms. However,

it is widely acknowledged that application to marine environments is complicated by the extremely large habitat volumes, physical dispersal of DNA fragments away from target organisms, and variable production and degradation rates. This overview presentation will attempt to provide a brief history of the current progress, identify current knowledge gaps and stimulate discussion on future avenues of research.

eCAP: Tracing capelin with environmental DNA (eDNA)

Christophe Pampoulie[1], Birkir Bárðarson[1], Davið Gíslason[2], Guðbjörg Ólafsdóttir[2]

[1]Marine and Freshwater Research Institute, Reykjavík, Iceland.
[2]Matís ohf., Reykjavík, Iceland.

There is increasing concern over the consequences of climate change on fish distribution and therefore on fisheries production and food security. Climate change can be an additional environmental pressure on top of the many other environmental factors (fishing mortality, loss of habitat, pollution, disturbance, introduced species) which fish stocks already experience. Examples of changes in distribution of fish and plankton due to climate changes are particularly striking because they are more rapid than the changes occurring in terrestrial fauna and flora. In Icelandic waters, such changes in fish distribution have already been observed with the well-known invasion of mackerel and other southern commercial species, and the significant changes in distribution and migration of capelin which has shown west- and northward regional shift.

Capelin is a very important resource for the Icelandic fishery, annual catches have varied from 0 – 1.5 million tonnes, and the total landed value in 2015 was over 12 billion ISK but dropped to 4.9 billon ISK in 2016. Changed or changing distribution of capelin around Iceland makes it 1) more difficult and expensive to assess the distribution of the stock with current methods, complicating further stock assessment and allocation of quotas, and 2) more difficult for the fisheries to harvest this highly valuable resource.

eCAP aims at developing a method using the recent advances performed in genetic which consist of: 1) collecting environmental DNA (eDNA) released by organisms in their environment and use it to estimate ecosystems diversity and ecosystems changes, and 2) use portable and practical on-board DNA technique which can be used on any type of boats called LAMP system (**l**oop-mediated isothermal **amp**lification). A key disadvantage of traditional PCR-based methods that are run in laboratory is the necessity for a generally non-portable, laboratory setting to undertake the time-consuming DNA-amplification protocols. LAMP now offers a logistically simpler and portable protocol: a relatively rapid DNA amplification reaction occurs at one temperature for an hour, and the products are visualized with a colour change within the reaction tubes visible with bared-eyes.

eCAP will develop this method to locate and trace capelin during the MFRI annual assessment surveys and will make it readily applicable to the Icelandic fishery fleets by collecting eDNA from capelin and develop a LAMP system for its detection. **eCAP** will therefore contribute to decrease the search time for capelin during annual surveys and fisheries periods, thus reducing the carbon emission of the Icelandic fleet while increasing their societal benefit.

Acknowledgments: eCAP is supported by a grant from the Technical fund of RANNIS for the next three years, and is a collaborative project developed by MFRI and Matís.

Marine eukaryotic biodiversity from COI metabarcoding of eDNA samples: new insights and knowledge gaps

Owen S. Wangensteen[1], Xavier Turon[2], Janne Søreide[3], Anna Vader[3], Judith Bakker[4], Stefano Mariani[5], Kim Præbel[1]

[1] Norwegian College of Fishery Science, UiT the Arctic University Of Norway, Tromsø, Norway
[2] Centre of Advanced Studies of Blanes (CEAB-CSIC), Spain
[3] The University Center in Svalbard (UNIS), Norway
[4] Florida International University, USA
[5] University of Salford, UK

Metabarcoding has become a well-established method for marine eukaryotic biodiversity assessment. However, most studies have used metabarcoding markers with low taxonomic resolution, such as ribosomal RNA 18S, which underestimate species richness and may only give a partial view of the true extent of eukaryotic diversity. Hypervariable markers such as mitochondrial cytochrome c oxidase subunit I (COI) are emerging as a novel way to obtain high taxonomic resolution datasets. Some recent applications enable the recovery of robust estimations for intra-species (haplotypic) diversity of hundreds of species simultaneously, opening the way to the emerging field of metaphylogeography.
The use of COI metabarcoding on marine environmental samples has been hindered by two technical limitations: primer bias and unspecific amplifications. Both caveats create a specificity/degeneracy trade-off which hampers the design of truly universal primers. Recently developed degenerate primers may overcome primer bias, but they may still produce many non-target amplifications. Incomplete reference databases are another handicap for COI metabarcoding, where Metazoans are fairly-well represented, but significant gaps still exist for other important eukaryotic groups. This prevents the accurate taxonomic assignment of many COI environmental sequences.
We present some recent results from COI metabarcoding of filtered seawater from a wide geographical coverage. Even though many sequences remain unassigned, COI metabarcoding still can provide unparalleled insights into molecular diversity of marine ecosystems. Thus, COI metabarcoding may become an invaluable tool for acquiring relevant ecological results, which can be used to efficiently monitor subtle changes in marine communities driven by climate change or anthropogenic disturbances.

Natural Sampler DNA

Stefano Mariani[1,2] & Charles Baillie[1]

[1]University of Salford, Salford, UK
[2]Liverpool John Moores University, Liverpool, UK

In the quest to screen ocean biodiversity ever more rapidly and efficiently, scientists are now aiding environmental DNA analysis with remarkable technological solutions, including underwater vehicles and robots that are able to process trace DNA in real time. Yet, at the other end of the spectrum lies a simpler, low-tech solution, which may offer an accessible, universal DNA collection tool for biodiversity assessment: natural sampler DNA (nsDNA), the DNA trapped and concentrated in the organs and tissues of scavengers and filter-feeders found in every corner of the world's oceans. Here we illustrate and discuss why and where this modified eDNA route can prove extremely valuable.

A molecular approach to study diet of *Pandalus borealis*.

Paulina Urban[1,2], Owen S. Wangensteen[1], Jan Dierking[2], Kim Præbel[1]

[1] Norwegian College of Fishery Science, UiT the Arctic University of Norway, Tromsø, Norway
[2] GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany

Species affect their ecosystems in different ways. One of them is predation, which inevitably shapes the structure and function of food webs. It is therefore important to assess the dietary composition of species, which is commonly performed using either stomach content analysis (SCA) or stable isotope analysis (SIA). A high level of taxonomic expertise is usually needed to identify the prey items (SCA), which is often challenging when the level of digestion is high. SIA, even though straightforward in application, does not allow for fine-scale dietary analysis and gives broad information about the species trophic position. In case accurate taxonomic dietary information is needed, the use of molecular approaches such as DNA-metabarcoding may alleviate these problems. In this study we evaluated the applicability of DNA-metabarcoding using universal primers for cytochrome c oxidase I, for studying the diet of the Northern shrimp *Pandalus borealis*, an omnivorous generalist and scavenger, with commercial value. We analyzed 169 shrimp stomachs from seven locations in the Barents Sea to investigate possible dietary differences. We present the results of the effects of sex, size and geographical location on the shrimp diet. Our results suggest that soft-bodied prey may play a more important role in the diet of *Pandalus borealis* than previously reported. Molecular SCA using metabarcoding can shed new light on trophic relationships of marine invertebrates, by providing accurate dietary information, especially for soft-bodied, easily digestible preys or tissue fragments, which are overlooked by microscopy-based methods.

Taking eDNA monitoring to the next level: Using unique genomic signatures for biodiversity and population structure assessments

Sissel Jentoft

Centre for Ecological and Evolutionary Synthesis, Department of Biosciences, University of Oslo, Oslo, Norway.

Generation of high-quality genomes in non-model organisms has steadily increased the past years and resulted in invaluable biological and comparative genomic insight in numerous of species including marine teleosts. One example is the recent discovery of four large chromosomal inversions (e.g. on LG 1, 2, 7 and 12) discriminate between Atlantic cod populations throughout its geographical distribution, i.e. dominating the observed genomic divergence by large allele frequency shifts, whereas the rest of the genome displays low levels of genomic differentiation. The most distinct separation is found between ecotypes of migratory and non-migratory costal cod, including the northeast Artic cod vs. Norwegian coastal cod as well as the frontal vs. coastal cod located in the Icelandic waters. Here, I will discuss the potential of taking advantage of this unique genomic information for eDNA monitoring of important marine resources such as the Atlantic cod and other marine species where genomic resources are already generated and/or underway.

A net with no holes: using environmental DNA to revolutionise the assessment of marine pelagic communities.

Marine Cusa[1,2], Christopher Brodie[1,2], J. van de Kooj[3], V. Creach, K. Yate[1] & S. Mariani[1,2]

[1]University of Salford, Salford, UK
[2]Liverpool John Mores University, Liverpool, UK
[3]Center for Environment, Fisheries and Aquatic Science, UK

The use of hydro-acoustic sounders to identify schools of fish has been an established survey method for decades. While the species 'identity' of fishes detected is indirectly inferred from the echo frequency, the schooling behaviour and size of the school, and the location of fish in the water column. Yet, validation is typically performed by means of expensive trawling activities, which often fail to accurately reflect fish populations in the wild. Environmental DNA (eDNA) could become a tool that revolutionises standardised surveys with its power to verify fish species and population composition. This study investigates the potential for using eDNA as a qualitative and quantitative tool for the assessment of marine pelagic communities. To investigate these possibilities, we collected eDNA seawater samples around the South coast of the UK, from surface water during trawls, and from a CTD Rosette at pre-determined sampling stations. We focused on economically valuable pelagic fish species including; European anchovy (*Engraulis encrasicolus*), Atlantic herring (*Clupea harengus*) and Atlantic mackerel (*Scomber scombrus*). DNA metabarcoding of 12S mitochondrial amplicons was used to target these, and other fish species, and the spatial faunal composition of species was compared to the hydro-acoustic and trawl data. The patterns obtained allow for a better understanding of pelagic communities around the South-West of Britain and may lead to improved approaches for the monitoring of marine biodiversity..

Environmental DNA exploration of Antarctic pelagic ecosystems: from species inventories to trophic webs

Charles Baillie[1], Lucia Lopez-Lopez[2,3], R. Collins[4], S. Fielding[5], M. Genner[4], G. Tarling[5], E. O'Gorman[2], O. Wangensteen[1,6] & S. Mariani[1,7]

[1]Salford University, Salford, UK
[2]Imperial College London, London, UK
[3]Oceanographic Centre of the Balearic Islands, Spain
[4]University of Bristol, Bristol, UK
[5]British Antarctic Survey
[6]University of Tromsø, Norway
[7]Liverpool John Moores University, Liverpool, UK

The Southern Ocean harbours diverse and abundant marine life, distributed along stark, latitudinal and depth gradients. Pelagic communities, here, are highly sensitive to rapid environmental perturbations that makes them flagship biotopes for climate change. It is imperative to fully appraise ecological structure and resilience to change of these ecosystems if we are to implement successful management to preserve Antarctic resources and ecosystem services. Yet, prospects to expand marine monitoring programs to collect baseline data of ecological trends are stymied by a lack of taxonomic expertise, high expedition costs and challenging logistics. Environmental DNA techniques have the potential to overcome these challenges and help us gauging ecosystem structure more rapidly than ever before. Here, we report on a depth-stratified eDNA survey of Antarctic fish and

zooplankton communities, conducted as a pioneering study for potential incorporation of eDNA into annual British Antarctic Survey monitoring. Seawater samples were collected at six locations between the Falkland Islands and South Georgia, from six depths between surface and 1000m per site. Immediately after seawater collection with CTDs the same sites were trawled using two different aperture nets at the same depth ranges, providing exceptional, contemporaneous comparisons between morphological and molecular methodologies. We present results from both datasets, highlighting strengths and limitations of this approach for the reconstruction of ecological networks and trophic webs.

Marine environmental DNA in the Anthropocene – Applications in the Arctic and sub-Arctic

Kim Præbel[1], Paulina Urban[1], Charlotte Havermans[2], Xavier Turon[3], Owen S. Wangensteen[1]

[1]Norwegian College of Fishery Science, UiT the Arctic University Of Norway, Tromsø, Norway
[2]Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research
Functional Ecology, Bremerhaven, Germany
[3]Centre of Advanced Studies of Blanes (CEAB-CSIC), Spain

Marine ecosystems are changing at a rapid pace due to the ongoing climate crises, increased organic and biological load from intensifying farming, and by increased human activities in the marine realm. This is especially true for Arctic and sub-Arctic marine ecosystems, where borealization and other ecosystem shifts are happening at unparalleled rates. The currently used surveillance and assessment methods halt behind in documenting and understanding these changing ecosystems. Here, I present some applications and results from our environmental DNA research and show how novel molecular techniques can provide insights and solutions for more timely ecosystem assessments. I will provide examples of mapping vertebrate communities, of boreal species that have unnoticedly expanded their range into the Arctic, of how organismal habitat preferences can be described over vast geographical distances and depths using a whole-ecosystem approach, and of how metabarcoding provides phylogeographic information for hundreds of species simultaneously. I will also provide a glimpse into the applications of biological samplers of environmental DNA in the Arctic. Finally, I will illustrate the quantitative value of metabarcoding, using aquaculture pathogens as an example. I will round up the presentation by discussing resources and approaches that we believe are essential for improving the application of environmental DNA in ecosystem assessments.

eDNA in preserved sediment trap samples: An observational approach to link climate variability, plankton diversity and marine ecosystem services in the Arctic.

Salter, I.[1,2], Metfies, K.[2], Bienhold[2,3], C., Boetius[2,3], A., Nöthig, E.M.[2], Wolff, C[2].

[1]Faroe Marine Research Institute, Tórshavn, Faroe Islands
[2]Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany
[3]Max Planck Institute for Marine Microbiology, Bremen, Germany

The Intergovernmental Panel on Climate Change (IPCC) has concluded with “high confidence” that climate-driven reductions in marine biodiversity will challenge the sustained provision of marine ecosystem services, especially in sensitive environments such as the Arctic. Currently however, it has been difficult to link climate trends, biodiversity and marine ecosystem services, due in part to variable

observational scales. One such important marine ecosystem service is the sequestration of atmospheric carbon dioxide by the biological carbon pump. In the present study we use a sediment trap archive from the Arctic to demonstrate that is possible to retrieve decadal-scale changes in plankton community structure through eDNA analysis of preserved ($HgCl_2$) samples. Particulate organic carbon fluxes measured in the same samples provide an indicator of the magnitude of carbon sequestration. Then, using a high-resolution particle backtracking model, we estimate the observational footprint of the sediment trap mooring (catchment area), and retrieve remote-sensing data products to assess changes in temperature, ice-cover and phytoplankton biomass over relevant spatial and temporal scales. Our analysis shows strong temporal variability in ice-cover and the temperature of Atlantic water flowing into the Fram Strait, previously termed the "warm water anomaly". Protalveolata, Diatoms and Rhizaria dominated sequenced export assemblages. The parasitic marine alveolate, Syndiniales, was a significant and ubiquitous component of sediment trap assemblages highlighting the potential importance of parasitoids in triggering bloom termination and export. Acantharia and Chaeoteceros were notably elevated in summer export assemblages compared to spring. Diatoms, especially Chaeoteceros, were associated with enhanced ice-cover and displayed significant reductions during the warm period, consistent with water-column observations. This community shift corresponded to a reduction in summer-time peak carbon fluxes. We also observed relative increases in *Phaeocystis* sp., confirmed by qPCR, and Miromonas following the warm period. Our study thus demonstrates a tractable eDNA approach to link climate-driven changes in plankton community dynamics to the sequestration of carbon. The approach could be feasibly applied to the large repository of global sediment trap archives. More specifically, our findings indicate that ongoing changes of Atlantification and reduced ice-cover in the Arctic are likely to modify plankton communities to such an extent that they could impact the provision of an important marine ecosystem service with measurable economic consequences.

iDNA - how to use a scavenger as a management tool

Paulina Urban[1,2], Owen S. Wangensteen[1], Jan Dierking[2], Kim Præbel[1]

[1]Norwegian College of Fishery Science, UiT the Arctic University of Norway, Tromsø, Norway
[2]GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany

Frequent and reliable biodiversity assessments help us to understand how global warming and human activities impact ecosystems. Currently, methods used for such assessments in marine environments are not only costly and labor-intensive, but can also be very destructive. As a result, frequent assessments can alter and damage habitats, and this is especially worrying when vulnerable ecosystems such as the Arctic are involved. Applications based on environmental DNA (eDNA) are proposed as a possible solution to this problem, because of their reduced impact and potentially high accuracy. Like all methods, eDNA has its possible drawbacks, among them the vulnerability to contaminations, requiring great care in the sampling and handling process. Our study focuses on invertebrate-derived DNA (iDNA) metabarcoding, a method that uses trophic samplers, e.g. species naturally sampling and accumulating eDNA. Through molecular stomach content analysis of scavenging invertebrates coupled with high-throughput DNA sequencing, fish assemblages of marine ecosystems can be analyzed. We applied iDNA-metabarcoding to assess stomach contents of 169 individuals of the Northern shrimp, *Pandalus borealis*, from the Barents Sea, using 12S primers (MiFish) designed particularly for amplification of vertebrate sequences. We present the results of fish-assemblages inferred by iDNA-metabarcoding compared to results obtained from bottom trawling as a traditional method of biodiversity assessment. The molecular stomach content analysis of scavenging, widespread and easily accessible invertebrates may serve as a less-destructive, powerful, and straightforward tool for frequent biodiversity monitoring of marine vertebrate communities.

Mapping marine phytoplankton in Iceland through environmental DNA metabarcoding

Mia Cerfonteyn[1,2,3], René Groben[1], Kristinn Guðmundsson[3], Pauline Vannier[1] and Viggó Þór Marteinsson[1,2]

[1]Matís ohf. Reykjavik, Iceland
[2]Faculty of Food Science and Nutrition, University of Iceland, Læknagarður, Reykjavik, Iceland
[3]Marine and Freshwater Research Institute, Reykjavík, Iceland

Marine micro-organisms play a crucial role in the food web and geochemical cycles, yet many questions regarding the dynamics of phytoplankton in Icelandic waters remain. Novel molecular methods can contribute to existing knowledge by increasing sample throughput and a better characterization of small and morphologically similar taxa. Metabarcoding environmental DNA (eDNA) extracted from seawater allows the characterisation of microbial communities by targeting certain genes. In addition, sequencing different parts of the genome can generate a clearer picture of autotrophic and heterotrophic community structures.

The aim of the "Microbes in the Icelandic Marine Environment (MIME)" project is to give a better understanding of the underlying mechanisms that control the marine food web surrounding Iceland. Seawater samples were collected annually from 19 coastal and oceanic stations around Iceland in May 2011-2018 and seasonally for one year (August 2017, February 2018 and May 2018). At the stations, seawater was sampled at several fixed depths from surface to bottom (n = 348) and environmental metadata were collected for each sampling event. DNA from May 2018 were amplified for regions common to autotrophic and heterotrophic eukaryotes (18s rRNA genes) and the 16s plastidial rRNA genes present in chloroplasts and cyanobacteria will also be amplified.

The advantages and biases of using metabarcoding for phytoplankton diversity studies will be presented, with a focus on taxa not previously identified in Iceland by traditional microscopic methods. The dynamics of cyanobacteria and autotrophic eukaryotes will be presented in context of time and physical oceanography.

Metagenomics of microbes in Icelandic marine waters.

Clara Anne Jégousse[1,2], Pauline Vannier[2], René Groben[2], Viggó Þór Marteinsson[1,2]

[1]Faculty of Health Sciences, University of Iceland, Reykjavík, Iceland
[2]Matís ohf., Reykjavík, Iceland

Sustainable management of marine resources requires us to explore and understand the ocean's ecosystem. At the very basis of this ecosystem are marine microbes: single-celled organisms organised in complex, interactive and dynamic communities that control the flow of energy and nutrients essential to the upper levels of the food web. But little is known about microbial communities in the ocean around Iceland. Here we propose to resolve the composition, the structure and the potential function of these microbial communities through high-throughput sequencing of DNA and metagenomics. Metagenomics is the study of genetic material recovered directly from an environmental sample (in this study, seawater), analyzing the genetic information of the whole microbial community together. The samples of seawater were taken at the surface and the bottom of the ocean at two hydrographic stations in the north and the south of Iceland. This study reveals differences in composition and structure in the marine microbial communities. These results will help us better explain microbial community dynamics and the impacts on the higher levels of the marine food web.

Microorganisms in groundwater spring sources in Iceland

Ragnhildur Gudmundsdottir[1], Agnes-Katharina Kreiling[1,2], Bjarni K. Kristjánsson[2], Viggó Þ. Marteinsson[1,3], Snæbjörn Pálsson[1]

[1]Faculty of Life and Environmental Sciences, University of Iceland, Reykjavík, Iceland
[2]Hólar University College, Hólar, Hjaltadal, Iceland
[3]Matís ohf., Reykjavík, Iceland

Two endemic subterranean freshwater amphipod species, *Crangonyx islandicus* and *Crymostygius thingvallensis* inhabit groundwater in Icelandic lava fields. Divergent mtDNA lineages of *C. islandicus,* in different regions of Iceland, indicate that the species survived the Ice age in Iceland, in separated groundwater watersheds. These findings show strong evidence for an old groundwater ecosystem in Iceland, which might be based on chemoautotrophy. Similar systems are found in various places around the world, commonly in limestone karst where concentration of sulphur is high in the groundwater. That is considerably different from the cold groundwater springs where *C. islandicus* is found, but they are mostly found at the edges of basaltic lava fields. With combination of eDNA and metabarcoding, the microbial diversity of this peculiar system can now be revealed. Results show that the microbial community of the amphipods are dominated by chemolithoheterotrophs possibly capable of utilising iron and manganese as energy source. These same groups are not found to be as abundant in the spring sources indicating they are confined to the amphipods. The microbial community in the spring sources reflects that this is a three-way ecotone with species from the surface water, groundwater and the overlaying terrestrial ecosystem present. The microbial spring community is shaped by pH at the local scale while on regional scale other factors have impacts such as geographical location, temperature and the presence of fish. Only few chemolithoautotrophic taxa are found in this system indicating that they are not the greatest contributors to the primary production.