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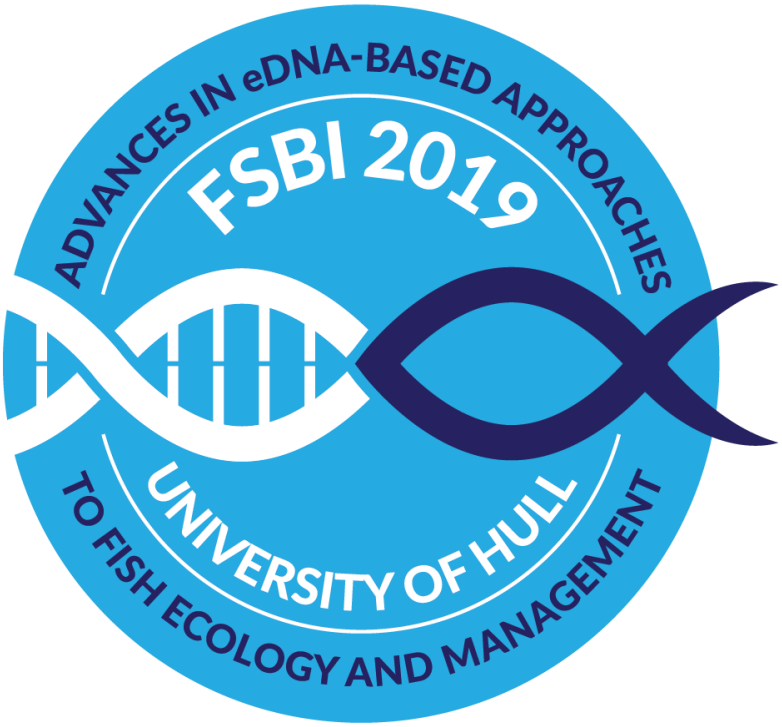

Book of Abstracts

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Keynote Speakers

Jack Jones Lecture

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Can we manage fisheries with the inherent uncertainty from eDNA? (K1)

Environmental DNA (eDNA), as a general approach in aquatic systems, seeks to connect the presence of species' genetic material in the water to infer the species physical presence. However, fisheries managers face making decisions with risk and uncertainty when eDNA indicates a fish is present, but traditional methods fail to capture the fish. In comparison to traditional methods, like nets, electrofishing, and piscicide, eDNA approaches have more sources of underlying error that could give rise to false positives. This has resulted in some managers to questioning whether eDNA can be used to make management decision because there is no fish in hand. As a relatively new approach, the methods and techniques have quickly evolved to improve confidence in eDNA. By evaluating an eDNA based research program through the pattern of the eDNA signal, assay design, experimental design, quality assurance and quality control checks, data analyses, and concurrent search for fish using traditional gears, the evidence for fish presence can be evaluated to build confidence the eDNA approach. The benefits for fisheries management from adopting an eDNA approach are numerous but include cost effectiveness, broader geographic coverage of habitat occupancy, early detection of invasive species, non-lethal stock assessments, exploration of previously inaccessible aquatic environments, and discovery of new species hidden beneath the water's surface. At a time when global freshwater and marine fisheries are facing growing threats from over-harvest, pollution, and climate change, we anticipate that growing confidence in eDNA will overcome the inherent uncertainty of not having a fish in hand and will empower the informed management actions necessary to protect and restore our fisheries.

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Environmental DNA is not a tool by itself (K2)

The detection of DNA in the environment ‘eDNA’ has gained immense momentum and its applications are steadily diversifying. Analysis of eDNA has developed towards an applied science because sampling and sequencing it is viewed as a simplifying method for detecting organisms in complex ecosystems. Consequently, ‘eDNA’ is often referred to as the tool rather than what it is, a mixture of polynucleotide molecules from organisms; not a tool by itself. Currently, we lack answers to many fundamental questions about the shedding rate and the behavior of DNA in the environment and this leads to uncertainty in its use for inferring a species detection. Thus, research on eDNA needs to move beyond the idea that it is a tool and focus on understanding the cycling of eDNA in space and time. If we accept the view that eDNA is a complex molecule worthy of fundamental study itself, then we are far more likely to make the discoveries and breakthroughs necessary to advance its use in a multitude of ways spanning the life and physical sciences. In my seminar, I will present what we currently know about the ‘ecology’ of eDNA and compel all to think outside the box for how we can use information gleaned from eDNA to infer species presence in space and time.

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Overcoming the challenges of environmental DNA for invasive species detection (K3)

In the last decade, non-invasive environmental DNA (eDNA) technologies have gained traction as a highly sensitive, cost-effective alternative to more traditional detection methodologies. eDNA holds considerable promise for surveillance applications such as the detection of high-risk organisms of biosecurity concern, identification of invasive species in the early phase of the invasion curve, or inferring the presence of endangered species. The potential for false positive or false negative detections, however, make eDNA surveys particularly susceptible to misinterpretation. Consequently, the development of standards and guidelines are required for the provision of adequate quality assurance. In order to overcome some of these challenges, our team have developed a framework to estimate the sensitivity of both the field and laboratory components of eDNA survey methods, and we have been able to demonstrate how these can be used to estimate the overall sensitivity of these methods for eDNA applications. We have applied this framework to species-specific eDNA surveys to estimate the sensitivity, or probability of detection, for native and invasive aquatic species present in Australia's freshwater environments. I will provide examples ranging from the detection of a reintroduced population of endangered corroboree frogs, identification of a redfin perch invasion front, and confirming the absence of European carp following eradication efforts. These examples highlight the potential for eDNA surveys to inform management decisions around the timing of reintroduction efforts, the location of species containment barriers, and the cessation of pest control efforts. Finally, I will focus on current advances in eDNA surveys such as real-time monitoring and point-of-site delivery that have the potential to greatly expand the application of this technology.

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Using eDNA to better understand fish ecology (K4)

Since its first application in 2008, several studies have demonstrated the performance and reliability of environmental DNA (eDNA) approaches for the monitoring of fish biodiversity in freshwater ecosystems and for the understanding of their ecology. However, as any survey method, it is important to identify its strengths and limits and it is crucial to understand the ecology of eDNA and its dynamics in aquatic environment. Knowing how the DNA is released by the organisms, in which form and its dynamics in aquatic environments will help to design adapted sampling strategies and to have more efficient sampling devices and analysis methods. The temporal and spatial representativeness of eDNA will inform about the community changes (e.g. presence of migratory species, introduction of invasive species, breeding season, etc.). In this talk, several studies that demonstrate the performances of eDNA approaches for fish monitoring will be presented.

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Environmental DNA metabarcoding enables a data-driven approach for fish community research in large spatiotemporal scales (K5)

Recent studies demonstrated the utility of eDNA for detecting fishes from various aquatic environments, including ponds, rivers, streams and seawaters. Most of the earlier eDNA studies focused on detection of a single or a few invasive and rare or threatened species, while a number of recent studies attempted simultaneous detection of multiple species in local fish communities and mesocosms. The latter approach is called “metabarcoding” and eDNA metabarcoding uses one or multiple sets of PCR primers to coamplify a gene region across taxonomically diverse samples. This is followed by library preparation with indexing and adapter addition, and the indexed libraries are analyzed by a high-throughput parallel sequencing platform. Recently Miya *et al.* (2015) developed universal PCR primers for metabarcoding eDNA from fishes (called “MiFish”). The MiFish primers target a hypervariable region of the mitochondrial 12S rRNA gene (163–185 bp), which contains sufficient information to identify fishes to taxonomic family, genus and species except for some closely related congeners. With the use of MiFish primers in eDNA metabarcoding (MiFish metabarcoding), my research group conducted a nation-wide study based on 528 samples from a length of the Japanese archipelago extending over 3,000 km along the northeastern coast of the Eurasia continent My research group has also conducted 45 biweekly samplings in Boso Peninsula located along the Pacific coast of Japan from August 2017 to June 2019. I will briefly introduce the latest results from these two projects and show the usefulness of these approaches to fish community research.

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Fish as predators and prey: assessing their role in food webs via molecular tools (K6)

DNA-based methods have significantly widened our ability to assess trophic interactions in both marine and freshwater systems. The technical advancement within the last decade offers nowadays a wide variety of methodological approaches to empirically examine complex feeding networks across various trophic levels. Different diet samples such as gut contents or faeces can be used as DNA source to disentangle consumed food. There are two main approaches of molecular diet analysis: 1) diagnostic PCR and 2) metabarcoding of food DNA. While the former is rapid and simple in its application, it is limited to a smaller range of taxa which can be tested for. Metabarcoding provides a much broader picture of what has been consumed but it is technically more elaborate. This talk will exemplify how molecular techniques have been employed to unravel food web interactions involving fish as consumers and prey. In addition to the exciting opportunities DNA-based approaches offer, current challenges and future prospects for assessing fish food webs will also be highlighted.

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The future of fish-based ecological assessment of European rivers: from WFD-compliant methods to eDNA metabarcoding-based indices (K7)

Most of the present Water Framework Directive -compliant fish-based assessment methods of European rivers are multi-metric indices computed from electrofishing occasions. But this method has known shortcomings, especially in large rivers. The probability of detecting rare species remains limited, which can alter the sensitivity of the indices. In recent years, eDNA metabarcoding techniques have progressed sufficiently to allow applications in various ecological domains as well as eDNA-based ecological assessment methods. Unlike other groups (macroinvertebrates, diatoms), the detection of aquatic vertebrates and especially fish, is based solely on the analysis of a rare and degraded extra-organism DNA. Consequently, fish-eDNA based assessment methods must consider specific constraints even if they yielded promising results in recent years (high taxonomic resolution of primers). A review of the 25 current WFD-compliant methods shows that 81% of the metrics used in river fish indices are expressed in richness or relative abundance and thus compatible with eDNA samples. However, more than half of the member states' methods include at least one metric related to age/size structure and/or metric based on absolute abundance / biomass. Almost all the fish-based assessment methods currently in use (WFD) will need to be revised before eDNA samples could replace electrofishing method: reference conditions, metric selection, pressure-impact relationships, intercalibration… It can be expected that fish-eDNA based indices would increase the sensitivity of metrics to anthropogenic disturbances (better detectability of rare sensitive species), reduce the uncertainties associated with their estimation (better reproducibility) and improve the spatial representativeness when compared to current fish indices based on electrofishing. Recommendations for the development of future fish eDNA-based indices, the associated eDNA water sampling strategy and their spatial representativeness in relation with downstream transport of eDNA in rivers are discussed.

Talks

Testing the performance of eDNA metabarcoding for surveying highly diverse tropical fish communities: A case study from Lake Tanganyika (T1)

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While recent studies have demonstrated the effectiveness of environmental DNA (eDNA) metabarcoding methods for surveying temperate aquatic fish communities, important questions remain surrounding the ability of these approaches to detect species across different habitats and scales. Despite much of Earth's freshwater diversity occurring in the tropics, few studies have attempted to test the effectiveness of metabarcoding methods in these ecosystems. We address this by applying an eDNA metabarcoding approach to survey the highly diverse littoral fish communities of Lake Tanganyika, East Africa. This system provides two unique challenges. Firstly, rocky sites contain a high local species richness, including species challenging to survey using traditional methods. Secondly, a large number of species, such as cichlid fishes, comprise evolutionary radiations resulting from rapid diversification and are difficult to identify using barcoding methods. To test the potential of eDNA metabarcoding in such a complex ecosystem we developed an extensive reference database (358 species) for the lake's fish species across three mitochondrial gene regions. Four separate primer pairs including a novel cichlid-specific primer are used to investigate the diversity of these fish communities across a 25km section of coastline. Resolutions obtained for the cichlid fishes varied greatly across the four primer sets with the greatest resolution, often down to species level, obtained with the cichlid-specific primer. Furthermore the universal fish primers enabled the identification of a number of non-cichlid fishes that are challenging to survey with traditional methods. The accuracy of eDNA detections are further assessed through comparisons made with visual survey data.

Monitoring the Mekong for fisheries management and conservation (T2)

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The Mekong is a productive freshwater system with high biodiversity that supports regional food security, and home to some of the most threatened iconic species. Monitoring for fisheries management and evaluation of the status of threatened species is essential for the biodiversity conservation and sustainability of the ecosystem. Monitoring freshwater species diversity is particularly challenging in this species rich, rapid moving turbid riverine and flood plain environment. This paper describes an eDNA monitoring system that combines cost effective species-specific assays and metagenomics. Using species-specific markers it is possible to regularly track commercially important and iconic endangered species for conservation action and adaptive management. Metagenomic approaches are used to generate a species inventory, providing less frequent but more intensive assessment of overall diversity and facilitating evaluation of temporal and spatial differences in species composition. The benefits of using eDNA monitoring approaches as part of an integrated monitoring system for fisheries management and biodiversity conservation are discussed, as well as the potential to investigate impacts of environmental transformation on species diversity, brought about by climatic and anthropogenic change.

Lake fish community monitoring with eDNA: a case study in England's largest lake (T3)

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Current established methods for monitoring lake fish communities are limited by the fact they are either destructive or highly selective. At present, there is no established method in place for routine lake fish monitoring in the UK, despite the fact that it is a legal requirement. England's largest lake, Windermere, is an exception. The fish community of Windermere has been been monitored regularly since the 1940s, providing an ideal long term dataset for comparison with eDNA. Since January 2015 we have carried out a number of eDNA sampling campaigns in Windermere to develop and refine an eDNA metabarcoding method for fish communities and to understand the spatial and temporal variation in eDNA distribution. I will summarise the results of our Windermere work, evaluate how well eDNA metabarcoding works for species detection and abundance estimation, and provide recommendations on how, when and where to sample for optimal species detection.

Optimizing detection of freshwater fish using eDNA metabarcoding in the Douro Basin (Portugal) (T4)

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The DNA from environment samples combined with high-throughput sequencing - eDNA metabarcoding - is a potentially useful approach for assessing freshwater biodiversity. While detecting threatened and introduced freshwater species is essential to inform managers and respond to Habitat and Water Framework directives, the widespread application of eDNA metabarcoding in freshwater monitoring is still hindered by the lack of standardised field, lab and bioinformatic protocols, and approaches for scaling up its use to regions. In the FRESHING project we aimed to build a freshwater fish DNA reference collection developed at InBIO-CIBIO, and examine the relevance of field sampling strategies and lab procedures on the eDNA metabarcoding detection of freshwater fish. We present eDNA metabarcoding using multiple samples of water filters and accounting distinct microhabitats, and using distinct lab protocols for DNA extraction and amplification. The watercourses for the Douro Basin (Spain and Portugal) were used as a case study since the region covers a wide range of environmental conditions and human stressors, which were sampled during early summer 2017 and 2018. The eDNA water filtering and electrofishing were done in order to compare results between methods from the same sampling site. Results show that eDNA metabarcoding detectability differs between sites and between distinct microhabitats within sites. Also, the biomass of each species captured by electrofishing seems to be related with the likelihood of detection when using eDNA metabarcoding. We further discuss the relevance of distinct field and lab strategies, and the need of spatial sampling replication.

Evaluation of freshwater fish communities with eDNA metabarcoding: a quantitative perspective (T5)

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Accurate data on species distribution and abundance are critical for conservation and management of aquatic resources. Several inventory methods, such as gillnets survey, are widely used to estimate those parameters. However, gillnets can be invasive, costly in terms of material and human resources, may cause unwanted mortality in the fish communities studied as well as been subject to size and species selection bias. Environmental DNA (eDNA) analysis, which consists of detecting DNA traces released by species in their environment, could be used as a non-invasive, more accurate and less costly alternative or complementary than so-called method. In this study, we evaluate the pros and cons between eDNA (eMetabarcoding) and gillnets for monitoring freshwater fish communities in terms of species richness, relative abundance and species abundance. Our main observations are : i) eDNA detected more species than gillnet; ii) detection sensitivity improves with the amount of filtered water; iii) sequence reads, qPCR, and catch per unit effort were correlated for the 3 main species. In sum, eMetabarcoding is a very valuable complementary, if not alternative tool for freshwater fish monitoring.

Water stratification in marine environments significantly restricts eDNA signal dispersal (T6)

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Effective environmental management requires an accurate description of the ecological community. While eDNA metabarcoding shows promise as a monitoring method for the vast and inaccessible marine environment, the lack of visual observation enables type I and type II errors. In light of accuracy, a few marine eDNA studies have reported modest eDNA dispersal through horizontal water movement. This result implies that spatially specific eDNA signals resemble in-field assemblages. However, marine communities are typically structured across a vertical depth range as well as horizontally. The scale at which eDNA signals differ according to depth and, hence, the accuracy of eDNA metabarcoding in assessing naturally stratified communities, have yet to be tested. In this study, we determined the vertical spatial resolution of eDNA metabarcoding surveys down a steep rock wall displaying well-described permanent zonal community patterns induced by water column stratification. Our eDNA survey detected 76 taxa across 10 phyla using three established assays. Ordination and cluster analyses show distinct eDNA signals between zonal community assemblages, suggesting dispersal of eDNA among communities and stratified water layers was limited. A comparison between eDNA detection and previously conducted photographic quadrat surveys revealed highly similar patterns. Our results demonstrate the accuracy of eDNA metabarcoding surveys in detecting distinct marine communities between depths. The high spatial resolution obtained in this study suggests conventional surface water sampling is not always sufficient to comprehensively sample marine biodiversity. Taking into consideration oceanographic processes within the area of interest when designing sampling strategies is essential for successful eDNA metabarcoding surveys.

eDNA metabarcoding reflects habitat preference of fish and mammal communities from the Arctic Northeast Greenland shelf (T7)

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Environmental DNA analysis is emerging as a powerful tool to monitor biodiversity of aquatic communities, but how biotic and abiotic factors determine the spatial distribution and the temporal persistence of eDNA signals still remains to be investigated. This information is crucial to infer robust local-scale species distribution patterns from eDNA surveys. To assess the spatial distribution patterns of eDNA from vertebrate communities along the undersampled Arctic Northeast Greenland coast, we performed eDNA-metabarcoding (using MiFish 12S primers) on filtered seawater samples collected from three geographical areas that differed in ecology and abiotic factors (Inshore, Offshore-North and Offshore-South). From each station, three different depths were sampled: shallow (1-3 m), midwater (12-67 m) and bottom (165-1040 m). We detected 47 vertebrate MOTUs (36 actinopterygians, 3 chondrichthyans, and 8 mammals). With some exceptions, patterns of eDNA presence/absence and abundance of metabarcoding reads closely reflected the known habitat preferences of the species (shallow-water, midwater, deep-water, or no preference). eDNA read abundances also reflected the known geographical distribution of the species, as most Boreal species appeared in the Offshore-South area, Arctic species appeared in the Offshore-North area, and coastal species appeared in the Inshore area. These results suggest that eDNA surveys provide an accurate, local-scale snapshot of the communities present in the sampled areas, both geographically and at different depths. Thus, eDNA metabarcoding is useful to study habitat preference of several species simultaneously, including identification of preferred feeding grounds and spawning areas, which is essential information to manage commercial stocks and to develop conservation plans.

Harnessing haplotype data from eDNA metabarcoding studies for conservation genetics and phylogeography (T8)

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Environmental DNA (eDNA) metabarcoding has emerged as a rapid, cost-effective, non-invasive monitoring tool for aquatic ecosystems. The main advantage over species specific assays is that the metabarcoding approach allows for inferring the presence of target species, as well as evaluating overall species diversity and community structure, simultaneously, from the same data, by utilising the power of so-called Next Generation sequencing technologies. Vast amounts of data are currently being generated, analysed and evaluated to verify that insights from eDNA based surveys can be translated directly into policies. Most recently researchers are beginning to explore another facet of eDNA metabarcoding data - the potential for extracting information on intraspecific genetic diversity and population structures. However, results have rarely been interpreted in a conservation genetics or phylogeographic context on a larger, e.g. pan-European, scale. I present a meta-analysis of haplotype data extracted from 150+ eDNA samples, obtained in the course of independent eDNA-based biodiversity surveys in three European drainage systems (Danube, Volga, Northern England). I discuss the recovered haplotype diversity, -structure and -distribution of 10 common European freshwater fish species, in the light of patterns expected based on (post-)glacial events, anthropogenic disturbances and methodological challenges (data filtering, excluding false positives/negatives).

From MOTUs to species richness: a clustering pipeline without exhaustive reference database (T9)

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All ecosystems are increasingly threatened by human activities, causing widespread decline in species diversity. Biological inventory is key to understanding their functioning under changing conditions, but remains challenging for elusive taxa or hardly accessible ecosystems. Environmental DNA demonstrates promising results, however reference databases to assign species are often incomplete, with less than 20% of all fish sequences available for the 12S region. Clustering algorithms, commonly used in metabarcoding studies, offer alternatives by delineating MOTUs instead of species, but their performances have rarely been evaluated. This study re-analyses 100 eDNA samples along 500km of river, for which the 12S local reference database is exhaustive to evaluate the performance of a complete pipeline using SWARM. All unique sequences are assigned to later ground truth obtained MOTUs from SWARM. Clustering performances compared to our ground truth is assessed for gamma, beta and alpha diversity at species, genus and family level. In combination with stringent filtering thresholds, SWARM delivers accurate estimates of species richness (R2 = 0.98). Gamma analyses reveal that 6 species are not represented by any MOTU with SWARM, all closely related to a more abundant species. Out of the 14 taxa represented by several MOTUs, up to 8 MOTUs are discarded when using the post-clustering cleaning algorithm LULU, and 3 belong to taxa currently under taxonomic revision. Beta-diversity patterns display similar trends, with more similarities for genus and family level. We conclude that SWARM clustering with appropriate filtering is a promising approach to deal with uncomplete reference database in eDNA studies.

Curating genomic inventories for eDNA surveys: A bottom-up model for obtaining, verifying, and accessioning species for genome assembly (T10)

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Technological advancements and decreasing DNA sequencing costs have led to a genomics revolution that has made possible the use of molecular tools to monitor biodiversity. Methods using environmental DNA (eDNA)—extraorganismal DNA found in the environment—are currently being applied to monitor freshwater species. These eDNA-based monitoring tools are typically developed as-needed using genetic information compiled piecemeal or sourced from genomic clearinghouses. This is a limiting factor for tool creation as genomic databases are incomplete and curating genetic data is a laborious process. Top-down initiatives aspire to create worldwide compendia of genomic data, but these efforts fail to take into account the regional expertise and labour required to generate comprehensive genomic data for a broad range of taxa. The Oregon Biodiversity Genome Project is working with regional agencies and institutions to develop a mitogenomic reference sequence database for all freshwater vertebrate species within the state of Oregon. Our pipeline harnesses agency expertise and capacity to curate whole-organism vouchers linked to tissue samples and genomic data. These data are publicly accessible to researchers everywhere to facilitate the development of eDNA-based detection tools. Our field to lab pipeline can serve as a blueprint to enable the expanded development of localized genomic collections worldwide. We anticipate that this distributed approach will spawn numerous regional biodiversity genomics catalogs that can be linked to provide genomic data to the international community to facilitate genetic research and biodiversity monitoring across the globe.

The application of CRISPR-Cas for single species identification from environmental DNA (T11)

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Development of simple and rapid techniques to monitor fish species for conservation strategies and detection of invasive species is vital to further the capabilities of environmental DNA. Adapting eDNA approaches to a biosensor device would enable onsite sample testing for rapid species assessment. Common eDNA strategies utilising PCR based amplification are challenging to adapt to a device due to the need for high temperatures and thermocycling. Isothermal amplification and detection offers a solution to this. An isothermal assay specifically targeting Salmo salar but with the potential for modification to any species of interest has been developed. This assay utilises eDNA extracted from freshwater and isothermal DNA amplification coupled to CRISPR-Cas detection. The assay provides attomolar sensitivity and rapid detection rates (<2.5 hours) at a single temperature and therefore enables easier adaptation to a microfluidic biosensor device than conventional PCR-based assays for simple, rapid, onsite monitoring of S. salar and indeed any target species.

Using environmental DNA to assess the current distribution of brown trout and native galaxiids in the Falkland Islands (T12)

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Brown trout (*Salmo trutta*) were introduced to the Falkland Islands on several occasions during the 1940-50's, mainly for recreational fishing. Since then, there has been a marked decline in the native freshwater fish fauna, which consists of only three species of galaxiids, endemic to the Southern Hemisphere (zebra trout *Aplochiton zebra*, *Aplochiton taeniatus*, and the Falklands minnow *Galaxias maculatus*). Given the threats to the long-term conservation of native galaxiids, a better understanding of the life history and movement ecology of brown trout, is urgently needed. Two presence/absence surveys, conducted 10 and 20 years ago, provide valuable snapshots of brown trout and galaxiid distribution in the Falklands and demonstrated that most zebra trout were concentrated within small refuges uninvaded by brown trout. However, the tendency of brown trout to display anadromy in the Falklands makes it likely that they could eventually colonise all the accessible rivers and, therefore, the risk of native galaxiid extinction is high. Our study assessed the current distribution of brown trout and native galaxiids using species specific environmental DNA (eDNA) sampling in order to monitor the extent of the brown trout invasion and provide valuable information for conservation and/or mitigation efforts.

Using environmental DNA detection for improving conservation management plan of critically endangered fish species (T13)

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The current populations of two critically endangered and endemic Greek fish species *Valencia letourneuxi* (Sauvage, 1880) and *Valencia robertae* (Freyhof et al. 2014) is decreasing. Due to anthropogenic habitat modifications and the spread of invasive species, populations of these two killifish have been drastically declining. Because of the low densities of remaining individuals and the need of avoiding any injury to the fish populations and their ecosystems, traditional monitoring methods (i.e. electrofishing or netting) are considered invasive and therefore, eDNA is being considered as strong alternative. We developed an eDNA targeted approach for both *Valencia* species, as well as for the invasive "eastern mosquitofish" *Gambusia holbrooki* (Girard, 1859), spreading in the same habitats. We conducted a mesocosm experiment in collaboration with The Zoological Society of London for investigating the efficiency of two different enclosed filters and the correlation between eDNA quantification and fish abundance. A first field sampling in Autumn 2017 was conducted in 6 Greek rivers for assessing the efficiency of the designed method and was followed by a second field sampling associated with an electrofishing campaign in Autumn 2018 in 20 Greek rivers. Using simplex and multiplex qPCR analysis we are presenting the results of this experiment and propose recommendation for improving the efficiency of future fish surveys.

Use of environmental DNA in Chile: problems and perspectives in freshwater fish ecology (T14)

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Traditional biological monitoring techniques have recently been superseded by cutting edge approaches involving the extraction of traces of DNA found in soil, water, sediment and other environmental samples. Collectively these new techniques have been based on environmental DNA, i.e. genetic material that can be obtained directly from environmental samples without any obvious signs of biological source material. This technique has great promise in fish ecology, e.g. for the monitoring of cryptic species, the early detection of invasive species and non-invasive surveys of species richness from many ecosystems. However, a number of issues can limit its widespread application in certain ecosystems. For instance, the Chilean 'freshwaters' (strictly continental waters) that support fishes are remarkably diverse. They range from bofedales and salares (High Andean peat bogs and salt pans at over 4 000 m above sea level), highly saline desert streams, Mediterranean-type ecosystems with high concentrations of dissolved particulate matter, through to oligotrophic rivers and lakes, among others. Here, we show Chile-wide results applying different eDNA-based techniques such as metabarcoding of freshwater fishes, qPCR and RFLP-PCR detection of invasive salmonid species and its interaction with methods of preservation and extraction of eDNA. We discuss the importance of testing different preservation methods compatible with use in remote sampling sites and having a thorough molecular database of native and introduced fishes, and suggest some perspectives to improve preservation, sampling and the application of eDNA-based techniques in our ecosystems for fish detection and monitoring.

Spatio-temporal variation in Neotropical riverine ichthyofauna inferred by eDNA analysis (T15)

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Despite showing an increasing need for intervention for species monitoring and management, biodiversity assessment remains a significant challenge in the Neotropical region due to the constraints imposed by infrastructure problems. To evaluate eDNA metabarcoding as a biodiversity assessment and ecological analysis tool in Brazilian rivers, we obtained sediment and water samples from 11 locations along the Jequitinhonha River catchment (South-eastern Brazil), sampling each site twice over a period of two months. The mitochondrial marker 12S (˜172bp) was amplified and the sequences obtained allowed the detection of 252 MOTUs, of which at least 34 were assigned to the species level, including endemic (*Wertheimeria maculata*) and introduced fish (*Astronotus ocellatus*, *Moenkhausia costae*), as well as new records for this basin (*Salminus brasiliensis*, *Lophiosilurus alexandri*). Contemporary spatio-temporal variation of fish assemblages demonstrated that communities can vary even within short time-frames (3 weeks). Only a few locations hosted the highest species richness during the first campaign, while the distribution followed a much more homogeneous trend during the second sampling. No correlation between β-diversities and longitudinal distance or presence of dams (barriers) was found. However, anthropogenic impacts might still influence fish assemblage distribution as sites located right near the dams consistently had the lowest species richness. The study area shows high endemism and many species remain unknown in reference databases, hampering their identification. Here we demonstrated that eDNA can contribute to fish biodiversity assessment in Brazil, detecting introduced species and providing data from localities often neglected by traditional sampling surveys.

Spatio-temporal distribution of eDNA indicates spawning activity and location of Arctic char in Windermere (T16)

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Understanding the timing and location of fish reproductive events is crucial for management and conservation purposes. Conventional methods to monitor such events are often highly invasive and, therefore, problematic. Novel and non-destructive tools are hence required for sensitive environments and endangered species such as Arctic charr at Windermere. This population has been studied since 1960 and locations and characteristics of its spawning grounds have been described in detail using traditional techniques. Here, we employed eDNA metabarcoding and a species specific approach to assess the spatial distribution of Charr throughout the year as well as to identify spawning locations and activity in conjunction with traditional netting surveys. Seasonal sampling was carried out through the course of a year along two transects in the lake covering putative and demonstrated spawning locations. In this study, eDNA metabarcoding enabled an accurate spatial and temporal localization of Charr spawning events as well as a description of fish community changes at those sites. Thus, our results demonstrated that eDNA metabarcoding is a broader and much more communicative tool than previously bespoken. Additionally, a subset of samples was chosen to perform quantitative PCR (qPCR) analyses in order to quantitatively support our metabarcoding evidence and compare the sensitivity of the methods. This study revealed the potential of eDNA metabarcoding to address ecological questions and investigate biological traits of Arctic charr, a species whose value, within Windermere, spans from evolutionary biology to local fishery and conservation.

Searching in the dark: environmental DNA based monitoring of the mysterious European weather loach (*Misgurnus fossilis*) (T17)

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Monitoring the distribution and abundance of endangered species is essential for developing conservation efforts to be successful. Especially for rare or cryptic species, eDNA approaches are expected to offer significant advantages over traditional field methods. European weather loach is such an example (protected by the EU Habitats Directive), showing a dramatic collapse of its populations throughout its entire distribution range. In Belgium, the species is presumed to reach a point of complete extinction and physical observations became very scarce last decades. To obtain an accurate overview of the remaining Belgian weather loach populations, we first adapted an extraction protocol, and developed and validated a new primer/probe assay for highly sensitive and specific detection. Second, we tested the detection resolution of qPCR- versus ddPCR-approaches on experimental and natural samples, and compared these results with metabarcoding data. Third, we applied a caging experiment in which we used different abundances of weather loach to establish an optimal sampling protocol in space and time. Finally, based on historical records and ecological parameters we selected, extensively sampled and analyzed 60 potential locations throughout Belgium. Surprisingly, 30% of these locations tested positive, including four sites where the species was not documented to occur before. Additional fishing interventions revealed that one of these sites harbored the largest remaining population in Belgium. Overall, our findings provide important insights towards eDNA based screening and monitoring of rare and cryptic species, and show the spatial, temporal and quantitative resolution of this methodology, which is extremely useful for conservation and management.

Assessing the impact of the threatened crucian carp (*Carassius carassius*) on pond invertebrate diversity - a comparison of conventional and molecular tools (T18)

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Fish species stocked for recreation and angling can damage freshwater habitats and negatively impact biodiversity, but this is not always the case. The crucian carp (*Carassius carassius*) is one of few fishes naturally associated with ponds and stocked for conservation management. This species may augment landscape-scale diversity; however, its impact on other pond biota has not been broadly assessed. Freshwater invertebrates comprise a large proportion of aquatic diversity, encompassing many rare and endemic species, but are difficult to assess due to small size and high abundance. Practitioners have typically employed sweep-netting and kick-sampling, but DNA and eDNA metabarcoding now provide alternate means to assess invertebrate diversity. We compared invertebrate diversity in ponds (N = 18) with and without the crucian carp using sweep-netting and microscopy, DNA metabarcoding, and eDNA metabarcoding. Five 2 L water samples and 4 min sweep-net samples were collected at each pond. Netted samples were identified to lowest taxonomic level possible by microscopy, and these inventories compared to DNA metabarcoding of bulk tissue samples and eDNA metabarcoding of water samples. Crucian carp presence minimally reduced alpha diversity in ponds, but positively influenced overall beta diversity through species and family turnover. Ponds with the crucian carp contained different invertebrate species and families to ponds without fish, resulting in different community composition. Our results will guide pond management in relation to conserving the crucian carp alongside other biodiversity, and freshwater invertebrate assessment using molecular tools.

Fish monitoring using eDNA vs. traditional methods (T19)

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Sound environmental management decisions - in accordance with the EU WFD for aquatic ecosystems – mainly depend on reliable species presence- and distribution- data. Here we compare the outcome of historical records of fish in 100+ freshwater bodies (lakes, dams and rivers) and in the Baltic Sea with eDNA metabarcoding data retrieved once to two times at each site between 2016 and 2019. The analysis revealed that eDNA detects between up to twice as many species of fish compared to traditional survey methods. This was especially true for rare, elusive and newly invasive species. The species presence from eDNA was confirmed by sportfishing records, fishing, and interviews. A sound eDNA pipeline including data collection-, extraction- choice of markers- and metabarcoding pipeline underpins the potential for a future paradigm shift for environmental monitoring over large geographic areas.

Environmental DNA for the enumeration and management of anadromous fish (T20)

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The first generation of eDNA science has shown that DNA from organisms can be extracted from many different sources in the environment and identified taxonomically. The next generation will use this information for environmental management. To do this, we need to test whether eDNA can give us quantitative information because it is very useful to know if a population of a species is large or small, and growing or declining. We tested the ability of eDNA to produce useful quantitative information for monitoring spawning eulachon (*Thaleichthys pacificus*) populations and Pacific salmon (*Oncorhynchus nerka* and *Oncorhynchus kisutch*) returning adults and outmigrating smolts. We quantified eDNA concentrations and stream flow rates contemporaneously with two years of daily salmon weir counts in Auke Creek, near Juneau, Alaska, and with five years of eulachon mark-recapture population estimates from the Chilkoot River near Haines, Alaska. We show that the daily flow-corrected eDNA rate (eDNA concentration x stream flow) closely tracks daily numbers of both returning and outmigrating salmon. Similarly, the peak and area-under-the-curve of the flow-corrected eDNA rate respectively explained 84% and 90% of the variance in eulachon population estimates. eDNA thus promises accurate and efficient enumeration, but delivering the most robust numbers will need high-resolution stream-flow data, at-least-daily sampling, and a focus on species with simple life histories. Given adequate calibration, eDNA-based methods could be used on large spatial or long temporal scales to monitor fish populations at a fraction of the cost of traditional methods.

Fish diversity assessment in the headwaters of the Volga River using eDNA metabarcoding and species-specific qPCR (T21)

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The headwaters of the Volga River retain large reaches of lowland river characteristics with near pristine conditions providing a rare ecosystem to conserve in its own right, but also to serve as a reference study area for broader-scale biological assessments. An eDNA metabarcoding survey for ichthyofaunal diversity was carried out in the upper Volga catchment area, encompassing 48 samples across 11 sampling sites ranging from small tributary rivers to the main Volga river. Three barcode markers (16S and 12S rRNA, cytochrome b) were utilized for the genetic analysis. A total of 23 fish species were detected throughout all samples and their frequency and spatial distributions are discussed with relation to environmental and methodological aspects. Several species that would be expected in the sampled reaches were not detected. Hypothesized explanations include primer mismatches, deficits in spatial or quantitative sampling (for rare species) and lack of reference sequences for individual species. In a follow-up study, the reference database has been extended and the metabarcoding results re-analysed. Additionally, single species approaches using TaqMan qPCRs were carried out on individual species using the same environmental samples in order to evaluate the sensitivity of the universal (multi-species) metabarcoding approach. The findings of the follow-up projects help to (re-)interpret the results of the initial metabarcoding study and add valuable knowledge about potential methodology-inherent pitfalls in ichthyofaunal biodiversity assessments using environmental DNA.

Le Cren Medal Talk

Shifting headlines? Size trends of newsworthy fishes (T22)

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The shifting baseline syndrome describes a gradual lowering of human cognitive baselines, as each generation accepts a lower standard of resource status as the new norm. There is strong empirical evidence of declining trends of abundance and body sizes of marine fish species reported from docks and markets. We asked whether these widespread trends are also detectable in popular media, or whether news writers, like many marine stakeholders, are captive to shifting baselines. Our reconstructed trends of relative size of newsworthy fishes over time yielded evidence of a shifting baseline syndrome in news media over the last 140 years: overall, the relative length of the largest fish worthy of a headline has declined over time. This pattern was strongest for large, charismatic fish species, which are now reported in the media at smaller relative lengths than they were near the turn of the 20th century, and are now on average only 56% of their species-specific maximum length. The continued use in the media of superlatives to describe fish that are now a fraction of the maximum size they could reach does reflect a shifting baseline. Given that media outlets are a powerful tool for shaping public perception and awareness of environmental issues, there is a real concern that such stories might be interpreted as meaning that superlatively large fish still abound

JSFS Student Talk

Distributional pattern of black sea bream *Acanthopagrus schlegelii* estimated from environmental DNA: seasonal changes in marine and river waters (T23)

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Black sea bream *Acanthopagrus schlegelii* is a popular fish for commercial and recreational fishing in the coastal waters in Japan. This study examined the distributional characteristics of this species by detecting its environmental DNA (eDNA) with species-specific real-ltime qPCR. First, to confirm emission of the eDNA, fish were reared in tanks from fertilized eggs to six-month-old juveniles. The eDNA was detected in water from egg to juvenile stages, and the eDNA concentration increased proportionately to the square of body length. Second, monthly surveys were conducted from May 2017 to April 2018 to collect water samples at 12 stations aligned from the river mouth to 19 km offshore in Tango Bay, Kyoto, and 6 stations from the river mouth to 3.3 km upstream of Isazu River. In the sea, the eDNA was detected throughout the year within 3 km from the coast; during the spawning season (May and June), the eDNA was detected even at the most offshore sampling station. In the river, the eDNA was distributed consistently within 550 m upstream from the river mouth, and was detected up to 1.5 km from the river mouth during summer (May to September). The present study thus indicated that eggs and larvae of black sea bream drift offshore, then they stay in coastal and estuarine waters, and also migrate upstream in summer. The eDNA distribution in the sea was consistent with the seasonal appearance of eggs and larvae which had been reported based on conventional net sampling

A Comparison of Environmental Metabarcoding Efficiency and Trawling Survey to Monitor Fish Communities in Marine Ecosystem (T24)

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Environmental DNA (eDNA) metabarcoding is a promising molecular tool that allows for non-invasive, comprehensive and cost-effective screening of the entire ecosystem. We compared eDNA metabarcoding and trawl catch data to evaluate their efficiency (i.e. presence) to characterize demersal fish communities in the Estuary and Gulf of Saint-Lawrence Canada (EGSL). DNA samples were collected in seawater at bottom depth at 97 stations in the EGSL. eDNA was extracted, the mitochondrial DNA 12S gene was amplified using indexed universal primers (MiFish-U/E) and sequenced with using Illumina Mi-Seq sequencer. The findings demonstrated that eDNA is more efficient to assess species richness compared to trawl survey. Out of 86 species that were detected in our study, 70 species were detected by eDNA and 63 species by trawling. Twenty-four families were found with both trawling and eDNA, while five families were found only with eDNA and ten families were found only with trawling. Three key commercial fish species for EGSL were the most abundant species in both eDNA reads and trawl catch but in different portions. A redundancy analysis (RDA) revealed that the number of eDNA sequence reads for each species at all sampling sites significantly correlated with trawling individual catch and biomass data. Also, eDNA could detect species known to be less vulnerable to fishing gear, and can help characterizing fish communities in non trawl-able areas. Our findings therefore suggest that eDNA metabarcoding should be useful as a complementary approach to traditional capture surveys to document demersal fish biodiversity in the marine environment.

Environmental DNA exploration of Antarctic pelagic ecosystems (T25)

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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 partly overcome these challenges and could prove a useful complement to existing survey strategies. 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 during cruise JR16003 of the RRS James Clark Ross 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 limitations of either methodology in the context of long-term monitoring and their strengths in combination.

Hidden fish biodiversity: Using eDNA to characterise the hidden diversity of coastal fishes in South Africa (T26)

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South Africa is the meeting point of the Atlantic and Indian Oceans, where marine biodiversity thrives and coastlines range from cool-temperate to tropical climates. This dynamic oceanographic regime supports over 2,000 fishes that utilise a range of habitat types including coastal seagrass meadows, mangrove forests and rocky and sandy shores. Here we describe the fish biodiversity of these near-shore habitats using a metabarcoding method that, when compared with traditional methods, provides rapid species lists with reduced bias and less reliance on taxonomic expertise. We applied an aquatic eDNA metabarcoding approach for describing the distribution of South African coastal fishes. Extensive troubleshooting and method optimisation led to a biomonitoring workflow that encompassed multiple coastal habitat types, and generated large-scale datasets with a seasonal component. We have established the baseline knowledge for eDNA-based fish distribution in the region, and demonstrated that eDNA metabarcoding is a useful biomonitoring tool for South African coastal fishes. It proved successful across large spatial scales with the use of a single method that was inclusive of different coastal habitat types and climates, and across varying levels of coastal development and marine protection. Furthermore, we are enhancing the ongoing efforts of including genetic data into spatial planning, something that has proven beneficial to conservation outcomes, by using our eDNA datasets for creating priority maps that are accessible to policy makers.

Spatial distribution analysis of fish larvae in the Celtic and Irish seas using environmental DNA (T27)

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Global marine fish stocks are under increasing pressure from rising consumer demand and, with the addition of climate change stressors, larval life stages of commercially important stocks are rendered particularly vulnerable. Rapid and feasible methods are therefore needed to monitor the temporal and spatial distributions of fish larvae. Traditional assessment of larval communities is time consuming: taxonomic identification is sometimes complicated by the absence of discriminating characteristics, and traditional plankton sampling requires specialist equipment. To establish whether environmental DNA could be used as a rapid assessment tool for larval assessment, we analysed DNA from water samples at 14 offshore sites in the Irish and Celtic seas, using a metabarcoding approach. Physical larval samples from the same sites were used as positive controls. Using detailed temperature, salinity and nutrient data, we then test which environmental variables drive the observed larval distribution patterns and ascertain whether environmental DNA metabaracoding has the sensitivity to monitor spatial community level shifts.

Integrated biodiversity assessment in Mediterranean Marine Protected Areas (T28)

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Marine Protected Areas (MPAs) are widely proposed as spatial management tools to protect marine biodiversity. Assessing whether MPAs deliver such ecological benefit requires multiple monitoring techniques, which may be inherently diverse in terms of resolution power, cost, feasibility and ability to catch the multifaceted components of biodiversity. By interweaving four data-gathering techniques, we investigated how effective MPAs are in protecting teleost fish diversity, along with documenting the contribution of each technique to such diversity assessment. Over the summers of 2017 and 2018, fish diversity inside and outside eleven Mediterranean MPAs was monitored using a raft of sampling techniques: Underwater Visual Census (UVC) (N=1288), Baited Underwater Video (BUV) (N=740), photosampling of Small Scale Fishery (SSF, trammel nets) professional catches (N=782), and environmental DNA (eDNA) metabarcoding (using a mitochondrial 12S gene marker) of seawater samples (N=143; only in summer 2018). Overall, fish diversity was consistently higher inside MPAs than in adjacent unprotected zones, whatever the technique used, with each of them contributing differently to the global picture. The highest relative contribution in species richness was provided by SSF catches, followed by eDNA, UVC and BUV, respectively. eDNA analyses exhibited the highest yield/effort ratio, contributing most to the overall species list, despite the lower sample size employed. We suggest eDNA can be a useful tool to investigate MPA effects on fish diversity, while still advocate the use of standard non-invasive techniques (i.e. UVC and BUV) to reliably assess the relative proportions of species inside and outside MPAs.

Environmental DNA based approaches for marine biodiversity monitoring and evaluation (T29)

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Due to global change, marine biodiversity is declining around the world, which calls for measures to ensure a sustainable use of the marine environment and its resources. Development of such measures requires biodiversity surveys, which are generally invasive and costly. Recent improvements in DNA sequencing have led to the discovery of new genetic tools for marine monitoring. Among the most promising ones is environmental DNA (eDNA), which has the potential of providing information about the macroorganisms inhabiting a certain environment without the need of catching or seeing them. In this study, we have assessed the potential of eDNA to ease marine macroorganism biodiversity surveys. For that aim, we have amplified and sequenced a region of the 12S ribosomal RNA gene from the eDNA extracted from hundreds of oceanic samples collected across the Bay of Biscay, including different depths. The taxonomic assignment of the millions of sequencing reads obtained allowed detection of fish and elasmobranch species expected in the area. The comparison of the biodiversity estimated through eDNA and other methods such as fishing and sightings highlighted the potential of the eDNA based marine biodiversity surveys, and additional analyses showed that eDNA could be also applied for obtaining marine management relevant information such as biomass estimations and intraspecific variability assessments. Our study has also revealed challenges associated to the data generation and analysis protocols as well as to the reference database construction, which have been considered to build a set of guidelines for eDNA metabarcoding based fish biodiversity studies.

Unity makes strength: combining DNA-metabarcoding, SIA and microscopy to study the diet of myctophid fish in the Scotia Sea (T30)

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Lanternfish (Myctophidae) are a dominant fish taxon worldwide and a key component of open pelagic ecosystems. Within the Scotia Sea (Southern Ocean), they are the most abundant mesopelagic fish and, as generalist predators, they are known to play a fundamental role channeling biomass from small crustacean zooplankton to top predators such as marine birds and mammals. Focusing on five abundant species of myctophids, we combined three different trophic methods (i.e., Stable Isotope Analysis, DNA-metabarcoding and microscopy) to obtain the best possible estimates of diet using MixSiar, a new generation of mixing models. DNA-metabarcoding provided a comprehensive prey list, while microscopy of the food remains provided quantitative estimates of prey consumption. Using these data as informative priors for the model, we could identify patterns of consumption based on the isotopic signature of the myctophid predators and their main prey. The set of prey identified by DNA-metabarcoding was roughly ten times more diverse than that identified using traditional microscopy of the stomach contents. The method proved particularly useful in identifying soft-bodied prey such as cnidaria, polychaeta or ctenophora, whose frequency of occurrence in the diets was consistently higher based on the molecular method. The mixing models indicated that gelatinous carnivores were one of the main prey groups for the myctophid fish, challenging the traditional view of these mesopelagic fish as generalist predators of crustacean zooplankton. Our study suggests that gelatinous carnivores are an important and neglected component of Southern Ocean food webs and highlights the potential of DNA-metabarcoding to improve our understanding of pelagic food-webs.

Metagenomic identification of diet in species targeted by South-western Atlantic commercial fisheries (T31)

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Determining dietary overlap between commercial fisheries species is important for understanding possible effects of fishing pressure on stocks of the species in any given region. However, traditional stomach content analyses often fail to identify dietary items to low taxonomic levels. Metagenomic analysis provides a reliable method for defining overlap using either taxonomic associations or, when lacking a reference sequence, MOTU assignment. Stomach samples were obtained from over 250 individuals during onboard sampling in early 2016 as part of a combined monitoring plan for Southern Brazilian pelagic fisheries. These represent 28 species, including not only the targeted pelagic species but also many benthic and demersal species. Where possible at least three stomach samples were analysed per species as a first pass to describing the trophic preferences of each species. Sequencing was performed via an amplicon approach using three primer sets (Chord-16S, Ceph-16S, and MiniBar-COI) to maximise taxonomic coverage, and run on the IonTorrent S5 platform. We present the results in terms of patterns of similarity and dietary overlap between individual taxa as well as between functional trophic groups and taxonomic groups of the commercial fish sampled. We also note that the information on biological diversity generated can also be used for future analyses of regional diversity trends and the identification of potentially undescribed taxa in conjunction with traditional molecular identification approaches such as DNA barcoding.

Predicting fish disease from eDNA (T32)

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Predicting epidemiological patterns across complex interconnected networks is challenging when information regarding parasite and host distribution is difficult to obtain. In this study, we harnessed the power of eDNA based detection to reveal drivers of disease outbreaks in wild salmonid populations. Proliferative Kidney Disease (PKD) has caused major declines of trout populations in Europe and causes major economic losses in aquaculture. The disease is caused by the myxozoan parasite *Tetracapsuloides bryosalmonae*, which uses freshwater bryozoans and brown trout as its hosts in Europe. The spores of the parasite are released into water and are readily captured by filtration approaches. We used spatial and temporal water sampling to detect and quantify the parasite and the bryozoan host eDNA in a single river catchment. This data was used to validate hydrologically based models that reconstruct the upstream distribution and abundance of the two target species throughout an entire river network. Our approach is generally applicable and can be used to predict biomass of any organisms across any interconnected network structures, given quantitative eDNA data. We show that epidemiological predictions of PKD outbreaks and prevalence across the catchment can be related to the bryozoan host biomass as detected via eDNA. Further, we show that infection prevalence in trout populations reflects the parasite load in water samples. Our general model of eDNA transport may thus be applied to predict the abundance of disease agents in the wild and provides a tool for future epidemiological predictions.

eDNA as a tool to monitor and assess disease risk from emergent parasites (T33)

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Species translocation leads to disease emergence in native species of considerable economic importance. Generalist parasites are more likely to be transported, become established and infect new hosts, thus their risk needs to be evaluated. Freshwater systems are particularly at risk from parasite introductions due to the frequency of fish movements, lack of international legislative controls for non-listed pathogens and inherent difficulties with monitoring disease introductions in wild fish populations. Here, we will use one of the world's most invasive freshwater fish, the topmouth gudgeon, *Pseudorasbora parva*, to demonstrate the risk posed by an emergent generalist parasite, *Sphaerothecum destruens* and discuss how eDNA detection of the pathogen could assist in disease monitoring and control. *P. parva* has spread to 32 countries from its native range in China through the aquaculture trade and has introduced *S. destruens* to at least five of these. We systematically investigated the spread of *S. destruens* through Great Britain and its establishment in native fish communities through a novel environmental DNA detection assay. Here, we present data on eDNA based disease detection, evaluate the risk of disease emergence from this cryptic fish parasite and provide a framework that can be applied to any aquatic pathogen to enhance detection and help mitigate future disease risks in wild fish populations.

Inland water fish skin microbiome is governed by the water chemistry and the fish species, according to a metabarcoding study of skin swabs (T34)

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Fish skin is a major site of protection against mechanical, chemical and biological insult. While the immune system is active in the skin mucus, some microbes are able to evade it and establish symbiotic relationships of various natures with the fish. This microbial community has been studied mostly in marine fish with high commercial impact, showing that the ambient microbial "seed bank", the fish nutrition and the fish species, are its main shaping factors. Interestingly, little fish-skin microbiome research has been carried out to account for the ecological well being of fish and their habitats in inland-water systems, although such systems can contain highly interrupted sites. We set out to study the factors shaping the skin microbiome in over 15 species inhabiting 16 sites in the Jordan River water system, and predict the possible function of these microbial communities, as far as knowledge of their taxonomic composition would allow. Several tilapine and cyprinid species are ubiquitous in the system and are found in sites with diverse chemical water characteristics (e.g. 0.3 - 3.1 PPT, 15-32°C). Our sampling approach was uniquely non-destructive, as fish skin was swabbed on site and fish were immediately released. The microbial water "seed bank", strongly correlated with the chemical attributes of the site, and the fish species, almost equally determined the skin microbial community composition. We propose that the environmental range in which a fish can be observed is partly determined by the skin microbiome composition which is enabled in each site.

FSBI Medal Talk

Exploring the mechanistic basis of social behaviour in fishes (T35)

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Individuals within species show tremendous variation in physiological and behavioural traits. Over the last decade there has been a surge of interest in the ecological and evolutionary importance of this diversity, but the vast majority of this work has been performed on isolated animals. In reality, however, most animals - from insects to mammals - do not live in a vacuum, but instead live within complex social structures. Social influences may override links between traits that exist in solitary animals. Conversely, an individual's standing within a group may be an important factor generating intraspecific variation. I will review some of our recent work examining the interplay between social behaviours and metabolic traits within various fish species and discuss how such links may be critical for understanding how fish will respond to anthropogenic environmental stressors.

eDNA metabarcoding as a tool for monitoring migratory fish in rivers (T36)

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Migratory fish in England (twaite and allis shad, Atlantic salmon, sea trout, European eel, smelt, river and sea lamprey) are protected under a variety of legislative drivers, yet they are difficult to monitor in freshwater systems. Here we present the results of a pilot study carried out by Natural England and NatureMetrics to assess the usefulness of eDNA as a tool for routine monitoring of migratory fish in rivers. Natural England staff collected multiple eDNA samples from each of three locations on the River Frome and three stations on the River Wye, using manual filtration. 12S metabarcoding was subsequently carried out by NatureMetrics following an established protocol. Migratory fish species detected included European eel (*Anguilla anguilla*), Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*), along with 25 other estuarine and freshwater species. Results provided information about the upstream dispersal ability of several marine species and also revealed a high level of consistency across samples in terms of both species composition and relative sequence abundance. This suggests that quantitative data, while imprecise, is nevertheless meaningful and replicable in lotic systems. The study also demonstrated the utility of eDNA metabarcoding as a surveillance tool for detection of invasive species, even when this is not the primary purpose of the monitoring. The invasive sunbleak (*Leucaspis delineatus*) was detected near the mouth of the River Frome.

PISCeS – Pathway to Increase Standards and Competency of eDNA Surveys: Canadian Experiences of Fish and Habitat Conservation (T37)

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Canada holds within its borders a panoply of aquatic habitats that is home to a diverse collection of fish species with concomitantly complex ecological interactions, including both native and non-native taxa. Constant monitoring of this piscifauna is essential to maintaining Canada's bountiful aquatic biodiversity. eDNA can expedite and provide novel sources of data for biomonitoring surveys, in addition to being able to triage species and habitats under intense anthropogenic pressure. However, conducting eDNA surveys is not trivial. The molecular biology, statistical robustness of the sampling design and deployment of personnel to perform such tasks have to be of a certain minimal standard so that the collected data is reliable, the surveys repeatable and are able to stand up to regulatory scrutiny. Thus, eDNA data can stand on an equal footing with contemporary biodiversity metrics, be adopted in global databases, and inform policy-makers, managers, stakeholders and end-users. In 2018, the inaugural PISCeS (Pathway to Increase Standards and Competency in eDNA Surveys) meeting discussed minimal mandatory standards for designing and conducting eDNA surveys within Canada – and beyond. We shall show how this discussion sought to improve eDNA surveys of freshwater fish populations within a Canadian context, a country whose aquatic habitats are facing a deluge of biotic and abiotic threats. Finally, we invite European and Global participation in the next PISCeS meeting (2020) to further codify the optimization of the process of designing eDNA-based surveys of fish biodiversity and bring further into regulatory alignment the use of this extremely powerful conservation tool.

Developing an eDNA tool to produce a WFD-compatible classification tool for lake fish in Britain (T38)

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The EU Water Framework Directive requires robust methods for assessing the ecological status of freshwaters using different biological quality elements (BQEs), including fish. While methods have now been developed and successfully intercalibrated for most BQEs, a suitable and cost-effective method for monitoring and assessing status of fish in lakes has yet to be developed in the UK, where established methods such as gill-netting are strictly controlled or locally unacceptable. Research in Britain, funded mainly by environment agencies, has demonstrated that eDNA metabarcoding can provide both qualitative and, to some degree, quantitative information on fish communities in large lakes, outperforming established methods in terms of the number of species detected. The use of eDNA to determine lake fish communities has been tested in a limited number of British lakes of contrasting size, productivity and fish density, with initial findings suggesting high detection rates and accurate performance across a range of conditions. This brings the prospect of a robust Britain-wide tool for lake fish assessment tantalisingly close, but full exploration of the now expanded regional datasets on fish eDNA is required to make this a reality. In this talk we discuss ongoing work, using recently collected fish eDNA data, to validate fish eDNA sampling protocols and to produce a classification tool suitable for reporting the status of lake fish in Scotland, North England and Wales.

Environmental DNA as a long-term ecological recorder (T39)

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Environmental DNA techniques have the potential to become default survey methodologies for the rapid assessment of ecological status or small-scale environmental impact. However, exploring how eDNA data are comparable to long-term ecological datasets is more challenging, particular with regard to fisheries. Here, we report upon a high-resolution time-series of eDNA samples collected from diverse marine and estuarine habitats around the United Kingdom. These sites are subject to long-term monitoring using standardised, traditional fish survey methods, thus enabling detailed comparison. Therefore, in the context of measuring ecological change, we describe powers and pitfalls, assess costs and benefits, and consider the exciting opportunities.

Measuring river connectivity: can eDNA help assess the impact of hydropower on freshwater biodiversity in the River Ness catchment? (T40)

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Dams on the Garry and Moriston rivers in the Ness catchment were developed for hydropower in the 1950s, with one at the bottom and one/two in the headwaters of each river. Moriston salmon runs are stable or increasing but adult salmon numbers ascending to the Upper Garry have declined >10-fold since the 1950s. Flooding/loss of access due to hydro dams has eliminated 40% of salmon habitat in the Upper Garry. A key objective of EU AMBER project is to develop tools for assessing the impact of stream barriers, and for measuring stream connectivity. An initial eDNA assessment of the Garry and Moriston rivers was undertaken, with sites selected above and below barriers throughout the catchment. Impacted and unimpacted tributaries were compared, taking into consideration habitat covariates such as temperature and sediment transport, with the aid of data processed from drone images. eDNA was queried against fish, invertebrate and diatom markers, with the aim of reconstructing a significant proportion of the freshwater community and identifying potential barrier-induced disturbance. Differences with respect to presence/absence and their associations with environmental variables are being used to inform a second round of sampling to further test and validate preliminary findings. Recommendations will be formulated for further assessment and monitoring and for practical actions to change barrier management, in order to improve the health and resilience of the upper Garry and broader River Ness ecosystem towards restoring habitat quality and productivity.

Speed Talks

Optimizing eDNA protocol for monitoring endangered Chinook Salmon in the San Francisco Estuary: balancing sensitivity, cost and time (S1)

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eDNA methodology has gained traction as a precise and cost-effective method for species and waterways management. Papers on protocol optimization for eDNA focus primarily on DNA yield. Therefore, inventory cost and time invested for researchers to isolate eDNA aren't the primary focus when designing or choosing an eDNA pipeline. At the same time, these two parameters are essential for the experimental design of a project. In this paper, we ranked different eDNA pipelines, balancing time, cost and DNA yield. For estuarine waters, which are troublesome for eDNA measurements, we conclude that using glass filters and magnetic beads with an extra step for inhibitor removal is the most streamlined protocol. We believe that our findings are applicable to most aquatic environments, and provide a clear guide for determining which eDNA pipeline should be used for a given environmental condition.

Applying eDNA metabarcoding to monitor fish communities in a biodiversity hotspot under threat, the Mekong River (S2)

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The Mekong River in Southeast Asia is the 10th largest complex transboundary river system in the World, supporting the second highest biodiversity wealth globally, only behind the Amazon basin. The fish biota, comprising more than 50% of migratory species, is vital for the annual productive inland fisheries, with an estimated 2.7 million tonnes captured and economic value of over $US 7 billion. Additionally, for more than 70 million people living in the basin, fishes are the primary protein source, with a 45 kg average per capita consumption. Significant gaps in knowledge remain concerning the migratory behaviour over the flood/dry seasons, the habitats explored and role of the seasonal flood regime in driving those migrations. These issues urge to be tackled in light of the increasing pressures posed by future water infrastructures development in the basin and climate change scenarios, which are expected to significantly modify the flood/dry season's flood regime, threatening habitat availability, the migratory pathway and food security for disruptions in the annual catches. Environmental DNA (eDNA) metabarcoding is a promising tool for biomonitoring and conservation in freshwater systems. Yet, few efforts have been applied to large tropical systems. Preliminary key findings suggest significant differences for the species composition between sites, with 76% of species in the wet season comprising residents and medium-distance migratory species. In the dry season, 81% of species detected perform medium to long-distance migrations, with the five most abundant species contributing 40% to the annual fisheries. Floodplain and lake-habitats were vital for the species detected in both seasons.

Can haplotypes be recovered from environmental DNA? (S4)

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Understanding population dynamics is imperative for conservation and management purposes. Recent development of the non-invasive environmental DNA (eDNA) technique allows for extracting organismal DNA from environmental samples such as water or soil. While successful in obtaining biodiversity data, few studies have proven the ability to obtain haplotype diversity. We aim to develop an eDNA approach for describing haplotypic variation in marine species of commercial and conservation interest, i.e. Pāua (*Haliotis iris*). We are developing a controlled laboratory experiment to obtain multiple haplotypes in varying ratios from water. Water will be spiked with differing ratios of Pāua PCR products at a concentration of 100 copies/µL, and eDNA methodology will be used to extract the DNA. Once samples are sequenced, we will develop a bioinformatics pipeline to retrieve population genetic data of these target species. Relative sequence abundance will be compared to initial haplotypic ratios. The relationship between haplotype abundance and relative sequence abundance will be analysed. Through developing a methodology for discerning haplotypes from water samples, we hope to widen the door for non-invasive genetic monitoring via eDNA.

Using eDNA to detect small populations of non-native fishes – how many samples are needed to achieve a high probability of detection? (S5)

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Environmental DNA (eDNA) survey methods have shown considerable potential to inform management of non-native fishes, often out-performing traditional survey approaches in the early detection of new invaders and the mapping of their distributions. These applications may require the detection of small fish populations, present at low density within a water body, but limited information is available as to what level of sampling is necessary to enable a high probability of detection. As the financial cost of increased sample replicates is an important consideration to environmental managers, eDNA surveying needs to be considered within the context of the suite of more traditional sampling methods available. To this end, field trials were conducted to assess the level of sampling needed to detect a small fish population in a pond, using three methods: eDNA, trapping, and electric fishing. With restrictions on stocking of non-native fish in the UK, two species were used for these trials: barbel *Barbus barbus* (a native cyprinid, representative of benthic feeders) and rainbow trout *Oncorhynchus mykiss* (a non-native salmonid for which stocking is legal, representing a shoaling pelagic species). Results of these trials, in which both fish species were stocked into six fishing ponds (0.2 to 3.1 ha) at a density of 100 fish per ha, will be presented.

Hunting for the pumpkinseed sunfish (*Lepomis gibbosus*) in Sweden (S6)

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The pumpkinseed sunfish (*Lepomis gibbosus*) is an alien invasive species (AIS) in Europe, endemic only to North America. In 2018 two reproducing stocks of the species was found in Sweden, something not previously reported. To make a threat assessment, we needed to determine if the species had spread to other sites in the immediate area. This was done using two different sampling methods, electrofishing and environmental DNA (eDNA). Results from electrofishing showed that the pumpkinseed had spread from the pond. Individuals were found in the creek which leads from the pond to a larger river downstream. Results from eDNA (ddPCR) were all negative. No discoveries of the species (either with electrofishing or eDNA) were made in any of the larger rivers downstream. Later, the pond was emptied by the landowner and the County Administrative Board. Notwithstanding, soon after the draining electrofishing caught several pumpkinseeds 1.5 km downstream of the pond. It should also be mentioned that the summer of 2018 was very dry, with several dried out riverbeds. Therefore, it is quite possible that individuals still remain in isolated hollows, and may spread further downstream at high tides. Recommendations from the authors are to follow-up previous samplings and analyses during spring 2019, in order to determine if the species still exists in the system. eDNA and electrofishing are good tools for this.

Using eDNA metabarcoding to monitor fish biodiversity and to assess fish passage success in the River Mersey (S7)

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Anthropogenic physical barriers such as dams and weirs are known to be a major disruption to species movements in freshwater ecosystems. This is particularly relevant for the migration of anadromous and catadromous species that use river systems in order to complete their life cycles. Fish passages built on weirs and dams can be used to monitor variety, abundance, dimensions and seasonality of multiple fish species using camera traps or by physically collecting specimens. Environmental DNA is an alternative non-invasive tool that can be used to screen fish biodiversity and the effective use of fish passages. The condition of the River Mersey, NW England, has been extremely poor since the Industrial Revolution, with fish only recolonizing the river only after strong remediation efforts. With improved water quality, Atlantic salmon (*Salmo salar*) started to be reported again and in 2001 the fish passage in Woolston Weir, Warrington was adapted as a fish trap, allowing for the capture of 158 adult salmon in a 10-year period. Periodical DNA metabarcoding of aqueous environmental samples collected above and below Woolston weir is being performed. The eDNA approach will expand the data retrieved in previous years from trapping and add information on the difference in fish communities upstream and downstream of the barrier (e.g. shad, lamprey and eel). This non-invasive technique could provide more comprehensive and reliable data which is fundamental for an effective management of overall aquatic biodiversity in the River Mersey and to contribute to the debate of the effectiveness of fish passages.

Environmental DNA as a non-invasive sampling tool to detect the spawning distributions of European shads (*Alosa* spp.) (S8)

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Quantifying the distributions of endangered, invasive or rare species is essential for developing conservation and management plans, but is often difficult using traditional sampling techniques due to low population abundances. The anadromous European shads *Alosa alosa* and *Alosa fallax* (*Alosa* spp.) have high conservation designations due to the vulnerability of their populations to human disturbances. Their vulnerability is especially apparent during their spring spawning migrations into freshwater, where river impoundments block their upstream migration, preventing access to spawning areas. As the capture and handling of spawning adults can be challenging and their spawning generally occurs at night, determining the spatial extent of their spawning migrations is inherently difficult. Correspondingly, assessing the spatial extent of Alosa spawning can utilise non-invasive sampling tools, such as environmental DNA (eDNA). Here, an eDNA assay for detecting *Alosa* spp. was successfully developed, based on the Cytochrome c Oxidase Subunit I gene segment and quantitative polymerase chain reaction (qPCR). Application in spring 2017 and 2018 to the lower River Severn basin (Western England) revealed high sensitivity in laboratory and field trials. Field data indicated their presence in the river between mid-May and mid-June. Whilst migrants were mainly restricted to areas downstream of the furthest downstream impoundments, the eDNA revealed some individuals did bypass these apparent migration blockages. These results highlight how eDNA detection methods can quantify the freshwater use of cryptic, adult, anadromous fishes, and emphasises the importance of using non-invasive methods to understand how blockages to anadromous fish movements can affect their spawning migrations.

eDNA methods matter for tropical conservation: a review and case study from threatened sawfishes (S9)

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Biodiversity conservation is a worldwide concern and management of threatened species depends on reliable and accurate data. Despite global applications of environmental DNA (eDNA) to ecological research and conservation management, the majority has been conducted in temperate regions. We reviewed the challenges for tropical eDNA studies and suggest that limited capacity and logistical challenges inherent to tropical environments (e.g. higher temperature, humidity, UV, and seasonal rainfall) contributed to the lack of conservation-focused studies (i.e. <5% of articles between 1993-2018). Sawfishes were once widespread in the tropics but are now one of the most threatened marine fish families (IUCN Red List 2006). Contemporary occurrence across the majority of their historic range is unknown due to rarity and scale of sampling efforts required. In light of this, we explored the utility of membrane filtered eDNA samples as an approach for advancing sawfish monitoring (i.e., increase detectability). Samples from turbid waterbodies were filtered using 1.2 µM, 5 µM, 10 µM, and 20 µM membrane pore sizes, which revealed that ≥ 10 µm filters are required to filter ≥ 2L of highly turbid water. Longmire's preserved filters extracted using a novel precipitate-lyse-precipitate method retained sawfish eDNA (i.e., 5 detections across 20 sites) whereas ethanol preservation and commercial kit extraction yielded no detections. This sawfish case study demonstrates that methodology requires careful consideration, especially when targeting threatened species with important conservation implications. Moreover, Longmire's preservative is particularly relevant for eDNA applications in remote or logistically limited locations (e.g., alcohol bans, impracticality of cold storage).

Testing non-invasive technologies to monitor sharks and rays – a combined approach using eDNA metabarcoding and BRUV (S10)

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Assessing the presence, abundance and population status of marine elasmobranchs of high conservation concern is challenging given the harmful effects of many traditional sampling techniques and the logistical difficulty of studying these generally elusive, highly mobile and low abundance taxa. Our project FindRayShark aims to contribute to the conservation of sharks and rays worldwide by implementing a non-invasive approach to assess their populations, by combining baited remote underwater video surveys (BRUVs; mono, stereo and spherical) with environmental DNA (eDNA) metabarcoding of two barcode markers (COI and ND2) to survey the presence and abundance of elasmobranch populations locally. The project proposes a methodological workflow to assess eDNA metabarcoding efficiency in detecting species diversity, including validation of the method under controlled conditions, prior to implementation in the field. Results will be shown on the species detected with eDNA from water samples collected during pilot tests performed on a public aquarium, and during a field trial conducted in Faial Island, Azores, where BRUVs were also performed. The two methods will be applied in the Berlengas Marine Protected Area (western Portugal) in the Spring of 2019, where knowledge on elasmobranch diversity and abundance is scarce but urgent. The non-invasive methods optimised in this project can be applied in other understudied sites worldwide, allowing the proposal of management actions and good practice guidelines adequate for the context of the study area.

Investigating the mangrove productivity paradigm in relation to socio-economically important us fisheries (S11)

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Two thirds of the human population live in or near coastal areas which have caused extensive damage to coastal ecosystems. In particular, mangrove ecosystems have undergone extensive damage due to anthropogenic pressure. Mangrove forests play an integral role for the sustenance of commercial fisheries and fuel such economies. Previous research has focussed mainly on the effects of abiotic fluctuations such as salinity and temperature on mangrove communities. However, relatively little is known about the nuanced interactions between mangrove ecosystems and associated organisms. This project aims to map out trophic interactions of heterogeneous subtropical mangrove ecosystems and identify sources of energy exchange and predict the consequent impact on coastal fisheries. The aims of the project will be achieved firstly through meta-barcoding of gut contents from key mangrove species using a combination of COI, 12S and 18S markers. Along with gut samples, sediment and water samples will be collected to create a reference database which will provide biodiversity information on organisms that occupy the surrounding waters. Carbon and nitrogen isotope ratios will be analysed to reveal the main carbon resource pool and determine trophic positioning. This research will be conducted in Estero Bay, adjacent to the Gulf of Mexico, in collaboration with Florida Gulf Coast University. Estero Bay is surrounded by mangrove forests which are fed by different rivers offering an ideal juxtaposition of contrasting habitats. The results obtained will unravel vital information about how food webs are influenced by mangrove ecosystem heterogeneity and possible impact on fisheries recruitment.

Implementing an eDNA based method for assessing the spread of a listed disease: The Irish National Crayfish Plague Surveillance Programme 2018-2020 (S12)

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Aphanomyces astaci (crayfish plague) is a World Organisation for Animal Health (OIE) listed disease which causes mortality in the white-clawed crayfish *Austropotamobius pallipes*, the only crayfish species native to Ireland, which is protected under the EU Habitats Directive and is considered endangered. The first confirmed outbreak of crayfish plague in Ireland was recorded in the Erne catchment in 2015. Since then, it has been detected in six additional geographically distinct catchments, with three different strains of *A. astaci* genotyped. This genetic diversity suggests there have been at least three separate introductions of the disease into Ireland. An eDNA based National Surveillance Programme, funded by the Irish National Parks and Wildlife Service, was implemented in mid-2018. This programme aims to assess all Irish catchments with known *A. pallipes* populations for the presence of *A. astaci* over a two year period. The first season of sampling is complete with 15 of 30 catchments surveyed. eDNA analysis of collected water samples is on-going, with samples initially being screened for the presence of *A. astaci*, with later work in year two to look for the presence of non-native crayfish eDNA. The programme aims to add to the knowledge on the extent of spread of crayfish plague in Ireland as well as understanding the possible sources of the disease and its routes of transmission. Such data will be critical to developing disease control measures to protect the endangered white-clawed Crayfish in Ireland. Results from year one of the programme will be presented here.

Exploring the impact of oxytetracycline on gut microbiome homeostasis in rainbow trout (*Oncorhynchus mykiss*, W. 1792) (S13)

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Intensification within the global aquaculture sector has led to an increase in disease outbreaks and a reliance on chemotherapeutants including antibiotics to maintain production. As up to 75% of the antibiotics used in aquaculture have been estimated to enter the surrounding environment, antibiotic agents used in these production systems are also biologically available for uptake by wild fish. Whilst antibiotic-associated disruptions in the gastrointestinal (GI) microbiota have been documented for a number of vertebrate species; their impact on the fish microbiome remains under explored. Likewise, further evidence-based studies are required to better understand how microbiome disturbances impact on fish health as the microbiome is thought to be important for host physiology and defence against invading pathogens. The following study therefore investigated the effect of a broad-spectrum antibiotic licensed for use in UK aquaculture, on the GI microbiome composition in rainbow trout. In this study, rainbow trout (n=39) at 152.8 ± 8.9g were exposed to a therapeutic dose of oxytetracycline (OTC), administered in the feed over seven days, followed by a 14-day withdrawal period. GI digesta from both control (fed non-medicated diet) and treated fish was harvested at six time points throughout the trial to characterise the GI microbiome composition before, during and after OTC treatment using 16S rRNA amplicon-sequencing. Results from this study could provide important evidence on the impact of antibiotics on the fish GI microbiome community which may inform better management strategies for fin-fish aquaculture, and reduce the impacts this sector has on wild fisheries and the environment.

Environmental DNA: A New Low-Cost Monitoring Tool for Pathogens in Salmonid Aquaculture (S14 + P25)

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Environmental DNA (eDNA) metabarcoding is a relatively new monitoring tool featuring in an increasing number of applications such as the facilitation of the accurate and cost effective detection of species in environmental samples. eDNA monitoring is likely to have a major impact on the ability of salmonid aquaculture industry producers and their regulators to detect the presence and abundance of pathogens and other biological threats in the surrounding environment. However, for eDNA metabarcoding to develop into a useful bio-monitoring tool it is necessary to (a) validate that sequence datasets derived from amplification of metabarcoding markers reflect the true species' identity, (b) test the sensitivity under different abundance levels and environmental noise and (c) establish a low-cost sequencing method to enable the bulk processing of field samples. In this study, we employed an elaborate experimental design whereby different combinations of five biological agents were crossed at three abundance levels and exposed to sterile pre-filtered and unfiltered seawater, prior to coarse filtering and then eDNA ultrafiltration of the resultant material. We then benchmarked the low-cost, scalable, Ion Torrent sequencing method against the current gold-standard Illumina platform for eDNA surveys in aquaculture. Based on amplicon-seq of the 18S SSU rDNA v9 region, we were able to identify two parasites (*Lepeophtheirus salmonis* and *Paramoeba perurans*) to species level, whereas the microalgae species *Prymnesium parvum*, *Pseudo-nitzschia seriata*, and *P. delicatissima* could be assigned correctly only to the genus level. Illumina and Ion Torrent provided near identical results in terms of community composition in our samples, whereas Ion Torrent was more sensitive in detecting species richness when the medium was unfiltered seawater. Both methods were able to reflect the difference in relative abundance between treatments in 4 out of 5 species when samples were exposed to the unfiltered seawater, despite the significant amount of background noise from both bacteria and eukaryotes. Our findings indicate that eDNA metabarcoding offers significant potential in the monitoring of species harmful to aquaculture and for this purpose, the low-cost Ion Torrent sequencing is as accurate as Illumina in determining differences in their relative abundance between samples.

Augmenting fish biodiversity assessments through the incorporation of eDNA metabarcoding surveys into existing monitoring programs (S15)

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Monitoring marine fish communities is essential to allow for proper management of fish stocks and accomplish sustainable fisheries. The inaccessibility and vastness of the marine biome, however, imposes complications for monitoring methods achieving detection through visual observation. Baited remote underwater video (BRUV) is frequently used as a non-invasive technique to observe cartilaginous (Chondrichthyes) and bony fish (Osteichthyes) communities. However, BRUV's bias towards visually conspicuous taxa might lead to mismanagement, due to underrepresentation or false-negative detection of imperceptible and cryptic taxa. Incorporating a non-destructive, genetics-based monitoring method, such as eDNA metabarcoding, might help with improving the detection of marine fish communities. In this study, we compared the fish diversity detected via BRUV with eDNA metabarcoding. Seawater was sampled prior to BRUV deployment at ten sites including a marine reserve, local fishery grounds, and an aquaculture zone (Paterson Inlet, Stewart Island, New Zealand). Video surveillance lasted for 30 minutes per site to allow detection of the fish community in the immediate area, while five 2L samples per site were amplified using two established metabarcoding assays targeting cartilaginous and bony fish taxa. Initial results suggest both approaches target partially overlapping but distinct groups of taxa, with eDNA metabarcoding surveys outperforming video surveillance for the detection of inconspicuous species, such as the family Tripterygiidae (triplefins). However, abundance estimates and population-level information through characterization of broadnose sevengill shark (*Notorynchus cepedianus*) individuals were only obtained by BRUV analysis. Our results demonstrate the benefits of incorporating eDNA metabarcoding surveys into existing monitoring programs.

Posters

FISHeEST – Development of an environmental DNA method for monitoring fish in estuaries (P1)

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Estuaries are among of the most productive and valuable aquatic ecosystems, in terms of their ecosystem service provision. They are of direct importance to fish by providing nursery habitats and forming part of the migratory routes of several anadromous and catadromous species. However, estuaries have been degraded by pollution, habitat destruction and overfishing. In the UK, the ecological quality of estuaries is assessed under the Water Framework Directive (WFD) by monitoring key ecological groups, including fish. It is assumed this will continue post-Brexit. Natural Resources Wales (NRW) uses several netting methods to survey fish in Welsh estuaries, although they are expensive and destructive. Metabarcoding of environmental DNA (eDNA) may prove to be a useful complimentary tool, however its application to estuaries is understudied. FISHeEST aims to develop eDNA metabarcoding to survey estuarine fish biodiversity for NRW. The initial results from surveys conducted on the Dee estuary (North Wales) during October 2018 are presented. Their aim was to assess the ability of eDNA to resolve the transition from a freshwater to marine fish community, down the estuary, to indicate its spatial representativeness. Replicate surface water and intertidal sediment samples were collected at 15 and 8 stations, respectively, around low tide in a transect corresponding to historical and contemporary fish sampling locations along the Dee. Samples were then extracted and metabarcoding performed by Illumina sequencing a fragment of the 12S rRNA gene. Investigations into the ecological differences between six Welsh estuaries (2011 to 2016), using WFD netting data, will also be presented.

Standardization of e-DNA metabarcoding approaches for assessing and monitoring changes in the ichthyofauna in the oligotrophic ecosystems of the eastern Mediterranean Sea (P2)

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The ecosystems of the eastern Mediterranean Sea are undergoing rapid changes, mainly due to the ongoing invasion of alien species from the Red Sea through the Suez Canal, the so-called Lessepsian migration. The effects are quite evident in ichthyofauna, where numerous invasive species, including toxic and venomous ones, have established populations, alter community composition and impact fisheries. The environmental DNA (eDNA) metabarcoding approaches, which are rapidly advancing, can be a powerful tool to study and monitor these changes, if properly standardised. In the current study, we are standardising a DNA metabarcoding protocol for the detection of fish species from e-DNA collected by filtering water from marine coastal ecosystems of the island of Crete. One technical challenge, may be the low abundance of fishes and hence their e-DNA, due to the oligotrophic conditions of the eastern Mediterranean Sea, which may require higher quantities of water to be filtered. Different primer pairs are currently tested (for mitochondrial 16S and COI and nuclear 12S DNA fragments) in samples collected from tanks of the Cretaquarium and coastal ecosystems. The DNA metabarcoding data will be compared with the actual fish species composition and abundance in the aquarium tanks and in the natural ecosystems (from fisheries data), in order to check the accuracy of the method. Once the methodology is standardised, it can be routinely applied for assessing and monitoring changes of the ichthyofauna through a network of genomic observatories that are been established.

Bigger and better: increasing eDNA detection probability with larger capsule filters for rare species or in turbid waters (P3)

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When eDNA is applied for early detection of invasive fish or monitoring rare fish we are searching for a very low number of eDNA copies. To increase detection probability we prefer to use higher capacity capsule filters than is generally necessary for eDNA metabarcoding of fish communities. Those bigger capsule filters are capable of filtering dozens of liters of regular freshwater or several liters of turbid water that else would immediately clog common disc filters or smaller capsule filters. We implemented in the field and lab a new larger type of capsule filter and optimized the DNA extraction protocol to improve eDNA recovery and outperform existing methods to extract eDNA from these type of filters that due to their size contain tens of milliliters of preservation and storage buffer that needs to be concentrated into few microliters of purified eDNA extract. With our optimized protocol we obtained much higher final concentrations of eDNA copies after digital droplet PCR for multiple target species on extracts from a pooled water sample originating from both fish mesocosm experiments and natural water bodies that contained eDNA from several rare and invasive fish species.

Can haplotypes be recovered from environmental DNA? (P4)

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Understanding population dynamics is imperative for conservation and management purposes. Recent development of the non-invasive environmental DNA (eDNA) technique allows for extracting organismal DNA from environmental samples such as water or soil. While successful in obtaining biodiversity data, few studies have proven the ability to obtain haplotype diversity. We aim to develop an eDNA approach for describing haplotypic variation in marine species of commercial and conservation interest, i.e. Pāua (*Haliotis iris*). We are developing a controlled laboratory experiment to obtain multiple haplotypes in varying ratios from water. Water will be spiked with differing ratios of Pāua PCR products at a concentration of 100 copies/µL, and eDNA methodology will be used to extract the DNA. Once samples are sequenced, we will develop a bioinformatics pipeline to retrieve population genetic data of these target species. Relative sequence abundance will be compared to initial haplotypic ratios. The relationship between haplotype abundance and relative sequence abundance will be analysed. Through developing a methodology for discerning haplotypes from water samples, we hope to widen the door for non-invasive genetic monitoring via eDNA.

eCAP: Tracing capelin with environmental DNA (P5)

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There is increasing concern over the consequences of climate change on fish distribution and on fisheries production and food security. Climate change can be an additional environmental pressure on top of the many other environmental and anthropogenic factors which fish stocks already experience. 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 80 million EUR but dropped to 35 million EUR in 2016. Changing distribution of capelin around Iceland makes it 1) more difficult and expensive to assess the distribution of the stock with current methods and 2) more difficult for the fisheries to harvest this highly valuable resource. eCAP aims at developing a method using the recent technological advances in genetic which consist of: 1) collecting environmental DNA released by organisms in their environment for genetic analysis and 2) using portable and practical on-board DNA technique which can be used on any type of boats called LAMP system (loop-mediated isothermal amplification). LAMP 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 in real-time during 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.

Determining mesophotic fish biodiversity using environmental DNA metabarcoding and baited camera assessments (P6)

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Comprehensive data on the existing status of marine biodiversity is required to establish effective marine spatial planning and invasive species management plans. Mesophotic coral ecosystems (MCEs) have been proposed as the “lifeboats for coral reef ecosystems” due to their potential to provide insulation from fishing pressures, the effects of climate change and localized anthropogenic stressors. Here we investigate the utility of environmental DNA metabarcoding (eDNA; 12S rDNA mitochondrial gene) to determine the spatial organization of fish biodiversity found on Bermudan mesophotic reefs. Our study incorporated sites from three locations ~10 km apart on the eastern portion of Bermuda’s upper mesophotic reef system (30 – 65 m). To assess the efficiency of the eDNA methodology, the diversity of fishes characterized using eDNA were compared to the diversity detected using baited remote underwater video systems (BRUVs). Whilst the eDNA detections emulated the visual sightings of the BRUVS; both methods identified a high level of spatial specificity at the site level. Environmental DNA also revealed the presence of pelagic species not observed on the BRUVs footage indicating a higher level of detection efficiency. These data demonstrate the utility of the eDNA metabarcoding approach as a mesophotic biodiversity monitoring tool. This suite of methods can be utilized by managers to aid in identifying knowledge gaps in biodiversity monitoring programs.

Assessment of fish fauna in the eastern part of South Sea of Korea using underwater visual census and environmental DNA (P7)

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The coastal region of Korean peninsula is the fastest warming region in the western Pacific Ocean where climate impact on the temperate-adapted marine ecosystem is already visible. In addition, the Tsushima Warm Current, a tributary of the Kuroshio Current, enters the South Sea, flowing northeastward through the Korea Strait to the East Sea. The objectives of the present study is to reveal fish diversity in the eastern part of South Sea of Korea for conservation and management using underwater visual census (UVC) and environmental DNA (eDNA). Underwater visual censuses conducted over 17 months (2016 Nov.-2017 Oct. and 2018 Jun.-Oct.) and eleven seawater samples for eDNA analysis were collected during the 5 months (Jun.-Oct.) of 2018 from the coastal regions in the eastern part of South Sea, Korea. Each 1L water sample was collected through a sterile ϕ0.45-μm Sterivex-HV filter using a sterile 50-mL syringe. The eDNA metabarcoding detected 157 fish species, of which 28% (44 species) were also observed by underwater visual censuses conducted over a 17 months. In total, 188 fish species were detected by both UVC and eDNA. The present study reveal that over 57% fish species were occupied by new and rare tropical and subtropical fishes in the eastern part of South Sea, Korea.

Biodiversity studies of mesopelagic fish populations on the Agulhas Bank, South Africa, using complementary direct imaging and eDNA approaches (P8)

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Research on consolidated benthic habitats from the mesopelagic zone (200 – 1000m) is limited due to logistical constraints limiting which sampling methods can be used and the extreme nature of the environment. Deep water baited landers fitted with video and lighting systems can collect observation data on habitats and fish and invertebrate communities, however, the absence of natural light at these depths means that the systems require an artificial light source. While this artificial light is essential to collect visual data, it may have the undesirable side effect of scaring animals that are not accustomed to light, away from the systems. Furthermore, only a small area will be illuminated on the lander systems limiting the possibility to see species beyond the illuminated area. The collection of water samples simultaneously with the imagery will allow the sampling of eDNA which will shed light on the accuracy and comprehensiveness of the data obtained from the video footage. To test this a baited stereo-camera lander system, fitted with 2 x 1.7L Niskin water samplers was deployed at 10 sites between 300 and 800m depth on the western edge of the Agulhas Bank in South Africa. All data are currently being processed and the comparative dataset and review the potential benefits of conducting paired visual and eDNA sampling in deep sea environments will be presented.

eDNA methods matter for tropical conservation: a review and case study from threatened sawfishes (P9)

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Biodiversity conservation is a worldwide concern and management of threatened species depends on reliable and accurate data. Despite global applications of environmental DNA (eDNA) to ecological research and conservation management, the majority has been conducted in temperate regions. We reviewed the challenges for tropical eDNA studies and suggest that limited capacity and logistical challenges inherent to tropical environments (e.g. higher temperature, humidity, UV, and seasonal rainfall) contributed to the lack of conservation-focused studies (i.e. <5% of articles between 1993-2018). Sawfishes were once widespread in the tropics but are now one of the most threatened marine fish families (IUCN Red List 2006). Contemporary occurrence across the majority of their historic range is unknown due to rarity and scale of sampling efforts required. In light of this, we explored the utility of membrane filtered eDNA samples as an approach for advancing sawfish monitoring (i.e., increase detectability). Samples from turbid waterbodies were filtered using 1.2 µM, 5 µM, 10 µM, and 20 µM membrane pore sizes, which revealed that ≥ 10 µm filters are required to filter ≥ 2L of highly turbid water. Longmire's preserved filters extracted using a novel precipitate-lyse-precipitate method retained sawfish eDNA (i.e., 5 detections across 20 sites) whereas ethanol preservation and commercial kit extraction yielded no detections. This sawfish case study demonstrates that methodology requires careful consideration, especially when targeting threatened species with important conservation implications. Moreover, Longmire's preservative is particularly relevant for eDNA applications in remote or logistically limited locations (e.g., alcohol bans, impracticality of cold storage).

Fishing for Genes: Surveying fish biodiversity in nearshore Caribbean ecosystems using environmental DNA (P10)

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The tropical mangrove-seagrass-coral reef complex constitutes some of the world's most ecologically and economically valuable, yet threatened, ecosystems in the world. In order to protect these ecosystems from further degradation, highly-sensitive and reliable biodiversity assessments are crucial to informing proper management and conservation. However, traditional biomonitoring largely depends on in-situ visual identification and counting, which tends to be expensive, time-consuming, invasive, and dependent on taxonomic expertise. An alternative strategy is utilizing environmental DNA (eDNA), the genetic materials that organisms shed as waste into seawater. This study examines Caribbean fish biodiversity by comparing results based on visual censuses to those derived from eDNA metabarcoding of samples amplified using COI primers. Visual fish surveys were conducted at the same time as eDNA water collections in a total of 17 mangrove, seagrass, coral reef, and sand bottom habitats in Bocas del Toro, Panama from May to July of 2017. In total, 141 fish species were detected by the two methods, with 36 species found in both the visual surveys and eDNA approach. In general, while the eDNA approach uniquely detected more pelagic fish species, the visual surveys detected more reef-associated fish species. Primer choice, site and species-specific variability, and the completeness of the reference database likely affected the eDNA results while observer biases and variation in taxonomic expertise likely affected the visual survey results. Differences in abundance detection, whether estimating absolute abundance or relating sequence abundance to biomass, yielded contradictory fish biodiversity results by habitat and designations of fish species as habitat generalists or specialists. Our results suggest that although a combination of biomonitoring methods yielded a more complete picture of fish biodiversity in this tropical lagoon, visual surveys and eDNA metabarcoding can be further optimized to provide an even more accurate biodiversity snapshot in ecological studies and biomonitoring efforts.

Using environmental DNA to revolutionise the assessment of marine pelagic communities (P11)

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Standardised monitoring of fisheries stocks is vital for their effective management. 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 (Hz), the 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 during the PELTIC 2018 survey on the Cefas Endeavour around the South coast of the UK, from surface water during trawls, and from a CTD Rosette at pre-determined sampling stations. The PELTIC survey is used for pelagic stock analysis of economically valuable fish species including; European anchovy (*Engraulis encrasicolus*), Atlantic herring (*Clupea harengus*) and Atlantic mackerel (*Scomber scombrus*). DNA metabarcoding of 12S mitochondrial amplicons is being used to target these, and other fish species, and the spatial faunal composition of species will be compared to the hydro-acoustic and trawl data. The patterns obtained will be examined to achieve a better understanding of pelagic communities around the South-West of Britain and to devise improved approaches for the monitoring of marine biodiversity.

Biomass-dependent emission of environmental DNA in jack mackerel *Trachurus japonicus* juveniles (P12)

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Although recent field and laboratory studies on environmental DNA (eDNA) have yielded promising results, most have been conducted based on freshwater species, and fewer have targeted marine fishes. The present study was conducted to test whether eDNA emission is dependent on fish biomass, using the marine carangid jack mackerel *Trachurus japonicus*. Wild juveniles of *T. japonicus* were transferred into tanks at 1, 3, 10 or 30 individuals per 500-l tank, with 5–6 replications each. Approximately 1 l of water was collected from the overflow of each tank 72 h after transfer, then was filtered on a GF/F glass filter. DNA was extracted using a DNeasy Blood and Tissue kit. Quantification of eDNA was carried out by quantitative PCR with a set of species-specific primers and probe. Concentration of eDNA was highly dependent on the wet mass of fish in tanks and increased almost linearly with the density of fish. This is the first study to show linearity between eDNA of a marine fish and its density-dependent mass in tanks. Although eDNA concentration was variable among trials, sufficient replication may counterbalance such variations to achieve reasonable accuracy and precision in abundance estimation using eDNA.

Fish communities in Czech reservoirs: a comparison of eDNA and traditional methods (P13)

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Complex fish monitoring in large water bodies has long-term tradition at the Biology Centre of the Czech Academy of Sciences. The current sampling scheme covers all habitats by combination of several methods, which was recently extended by environmental DNA sampling (eDNA). The poster introduces and summarizes preliminary results of a project aiming to compare the structure of fish communities in three large Czech reservoirs based on traditional methods (electrofishing in littoral, trawling in open water and gillnetting in all habitats) and eDNA signal (DNA metabarcoding). The traditional methods are size and species selective. Moreover, the netting is significantly time demanding and invasive, and in case of gillnetting even highly destructive compared to eDNA. However, eDNA signal is not standardised yet, several methodological issues (e.g. impact of light, temperature, water flow) are unanswered and some population parameters such as fish size distribution cannot be provided by the eDNA. The combination of traditional methods and eDNA will be utilised to get the true picture of eDNA and fish communities.

ePIKE - The applicability of eDNA for monitoring pike abundance in Sweden (P14)

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Recent advances of eDNA technology suggest that quantitative eDNA can be an important complement to traditional fish monitoring methods. However, methodological issues remain before quantitative eDNA can be used practically; molecular techniques need to be developed and optimized, and eDNA-biomass relationships need to be described. Development of eDNA techniques for fish monitoring is particularly important for species currently lacking suitable monitoring techniques. The northern pike (Esox lucius L.) is one of the most important recreational fish species in Sweden. Pike is also a keystone predator important for ecosystem health and functioning, in both inland and coastal waters. Despite its ecological and socioeconomic importance, pike has been neglected in monitoring. One reason being the low catchability of pike using traditional monitoring methods, thus precluding accurate population parameters to be collected, leading to a poor scientific basis for management and uninformed decision-making. To increase our understanding of pike ecology and to allow management to make informed decisions, novel fish monitoring methods are needed. In this project, we aim to develop and carefully evaluate the performance of a non-invasive monitoring protocol for northern pike using eDNA. As a first crucial step, we start from comparative evaluations of the techniques and testing of emerging technologies, then, move from small-scale mesocosm experiments to controlled seminatural habitats and finally to natural habitats. We believe that our project represents a state-of-the-art approach which is expected to integrate eDNA methodology with non-lethal estimation of fish abundance

Application of environmental DNA (eDNA) based monitoring to assess the distribution of eels and coarse fish in pumped catchments (P15)

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The European eel (*Anguilla anguilla*) is a catadromous fish species with population trends presenting significant declines over the last three decades. Currently classified as 'critically endangered', concerns are so great that the European Union has specific legislation in place (The EC Eel Regulation, 1100/2007) regarding their protection from anthropogenic activities. *A. anguilla* must undertake two transatlantic migrations between their inland occupancy range and the Sargasso sea. With recruitment being so low, it is important that mature 'silver' eels are able to achieve passage out of river catchments to spawn. This research applies eDNA metabarcoding as a tool to monitor the distribution of eels and coarse fish in relation to pumping stations - which may disrupt migration and lead to fish kills through entrainment. Here the output from a preliminary study reveals the differences in species composition upstream of pumping stations. Findings suggest that eDNA surveys are more sensitive than traditional methods in the main river channel, and highlights the disparity between pumped tributaries and the main watercourse. Followed by discussion of using eDNA as part of an effective monitoring framework to inform the prioritisation of pumping stations - with the potential to enable more targeted management at such barriers.

Environmental DNA metabarcoding as an effective and rapid tool for fish monitoring in canals (P16)

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Environmental DNA (eDNA) metabarcoding has revolutionized biomonitoring of aquatic habitats. Man-made canal systems are among the least-studied environments in terms of biodiversity in Britain. In this study, we took advantage of a Canal and River Trust-commissioned electrofishing survey conducted over three stretches (i.e. between locks) of the Huddersfield Narrow Canal. This is listed as a SSSI site but there is a lack of baseline information about the biodiversity present in the canal. We compared eDNA metabarcoding with two types of electrofishing techniques (wade-and-reach and boom-boat). In addition to corroborating data obtained by electrofishing, eDNA provided a wider snapshot of fish assemblages (9 species with electrofishing, 16 species with eDNA). Given the semi-lotic nature of canals, we encourage the use of eDNA as a fast and cost-effective tool to detect and monitor whole fish communities.

Monitoring fish biodiversity with environmental DNA metabarcoding in small streams in the Brazilian Amazon (P17)

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The Amazon basin is home to the highest biodiversity of freshwater fishes on the planet. Such extraordinary richness of species occurs in large rivers, floodplains, and also in small tributaries. In the aquatic realm, the efficiency of environmental DNA metabarcoding has been widely tested for biodiversity monitoring, but tropical systems present additional challenges (high temperature, low pH, high turbidity and lack of references sequences). The efficiency of eDNA metabarcoding for fish monitoring (in addition to other biota) will be evaluated in the Reserva Florestal Adolpho Ducke, Amazonas, Brazil. The reserve is traversed by tributaries flowing into the Rio Negro, characterised by black waters containing humic substances, extremely acidic pH, and poor in nutrients. Given that long-term ecological monitoring started in 2004, the reserve represents the optimal location to compare traditional and molecular monitoring techniques. Fish presence will be obtained by DNA metabarcoding in January 2019 from four sites, subdivided in three transects (0, 25 and 50 m) from water and sediment samples. Results will be compared with traditional netting and long-term data, to compare and contrast the efficiency of eDNA to monitor fish biodiversity in black waters in the Amazon Basin.

From rare species detection to whole-community diversity using high-throughput sequencing of freshwater eDNA (P18)

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Understanding natural communities and ecosystems, and the services they provide to humanity, is highly dependent on knowledge about species composition and diversity through space and time. This is especially difficult in aquatic systems, where traditional census methods provide species compositions that usually lack rare species, which tend to go undetected. Detection of rare species is highly relevant when they are either threatened, or invasive at the earliest stage of invasion. One recent approach allowing detection of rare species uses environmental DNA (eDNA), present in water or soil, as traces of their existence. Here we propose to make use of recent technological developments in the area of high throughout sequencing to characterize freshwater fish communities and detect rare species, using a combination of eDNA metabarcoding and bulk eDNA metagenomics. This study will be conducted on the River Tagus (Portugal), which is inhabited by several rare fish species including both native and introduced taxa. In addition, the applicability of eDNA metagenomics for estimating the genetic diversity of populations will be assessed by comparing its results against those produced by traditional genetic screening of individual fish samples. For this purpose, two case study species will be studied: one highly endangered and rare species (the Lisbon arched mouth nase *Iberochondrostoma olisiponense*) and one recently introduced species (the European catfish *Silurus glanis*).

Development and establishment of molecular genetic (eDNA) monitoring methods for fish in waterbodies in the alpine region, and comparison with traditional ecological status assessment methods (P19)

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Within the Eco-AlpsWater project, the implementation of recently developed monitoring approaches aims to complement traditional methods for ecological status assessment for waterbodies in the alpine region with novel molecular methods and to provide a fish census of lake and river biodiversity. Ecosystem services provided by lakes and rivers are facing serious threats under the pressure of anthropogenic impacts, climate change, loss of biodiversity and occurrence of invasive species. The assessment of impact caused by these pressures are based on the criteria of traditional ecological status assessments, which are regulated by the Water Framework Directive (WFD). These traditional approaches are known to be expensive, selective and time- consuming. By using next generation sequencing technologies to analyze environmental DNA samples, collected in rivers and lakes in alpine regions, a fast and cost effective tool will be implemented to create taxonomic inventories at an unprecedented level. Furthermore, spatial distribution as well as seasonal changes in the fish community will be assessed. Various DNA extraction protocols were tested and optimized and the most promising protocol will be presented in the context of my contribution. The data acquired by the new approach will be compared to comprehensive data sets based on traditional fish ecological status assessment survey, to identify the limitations of the different approaches.

Environmental DNA as a non-invasive sampling tool to detect the spawning distributions of European shads (*Alosa* spp.) (P20)

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Quantifying the distributions of endangered, invasive or rare species is essential for developing conservation and management plans, but is often difficult using traditional sampling techniques due to low population abundances. The anadromous European shads *Alosa alosa* and *Alosa fallax* (*Alosa* spp.) have high conservation designations due to the vulnerability of their populations to human disturbances. Their vulnerability is especially apparent during their spring spawning migrations into freshwater, where river impoundments block their upstream migration, preventing access to spawning areas. As the capture and handling of spawning adults can be challenging and their spawning generally occurs at night, determining the spatial extent of their spawning migrations is inherently difficult. Correspondingly, assessing the spatial extent of Alosa spawning can utilise non-invasive sampling tools, such as environmental DNA (eDNA). Here, an eDNA assay for detecting *Alosa* spp. was successfully developed, based on the Cytochrome c Oxidase Subunit I gene segment and quantitative polymerase chain reaction (qPCR). Application in spring 2017 and 2018 to the lower River Severn basin (Western England) revealed high sensitivity in laboratory and field trials. Field data indicated their presence in the river between mid-May and mid-June. Whilst migrants were mainly restricted to areas downstream of the furthest downstream impoundments, the eDNA revealed some individuals did bypass these apparent migration blockages. These results highlight how eDNA detection methods can quantify the freshwater use of cryptic, adult, anadromous fishes, and emphasises the importance of using non-invasive methods to understand how blockages to anadromous fish movements can affect their spawning migrations.

Evaluating spatial distribution of aquatic animals by modelling water-borne environmental DNA (P21)

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Environmental DNA (eDNA) analysis, as a tool for assessing and monitoring aquatic animal distributions, depends on our understanding of biotic and abiotic factors that may affect the degradation and transport of water-borne DNA. The mechanisms of dispersal of cellular material and particle-bound DNA across open water systems, such as the ocean, remain largely unexplored. Yet, wave action, currents and tides are likely to affect spatio-temporal estimates of species distributions inferred from aqueous DNA. In this study the hydrodynamic model ROMS (Regional Ocean Modeling System) with the model's Lagrangian floats module, together with added eDNA persistence data, was used to simulate degrading material, aiming to predict the dispersal pattern of eDNA fragments. To validate this model, we chose the brush-clawed shore crab (Hemigrapsus takanoi), living mainly on oyster reefs in the Wadden Sea. Environmental DNA samples were taken along a regular grid centred at the reef, and over the area across which eDNA could disperse according to the model. Crab eDNA concentrations at each sampling point were determined using a qPCR assay. We were able to localize the eDNA source by retrograde calculation, suggesting that hydrodynamic models can successfully be coupled with eDNA persistence data to infer eDNA dispersion within shallow marine systems.

Investigating the mangrove productivity paradigm in relation to socio-economically important US fisheries (P22)

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Two thirds of the human population live in or near coastal areas which have caused extensive damage to coastal ecosystems. In particular, mangrove ecosystems have undergone extensive damage due to anthropogenic pressure. Mangrove forests play an integral role for the sustenance of commercial fisheries and fuel such economies. Previous research has focussed mainly on the effects of abiotic fluctuations such as salinity and temperature on mangrove communities. However, relatively little is known about the nuanced interactions between mangrove ecosystems and associated organisms. This project aims to map out trophic interactions of heterogeneous subtropical mangrove ecosystems and identify sources of energy exchange and predict the consequent impact on coastal fisheries. The aims of the project will be achieved firstly through meta-barcoding of gut contents from key mangrove species using a combination of COI, 12S and 18S markers. Along with gut samples, sediment and water samples will be collected to create a reference database which will provide biodiversity information on organisms that occupy the surrounding waters. Carbon and nitrogen isotope ratios will be analysed to reveal the main carbon resource pool and determine trophic positioning. This research will be conducted in Estero Bay, adjacent to the Gulf of Mexico, in collaboration with Florida Gulf Coast University. Estero Bay is surrounded by mangrove forests which are fed by different rivers offering an ideal juxtaposition of contrasting habitats. The results obtained will unravel vital information about how food webs are influenced by mangrove ecosystem heterogeneity and possible impact on fisheries recruitment.

Environmental DNA applications, research, regulatory acceptance and standards: a fisheries and oceans canada perspective (P23)

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As measured by the number of peer-reviewed scientific publications and newly funded federal projects within Fisheries and Oceans Canada (DFO), the use of environmental (eDNA) technologies in Canada is well established and growing. DFO enjoys a strong reputation in fisheries, aquaculture, and aquatic environmental science and research, and new tools such as eDNA technologies potentially can generate an unprecedented ability to detect species for biosecurity, biosurveillance, aquaculture, commercial fishery, and aquatic conservation management. Concomitant with increasing awareness of eDNA and evolution of its associated technologies, is the growing need to identify and address the challenges that government agencies face regarding the application of eDNA tools for permitting, decision-making, and regulatory purposes. There are important technical considerations that need to be better understood by DFO scientists and managers alike, and there is a need to make DNA-based protocols more transparent and understandable to non-geneticists in order for this technique to become a regular biomonitoring tool. Here, we showcase several large and new eDNA projects being implemented across Canada, and describe the beginning of a national dialogue on research priorities and other science needs that may be implemented across Canada in a cohesive approach to strengthen future eDNA research, regulatory promotion and acceptance, and eDNA standards.

Shifts in trophic feeding ecology of sunfish in Taiwan inferred from stable isotope analysis, stomach contents and reproductive biology (P24)

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Sunfishes of the family Molidae inhabit tropical and temperate ocean regions and generally feed on gelatinous species. Their feeding ecology may be influenced by environment variables, body size, and reproductive activity. To better understand drivers of feeding ecology in sharptail mola (Masturus lanceolatus), reproductive condition, stomach content analysis (SCA) and stable isotope analysis (SIA) were incorporated to model changes over temporal scales and body sizes off Taiwan. SCA showed sharptail mola consume various species and that the diet varied among season and body size. Planktonic (gelatinous) species (about 80%) were primarily consumed in autumn to spring and both planktonic (65%) and benthic (35%) species consumed in summer. SIA indicated that larger sharptail mola showed significantly higher nitrogen isotope values than smaller individuals. Histological analysis results suggested that the spawning period of sharptail mola in Taiwan is probably in spring (April to June). In brief, the combined reproductive and SIA approach suggested that sharptail mola had heterogeneous diets, and that they fed on diverse species during the spawning season. The shift in feeding ecology appeared to be influenced by body size, reproductive activity, and environmental features.