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Theme session Report

Growing together: prospects and opportunities for environmental DNA and Fisheries sciences

Conveners: Stefano Mariani (UK), Sofie Derycke (Belgium), Tommaso Russo (Italy)

Content

After the explosive advent of environmental DNA science in almost every area of ecology, it was highlighted that the discipline was now becoming mature enough to allow for new integrative research avenues to be pursued (Fig. 1): Session P explored one of these avenues: the blending of eDNA tools in the broader context of fisheries science. The session included 20 presentations and a handful of posters that truly represented the diversity of ongoing research efforts that are bringing these fields together (Fig. 2).

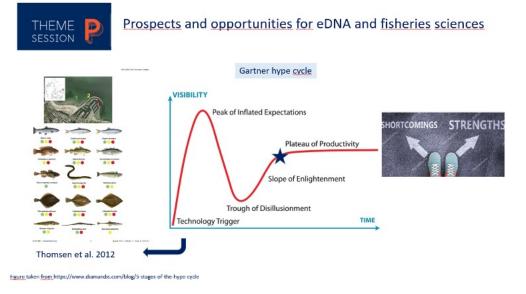


Figure 1: A visualization of the temporal evolution of a discipline: eDNA science conformed to the rapid burst of excitement shortly following its inception (about a decade ago), and may now stabilize into a variety of robust, promising research avenues, among which its interaction with fisheries.

These diverse contributions showcased the application of eDNA as a quick way to identify the composition of commercial catches, with examples ranging from the identification of pelagic catches to the investigation of illegal, unreported and unregulated species. In addition, several contributions illustrated that eDNA detects fishes and macrofauna that are not found with traditional trawl, video capture or acoustic data, thereby highlighting the complementarity of eDNA to traditional methods. The use of eDNA for fisheries is continuously evolving, and new methodological opportunities were shown at the level of eDNA collection, with passive filters/probes and at the level of DNA sequencing using handheld devices such as the MiniION sequencer. Finally, several contributions investigated the use of eDNA for biodiversity monitoring and illustrated that environmental conditions, habitat type, an adequate sampling design, contamination, spawning period and type of fishery need to be considered to generate reliable interpretations of eDNA data. The use of eDNA for monitoring temporal changes in fish communities and to look at foodweb structure were also demonstrated.



Figure 2: overview of the main topics that use eDNA in a fisheries context, as represented by the contributors of session P.

The session was without doubt a resounding success, with 118 attendees, the vast majority of which were there in person. The remarkable aspect of the event was the substantial engagement of nongeneticists, who flocked the session with the genuine desire to contribute to this burgeoning field. The "flash talks" helped the audience settle on the topic, while the recorded talks provided a useful resource that could be accessed at any time during the conference. However, the actual session was run as an open forum, in order to carry out a "SWOT" analysis (Strengths, Weaknesses, Opportunities, Threats) in the context of the synergy between eDNA approaches and fisheries science.

Strengths and Weaknesses were seen as intrinsic features of the eDNA processes and workflows, while the targets, priorities and constraints of fisheries were framed as the extrinsic factors. We assembled a considerable number of concepts that can arguably guide the path of interactions between these fields and ultimately produce brand new avenues for both scientific advancement and practical applications. The material that arose from the session is now being collated to produce a "perspective" article, with many of the contributors to the session involved as co-authors.

Conclusions

Those who actively carry out eDNA research (including the conveners) appeared elated by the enthusiasm and constructive energy that erupted from this session. It was a rather refreshing demonstration that when researchers do not work in "silos", true advancement of knowledge is produced. There was a palpable sense of timeliness about the discussion, which strongly suggests that we should pursue this line of scientific integration with confidence and optimism. The prospects for eDNA applications in fisheries science appear vast: from rapid catch composition assessment and by-catch detection, to stock distribution analysis, ecosystem impact evaluation, food web studies and participatory and citizen science. Importantly, at the end of the session, a number of ICES representatives contacted us to consider further expansion of this field within the ICES community. We are currently garnering feedback from several experts, with the aim of contacting the SCICOM Chair with some ideas regarding the ways we can bring these works forward.

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<u>CM 31</u>: Using environmental DNA to reconstruct target and byproduct catch composition for fisheries vessels

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Fisheries are an important source of food, income and cultural connection for millions worldwide. The pressure from fishing activity can have detrimental impacts on marine ecosystems and coastal liveihoods if not properly managed. Establishing appropriate management measures relies on understanding specific aspects of the fishery, for example what species were caught and recorded in logbooks. There are a wide range of reasons for wanting to reconstruct catch data from fishery independent data. Logbook records may be unavailable or inaccurately report landed species and biomass. Vessel operators or crew may take unregulated or prohibited species, either for sale or for personal consumption. Policy makers during international conventions (CMS, CITES) may make significant binding decisions and treaties with incomplete data. Unfortunately, trained observers who collect crucial independent data onboard vessels cover only a small percentage of total fishing activity. This leaves a significant opportunity for Illegal, Unreported and Unregulated (IUU) fishing practises, the likes of which pose a risk for the management and protection of vulnerable species.

This presentation will introduce a novel eDNA method for forensically reconstructing catch stored in the brine tanks of commercial fishing vessels. Our method allows for a small volume of water to be collected, sequenced and analysed to identify species and rank order abundance. The eDNA collected on-board fishing vessels represents animals that have been in the hold since it was last emptied, providing a time-integrated record of species catch and transport. We propose the application of our eDNA sampling protocol is a cost-effective tool for monitoring and surveillance, particularly for protected or quota species and in under-resourced regions.

Keywords: Fisheries monitoring, Improving logbook data, Genetics, eDNA

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<u>CM 118</u>: Comparison of three methods to monitor species diversity on soft bottom habitats

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Increasingly, non-extractive techniques are used by scientists to study marine communities. For instance, in several European countries, underwater video is now used for Nephrops stock assessment instead of scientific trawling. However, in muddy habitats such as those suitable for Nephrops, videos are often challenging to analyse and require large amount of time to get extensive data on marine communities. The taxonomic resolution is also often compromised due to water turbidity inherent to these habitats. In that context, environmental DNA is seen as a revolutionary tool to study species richness. To determine which method is the most appropriate to describe species diversity on soft bottom habitats, we compared three datasets available for the Grande Vasière area, a heavily fished area in the Bay of Biscay. The diversity obtained from bottom trawl hauls, video transects and seawater eDNA was compared. We determine the number of species common to each technique as well as the ones that are only obtained from one particular technique. Our results reveal that even if eDNA is powerful to describe marine communities, some species that are of importance for the ecosystem such as sea pens are not detected, probably due to their absence in gene databases or due to few DNA release by these species in the environment. We conclude that the different techniques provide different information and that no technique is exhaustive enough for the moment to be used alone.

Keywords: eDNA, underwater video, trawling, Bay of Biscay, species richness

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<u>CM 126</u>: Environmental DNA-based food web analysis as a tool for fishery impact assessment

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Monitoring exploitation of marine resources and assessing the status of marine stocks and communities are key activities in fisheries science. Most approaches to assess fisheries impacts are still based on single-species evaluation or on limited and often expensive monitoring of habitats and communities, while rapid and comprehensive evaluations of ecosystem structure changes in response to environmental perturbations remain few. Fishing activities are a major source of disruption to marine food webs, both directly, by selectively targeting components at specific trophic levels, and indirectly, by altering habitats and production cycles. A closer monitoring of food webs metrics, such as number of nodes, trophic links, trophic position, omnivory, connectance and redundancy can yield important information on the overall status of marine ecosystems and how they are affected by commercial fishing. Recently, new technologies have been developed to easily, quickly and costeffectively collect environmental DNA during fishing activities, opening new avenues for fisheriesdependent data collection. Commercial trawlers also tend to operate in highly impacted areas, where the assessment of ecosystem status is particularly urgent. By generating large, multi- marker metabarcoding data from eDNA samples obtained from commercial trawlers, it is possible to produce exhaustive taxonomic inventories for the exploited ecosystems, which are suitable for food-web reconstruction. Here, we use eDNA metabarcoding to assess patterns of biodiversity and reconstruct Mediterranean demersal food webs via a metaweb-based approach. We reconstructed food webs structure in nine locations along the southern coast of Sicily, based on the alpha-diversity obtained from eDNA samples collected during experimental survey operations. Network analysis of 9 sites characterized by different fishing pressures, revealed dense ecological networks composed of 125 (Site C-19)- 140 (Site B-19) species/nodes and a community that is highly packaged and modular (densely connected). Changes in architecture of complex species interactions between sites coincides with topological variations, followed by a variation in connectedness among nodes, and an increase in vulnerability of co-occurring species. We also note a shift in trophic levels of certain species that are likely associated with ecosystem alteration due to fisheries impact. Taken together, these findings demonstrate the power of eDNA metabarcoding to rapidly reconstruct informative food web models and serve as a descriptor of disturbance levels caused by anthropogenic activities such as commercial fisheries.

Keywords: eDNA, food-webs, fishery impacts, fommercial fishing, network analysis, metaweb-based approach, alpha-diversity

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<u>CM 131</u>: Nimble, low-cost, trawl-associated eDNA samplers upscale the assessment of fishing impacts on demersal ecosystems

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In the present context of rapid biodiversity loss and climatic changes, the need for effective management programs to monitor the status of environmental resources is a key point for their sustainable use. Nowadays, marine ecosystem monitoring mostly relies on ocean surveys still based on costly and time-consuming capture-based techniques, constraining our ability to understand the distribution and status of marine populations and communities. Fishery-dependent approaches, in which biological and environmental data are collected directly from fishing vessels, have the potential to drastically expand the reach of ecological monitoring, whereby fishing vessels operating across the oceans may serve as opportunistic scientific platforms to increase the strength and granularity of marine biodiversity data. We recently demonstrated the possibility of assessing catch composition of individual hauls carried out by trawlers by gathering environmental DNA aboard commercial fishing vessels using custom-made rolls of gauze tied to a hollow perforated spherical probe (henceforth the "Metaprobe") placed inside the fishing net. Here we illustrate catch and community composition reconstructed from eDNA captured in 24 sampling sites in the central Tyrrhenian sea, using a combination of a fish-specific (Tele02 12S) and a metazoan universal (Leray-XT COI) metabarcoding markers. The overall structure of the assemblages across the studied sites reflected expected differences linked to factors such as depth, distance from the coast, and fishing effort, which are known to be major drivers of community structure in Mediterranean demersal ecosystems. DNA metabarcoding data also returned a biodiversity 'bonus' of species not catchable by bottom trawl but present in the surrounding environment that can directly or indirectly interact with the net. Interestingly, 'bonus' taxa were mostly mesopelagic species, usually not targeted by bottom trawlers. Our results strengthen the idea that these low-cost eDNA-based tools can play a major role in upscaling the gathering of large data sets germane to both catch composition and the status of marine communities that sustain trawling activities.

Keywords: marine biodiversity, marine communities, fisheries, Mediterranean Sea, environmental DNA

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<u>CM 156</u>: Optimising eDNA sampling design for monitoring fish community composition in Arctic fjords

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The current marine biodiversity crisis has prompted decision makers to take action to reduce biodiversity loss. Biomonitoring is the key component for assessing ecological resources and it allows decision makers to adjust policies and management plans towards targets and sustainable use of marine resources. Multiple studies encourage the use of environmental DNA (eDNA) in biomonitoring, but optimized and standardized sampling protocols are needed for robust identification of fish community structures. Although significant improvement has been made on several workflow steps of eDNA metabarcoding such as laboratory and fieldwork protocols, the issue of optimal sampling design requires further research. In this study, we sampled three localities (fjords) with samples distributed among three depth categories (surface, pycnocline, and bottom) where we amplified a fragment of the mitochondrial 12S rRNA gene using the MiFish-U universal primers. Subsequently we: (i) estimated the contribution of each sampling station to detect fish community differences among localities; (ii) investigated significances of each sample depth category for vertical discrepancy of fish communities; and (iii) applied horizontal distances among sampling stations for spatial discrepancy. Additionally, we examined the influence of metabarcoding data treatment approaches such as qualitative (presence-absence) and semiquantitative (relative abundances) for inference of fish community differences. We found that: (i) contribution of each added station for finding significance showed fast diminishing return, added significance of each station accelerated rapidly within the first stations and then slowed down over the remaining stations; (ii) overall, samples taken in pycnocline depth were not significantly different from the other two depth categories and can therefore be removed without compromising the robustness of the study, and (iii) deploying samples with a distance between 5 and 15 km among each-other increases the detection of the variability within the fjord. Additionally, semiquantitative approach revealed significantly higher robustness for distinguishing fish community patterns compared to presence-absence approach.

Keywords: eDNA metabarcoding, sampling design, fish, spatial distribution.

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<u>CM 183</u>: It's time to take samples! Assessing the effect of time to improve understanding and monitoring of the biodiversity of Amazonian vertebrates

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Hydropower is the main source of renewable energy in the world and a key element in the fight against climate change and the transition to a low carbon economy. However, the construction and operation of hydroelectric plants may generate significant environmental consequences, especially in a region like Amazonia which harbors the world's most speciose freshwater and terrestrial biotas. Aiming to quickly and widely monitor changes in the biodiversity and sustainable management of natural resources, it is imperative to investigate new fast and low-cost methods that can easily bring reliable and robust results and information. It is exactly in this context that approaches based on the recovery and screening of the environmental DNA (eDNA) present in the water column stand out. Here, we explored the temporal variation in vertebrate richness, abundance, and composition detected in eDNA samples collected every 2 hours during 24 consecutive hours in the fish transposition system of the Belo Monte Hydroelectric Complex, located on the Middle Xingu River, Brazilian Amazon, using a brand-new sampling approach to collect eDNA. Namely, two rolls of gauze tightly fixed to a self-made plastic tube, that aims at funnel river water to maximize the potential amount of eDNA passing through the samplers, were used to gather eDNA from the surrounding environment. Then, a fishspecific (Tele02 12S) marker was applied. We registered 83 taxa on average, including freshwater fishes, terrestrial mammals (e.g., humans, dogs, and sheep), birds (e.g., antbirds, chickens), and reptiles (lizards). The richness and abundance trends registered in each of the two probes were not always highly correlated, showing that many external factors can influence the collection of DNA and even adjacent eDNA may provide different views of the community. Interestingly, temporal trends fitted with hierarchical generalized additive models indicated significant nictemeral changes with picks of richness and abundance during sunset and sunrise periods. Our results showed that temporal sampling designs are needed for eDNA studies to have a better representation of diverse communities. Although there are reservations, the results indicate that eDNA is a promising approach and that will be a valuable tool to study and monitor the composition of vertebrates in freshwater rivers with minimal impacts on organisms and habitats.

Keywords: hydropower, Amazon, biodiversity, vertebrates, environmental DNA, fisheries sciences, conservation

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<u>CM 193</u>: Assess without harvest: Pros and cons of eDNA seawater samples and video analysis to assess marine biodiversity

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Scientific studies, either for stock assessment or biodiversity surveys, are traditionally based on extractive techniques such as trawling or grab but are progressively being replaced by non-extractive techniques. For instance, while fish abundance indices can be obtained from baited remote underwater video, deep learning algorithms are not efficient enough to bypass the manual validation by human—a process highly time consuming. For biodiversity studies, eDNA seems appropriate and has provided solid results to describe species richness. In parallel, the recent advances in high-definition cameras now allow identifying species at a low taxonomic level (such as sponges, ascidians or crustaceans). Several of these species are poorly documented in gene databases or with sparse DNA in seawater samples due to limited DNA releases. To assess to which extent eDNA describes the entire community, we compared video and seawater eDNA samplings at several sites in the Bay of Biscay. The Ifremer ROV Ariane was deployed to record videos and to sample seawater at 1 meter above the seafloor.

Preliminary results show that a total of 127 taxa were found in the eDNA samples among which 25 were assigned to zooplankton species and were not used for comparison. 132 taxa were determined in the videos. The best represented phylum for both methods is Chordata with 37 Actinopterygii detected on videos *vs* 28 with eDNA. eDNA failed to detect Bryoza and Cnidaria that were well described using videos. The major difference for Echinodermata and Mollusca is the low detection of respectively Asteroidea and Cephalopoda in seawater samples. The phylum for which eDNA is the most efficient at detecting species is Annelida (23 species *vs* 7 in video). Our results suggested that both methods are complementary to describe a whole community.

Keywords: video, eDNA, species richness, Bay of Biscay

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<u>CM 200</u>: Can environmental DNA complement acoustic-based fisheries assessment in the mesopelagic? First insights from the Northeast Atlantic

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In recent years there has been an increased interest in exploring the mesopelagic realm (200m - 1,000m). This interest is not only driven by research questions regarding the ecosystem, its functioning and diversity, but also by a commercial interest in potentially valuable species such as Mueller's pearlside (*Maurolicus muelleri*). Much of the on-going research looking to investigate distribution and abundance of mesopelagic species is utilising hull-mounted acoustic data collection at several acoustic frequencies with 18, 38, 70, 120 and 200 kHz being most commonly applied. Collecting, processing, and interpreting acoustic data require a very specific set of skills and the processes are thought to be highly subjective and involve many environment-dependant ad-hoc decisions. Even trained and highly experienced acoustic signals are assigned to a particular group of organisms or even single species. Clarity about the nature of an acoustic signal on an echogram is often only brought about by complimentary biological sampling. This involves the deployment of various trawls and nets to great depth.

Biological sampling is, however, particularly challenging to conduct in the mesopelagic environment and common problems encountered include gear selectivity, the condition of (especially) fish when they reach the surface and the cost and time of carrying out biological sampling. The collection and analysis of environmental DNA (eDNA) may provide an unambiguous and time as well as cost effective way to complement on-going efforts in acoustic data interpretation. During the 2021 Irish Blue Whiting Acoustic Spawning Stock Survey we conducted depth-stratified sea water sampling at 5 stations in the Northeast Atlantic off the Irish shelf. Following eDNA extraction, Illumina High Throughput Sequencing using two mitochondrial DNA markers (12s and cytochrome b) and initial bioinformatics analysis we found that: (i) fish community profiles could be defined using eDNA and differed across depths and stations, (ii) our field controls contained a lot of contamination, (iii) spawning blue whiting caused "clogging" of DNA reads, (iv) over 50% of the sequence reads were not matched to entries in publicly available sequence libraries. We conclude that eDNA analysis shows great potential to enhance acoustic-based fisheries assessment, can provide insights into biodiversity of the deep scattering layer and may be used to confirm the presence of key mesopelagic species. Following form this study, important next steps are the adaptation of protocols to reduce crosssample contamination in the field and the expansion of mesopelagic DNA sequence reference data.

Keywords: mesopelagic, fisheries acoustics, environmental DNA, high throughput sequencing, deep scattering layer, biodiversity, Maurolicus muelleri

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<u>CM 235</u>: Developing novel eDNA metabarcoding tools for *in situ* fisheries and megafauna biodiversity monitoring

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Environmental DNA (eDNA) comprises intra- and extra-cellular DNA molecules that are released in the environment and can be traced back to the source organism by analysing an environmental sample, such as water or sediment. While many limitations still exist, the coupling and integration of eDNA sampling with both traditional methods (direct capture) and/or other non-invasive approaches (imaging and acoustic data) has great potential to enhance monitoring capabilities of the marine environment. In addition, emerging technologies (including portable sequencers) are enabling the development of protocols for near real-time *in situ* applications of eDNA analysis.

Our aim is to develop, test and implement eDNA metabarcoding protocols capable of being installed onto marine platforms such as marine fixed or mobile infrastructures (e.g. fixed observatories, platforms, research vessels) to provide real-time data of support to fisheries and other megafauna monitoring programs. An initial protocol has been already tested in laboratory conditions using a MinION portable sequencer (Oxford Nanopore Technologies), with preliminary results showing promising results in generating fish community profiles from eDNA samples collected at depth along the Northeast Atlantic continental shelf. Furter protocol development is ongoing and will include complete processing of environmental samples, from collection to High Throughput Sequencing (HTS) and bioinformatic analysis, in remote conditions. Specifically, results of protocol validation and implementation will be presented after execution in three contexts: (i) an aquarium with known species richness (Galway Atlantaquaria, Ireland); (ii) an open water fixed observatory equipped with a full suite of sensors including video (the Acqua Alta platform (Northern Adriatic Sea, Italy)); and (iii) offshore on board a research vessel during acoustic fisheries surveys (the Marine Institute's RV Celtic Explorer, Ireland).

The inclusion of eDNA in the monitoring and assessment of commercially and ecologically important species has the potential to drastically change how monitoring programs function by increasing the level of detail obtained and reducing the need for costly and destructive assessment techniques. The proposed methodological developments will make eDNA metabarcoding a more accessible tool to researchers and stakeholders, enabling near real-time data acquisition for augmented biodiversity monitoring and evidence-based fisheries surveys, and extending beyond the more traditional optoacoustic imaging technologies.

Keywords: eDNA, metabarcoding, MinION, biodiversity, fisheries, augmented ecological monitoring

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CM 240: Can eDNA predict catches in fisheries for small pelagics?

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Pelagic fisheries for sprat (Sprattus sprattus) and sandeel (Ammodytes sp.) aim to maximize catch per unit effort (CPUE) by diminishing search and trawl times. Environmental DNA (eDNA) based methods are increasingly being applied for monitoring of marine biodiversity, but so far, few studies have investigated the potential use of eDNA in the fisheries to track and predict offshore fishing catches prior to making the choice about fishing in a specific location. The aim of the present study was to examine whether quantitative PCR based (qPCR) analysis of water samples, collected by the fishery before and after fishing, was related to realized catches, and thus whether on-site eDNA analysis may constitute a potential means to maximize CPUE. Water samples were collected on-site by fishermen in connection with the two industrial fisheries for sprat and sandeel in the North Sea region between 2015-2016. All samples were filtered and DNA extracted. Sample concentrations of eDNA were analysed using species-specific qPCR and tested for correlations with catch weight reported by the specific vessels, as well as CPUE estimated based on the weight of the reported total weekly catches in a larger area around the sampling sites $(0.05 \times 0.05 \text{ degrees C-square})$. Finally, we also examined whether eDNA from water samples could be used as a predictor of the occurrence of bycatch of Atlantic herring (Clupea harengus), which is a bycatch species in the sprat fisheries and a potential 'choke species'.

Overall, the observed variation in qPCR results corresponded with fisheries targets and their herring bycatch. However, the correlations between eDNA and catch or CPUE were weak. Several factors can explain the observed variation and we discuss these and point to opportunities for improving the eDNA method for future applications in the fisheries.

Keywords: DNA quantification, pelagic fisheries, quantitative PCR, *Sprattus sprattus, Ammodytes* sp., *Clupea harengus*

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estuarine systems Using eDNA

Alison W. Watts, Laura C. Crane Jason Garwood, Jason S. Goldstein, Megan Lamb, Christopher Peter, Yoshimi Rii, Shon Schooler

eDNA monitoring is becoming more common in estuarine assessment, and has great potential for standardized biological monitoring across sites and regions. At the same time, application and interpretation of eDNA results can be challenging, and resource managers must have a clear understanding of both the strengths and limitations when evaluating eDNA-based monitoring data. To assess the viability of a standardized eDNA monitoring approach we are collecting water samples in coordination with existing long term water quality and fish monitoring programs in 10 estuaries in the United States. These sites represent very different conditions (e.g., latitude, geomorphology, temperature, salinity) and fish communities. By applying the same sampling and analysis method at each location we are able to compare the results from a standardized method to better understand the practical use of eDNA monitoring in estuarine systems. We seek to answer three questions posed by resource managers: *How many fish species are detected by eDNA sampling in a given estuary? How does eDNA-based monitoring compare to traditional fish surveys?* And *what is the relative cost of these methods?*

Initial surveys from 5 sites in New England, the Gulf coast, the Pacific coast and Hawaii found that results varied; in some estuaries the number and type of fish species was consistent with expected occurrence, and eDNA analysis detected fish that often eluded traditional sampling methods. In warm turbid waters, however, we detected fewer species than expected, and additional sample processing is required at these sites to improve detection. The overall cost of eDNA monitoring is generally lower than traditional fish surveys, and is most cost efficient if applied in coordination with existing water monitoring programs to reduce additional travel time, boat costs etc. In 2022 we are expanding from 5 sites to 10, and we will use this data to develop draft recommendations and best practices for incorporation of eDNA into regional fisheries monitoring programs, with a focus on standardization and transferability of methods and results. Managers interested in applying standardized eDNA monitoring across a range of sites may want to consider a decision tree protocol, where a baseline methodology is developed for all sites, enhanced by additional laboratory or analysis steps when specific conditions are present.

Keywords: Estuaries, eDNA, fish, managers

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<u>CM 245</u>: An eDNA based approach for quantitative assessment of bycatch in pelagic fisheries.

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Precise information about bycatch quantities is needed to make sound assessments of exploitation rates in commercial fisheries, which is specified under the EU Landing Obligation. Likewise, bycatch information is important for the fish processing industry to document the composition and quality of their products, and as a means for individual fishermen to document their catches in relation to vesselbased quotas. Reliable assessment of species composition in large catches (>500 T) is especially challenging, and the current practice of visual assessment of sub-sampled catches is time-consuming, requires extensive manpower, and has low resolution. In recent years, the analysis of environmental DNA (eDNA) has significantly improved, allowing for high-resolution species identification and possible quantification from water samples. In view of these developments, we explored the opportunity to apply eDNA based methods for studying catch composition and thereby derive quantitative bycatch information with high precision and at low cost. We investigated the case of the pelagic North Sea herring fishery with bycatch of mackerel and test fisheries-derived process water onboard vessels and at the processing factory for its suitability for reliable eDNA based bycatch quantification. We hypothesized that the water surrounding the fish will provide a well-mixed source of eDNA, thus providing an integrated signal of the catch composition. In our approach, we first experimentally simulate catches for a series of known weight proportions of herring and mackerel. These mock samples are analyzed using species-specific quantitative PCR (qPCR) targeting mtDNA, in order to estimate the relationship between input weight proportions and eDNA derived proportions. The relationship is subsequently used to estimate the weight of mackerel bycatch from eDNA from fisheries process water collected from different herring catches, both onboard the vessels and at the processing factory. Precision and accuracy of the eDNA-based estimates were compared to other commonly used metrics for bycatch estimation (logbook and visual catch assessment). We investigate if and how eDNA based estimates of catch composition are affected by: i) the distribution of the catch hauls in different holding tanks onboard the fishing vessel and ii) mixing of the process water at the factory, during the unloading process. We show that eDNA-based bycatch estimates provide robust reproducible quantitative results, and thus can improve quality and reduce costs of collecting fisheries-dependent data and thereby contribute to securing sustainable fisheries.

Keywords: bycatch, quantification, landing obligation, species-specific quantitative PCR

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<u>CM 281</u>: Cost-effective and non-intrusive monitoring of continental shelf biodiversity

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Rhodolite beds are aggregations of free-living red coralline algae that can extend down to 100 m in sedimentary continental shelves; these are also known as maërl beds. These beds are highly productive and an important biogenic deposit of calcium carbonate in the planet. The regenerative capacity of maërl-forming species is relatively low and these habitats are particularly sensitive to increases in suspended sediment, physical disturbance by bottom fishing gears and acidification due to climate change. In the Mediterranean, their protection is deficient and maërl beds frequently overlap fishing grounds. However, maërl beds in the Menorca Channel (Balearic Islands) have been partially protected due to an exclusion area around an underwater cable. In 2016, this protection was extended thanks to a trawling ban established in a large proportion of the channel. In consequence, there is an impact-recovery gradient that provides a unique case study to test novel techniques for the monitoring of key but hardly accessible mesophotic ecosystems, including environmental DNA (eDNA) and stationary video recording. Video records are useful to assess the distribution of habitat forming species, sedimentary features and biological traits of conspicuous fauna. eDNA provides information on those organisms present in an environment, including cryptic and rare species, which are not generally captured in video surveys. The complementarity between the two techniques allows non-intrusive biodiversity inventories linked to biogenic habitats at large scales over continental shelves and in the deep sea. To test the effectiveness of these techniques, a set of ten 1km-transects were selected covering the gradient of impact: sites close to the underwater cables; sites in the area that was closed to trawling in 2016; sites currently open to trawling activities. These transects were surveyed with stationary video and eDNA that allowed a remote characterization of habitat and associated fish biodiversity under different protection levels. The information provided contribute to feed ecological indicators of seabed heath status that can better guide large-scale cost-effective monitoring schemes in continental shelf ecosystems.

Keywords: non-intrusive sampling; video sampling; maërl beds; benthic communities; fishing impacts; seascape approach.

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<u>CM 284</u>: Characterization of reef fish communities in the Gulf of Mexico using eDNA metabarcoding

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Coral reefs are some of the most biologically diverse and productive ecosystems in the world. Not only do reefs provide biological support for the ocean, but they also provide physical support as they serve as areas for shelter, nursing, and feeding for many species of fish, including commercially-valuable as well as threatened and endangered species. Reef fishes, some of the more imperiled groups of marine fishes, play an important role ecologically and economically, therefore it is crucial to monitor their biodiversity and conservation status. How reef complexities can change the characterization of reef fish communities is important information for effective fisheries management. Effectively monitoring reef fish communities can be challenging due to the complexity of reefs and cryptofauna. Environmental DNA (eDNA) is a tool that does not require direct contact with target organisms and can provide an efficient way to detect species that may have recently been present at chosen sampling sites but have since moved on and would therefore not be captured with conventional techniques. Additionally, eDNA may detect small-bodied, cryptic, or benthic species that are conventionally difficult to sample and are not generally documented. The objective of this study was to utilize eDNA metabarcoding to characterize species richness of reef fish communities according to habitat type in the Gulf of Mexico. Specifically, how reef fish communities differ by reef relief strata, habitat complexity, and region was investigated. eDNA samples were collected on a NOAA Reef Fish Video (SRFV) survey cruise from predetermined, randomized sites in the Gulf of Mexico. Surface and benthic water samples were preserved using the precipitation method (precipitated and preserved 3M sodium acetate and 95% ethanol). DNA was extracted from the eDNA samples, followed by PCR amplification of a partial region of the 12S rRNA mitochondrial gene using universal MiFish primers. A total of 313 samples from 61 different sites were sequenced with high-throughput sequencing. Variation in reef fish species richness between different reef complexities was examined. How commercially important/exploited fish species are distributed among different habitat types were also assessed. As limited studies have examined the characterization of reef fish in the Gulf of Mexico, the findings from this study can be used to inform fisheries management and conservation efforts.

Keywords: environmental DNA, eDNA metabarcoding, reef fishes, coral reefs

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Hydroacoustics is a non-invasive, relatively cheap and largely autonomous tool, which provides a high resolution insight into organisms residing in the water column. In fisheries research it is routinely deployed from research vessels to map and quantify aggregative species like pelagic fish. However, despite decades of innovation in this technology, species identification remains challenging and ground-truthing, most commonly using (mid-water) trawling, is often required to verify the identity of the species detected from the acoustic backscatter. While hydro-acoustics can be deployed from most platforms, trawling requires specialist equipment and operators, takes time, can be destructive and is therefore expensive and restrictive. Moreover, the catch-composition of the trawl may not be a true representation of the organisms in the water column, due to different catchability rates among species. Environmental DNA (eDNA) is a non-invasive, rapid tool that identifies the presence and abundance of aquatic species by collecting the DNA footprint left by organisms residing in the water. Recent studies have shown a positive relationship between trawled fish biomass and the relative abundance of eDNA sequence copies ('reads'); yet, it is unclear whether eDNA can replace trawling to verify the species composition of fish schools from hydroacoustic data. In this study we collected eDNA samples during existing hydroacoustic pelagic monitoring surveys around the South-West coast of the UK, over two consecutive years, in 2018 and 2019. We first tested the similarities between hydroacoustic derived relative abundance against the relative abundance of fish species found from eDNA sampling using a mixed effect model and linear regression. Then, we compared the performance and accuracy of trawling and eDNA in identifying the fish species constituting the shoals of fish detected by hydroacoustics, focusing on the five most found pelagic fish species (sprat, sardine, herring, anchovy and boarfish). We find that, with some adjustment, eDNA alone can reliably capture the species composition responsible for the acoustic signal, opening the possibility for automated pelagic fish assessments and consequently increasing the range of surveys, over space and time, at reduced costs.

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<u>CM 343</u>: Lessons learned from the application of environmental DNA based approaches to fisheries management

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Ongoing research suggests that the analysis of environmental DNA (eDNA) has the potential of providing information about the macroorganisms inhabiting a certain aquatic environment (including their interactions) without the need of seeing or sampling them. Accordingly, an increasing number of studies are sampling and analysing eDNA from diverse marine and freshwater environments. However, evidence of the effective integration of eDNA sampling into marine fisheries monitoring programs is scarce, most likely due to a lack of i) standardized procedures, ii) a thorough evaluation of eDNA derived measures as indicators, iii) communication with relevant stakeholders and iv) technology transfer to service providers. Here we will present an integrated view of the results obtained during the course of several projects tackling these issues. In total, we have analysed more than 500 samples spanning three different aquatic environments (rivers, estuaries, and surface to deep ocean) focusing on diadromous fishes, commercial marine fishes and deep-water fishes, including diet DNA, and using community (metabarcoding) and species-specific (quantitative PCR) approaches. The results of our individual projects support the potential of eDNA for providing fisheries management relevant information through improving species detection, providing abundance estimations, evidencing temporal, horizontal and vertical variation, detecting reproductive phenomena, and revealing trophic interactions. Yet, they have also highlighted challenges associated to the data generation and analysis protocols (including inaccuracy and incompleteness of reference databases), which have been considered with the final aim of developing and evaluating eDNA based indicators to be integrated into the fisheries management process.

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<u>CM 360</u>: Deep-sea elasmobranchs monitoring and biodiversity assessment in the Azores: a comparison of different methodological approaches

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With the expansion of fisheries to deeper waters, more knowledge about the structure and status of the communities inhabiting those habitats is needed. Effective monitoring and management tools need to be built in order to ensure the sustainability of deep-sea fisheries.

Deep-sea elasmobranchs are characterized by low reproductive rates, slow growth, late maturity, which makes them particularly vulnerable to changes in their environment such as those caused indirectly or directly by fishing, even if only caught as bycatch in fisheries directed to other, commercially important, demersal species sharing the same habitats.

In the Azores, despite a fishing prohibition implemented on several species of deep-sea elasmobranchs, they remain a common bycatch of deep-sea longline fisheries. Monitoring of the demersal stocks in the region has been undertaken annually since 1996 by traditional scientific fishing surveys using bottom longlines, but limited catch has rendered difficult to draw reliable conclusions about stock status. Furthermore, due to the high vulnerability of deep-sea elasmobranchs, finding alternative non-lethal methods which can provide information for fisheries monitoring and management and promote their conservation appears necessary.

This work aims to compare the efficiency of two non-invasive techniques: environmental DNA (eDNA) and Baited Remote Underwater Video (BRUV) surveys, with traditional methods (longline fishing scientific surveys) to study the biodiversity and abundance of deep-sea elasmobranchs in the Azores. Sampling was conducted from 2019 to 2021 during annual bottom longline scientific surveys, spanning every island and several seamounts of the archipelago of the Azores, including the Condor Seamount marine protected area. Our data includes 58 bottom longline fishing sets, deployed along a depth gradient going from depths as low as 30m to a maximum depth of 800 to 1200m, resulting in the catch of 809 elasmobranchs belonging to 16 different species. BRUV surveys were carried out at depths between 700 and 800m in the vicinity and at the same time as the fishing set, recording an average of 5 hours of bottom time each, and resulting in 26 hours of video with 5 different species identified. For eDNA analysis, 53 water samples were collected after the associated longline fishing set and at its maximum depth.

To compare the biodiversity from each method we analyzed taxonomic presence-absence data. For relative abundance, we related CPUE values and eDNA metabarcoding reads, as well as maxN from video frames.

Keywords: deep-sea sharks, longline fishing, biodiversity monitoring, BRUV, eDNA

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<u>CM 419</u>: Unravelling the composition of vulnerable marine ecosystems in the deep Eastern Atlantic Ocean through eDNA tools

Alejandra Mejia, Joana R. Xavier

Vulnerable marine ecosystems (VMEs) are rare or unique ecosystems characterised by high functional significance, fragility, and structural complexity, together with low resilience to disturbance. In the deep sea, the vastest component of the ocean, VMEs encompass cold water coral- and spongedominated communities, such as Desmophylum pertusum fields and Pheronema grounds, respectively. Slow recovery rates make them extremely susceptible to human activity and are therefore of high conservation priority. However, conservation efforts are hampered by large information gaps on the distribution of these ecosystems, as seafloor exploration entails intricate logistics and vast economic means. Benthic studies in the deep sea typically rely on camera footage to detect and characterise communities. In addition to high acquisition costs, visual survey methods inherently have a limited taxonomic resolution. Metabarcoding of environmental DNA (eDNA) i.e. shed genetic material, is emerging as a powerful, fast and more affordable tool to aid biodiversity assessment and monitoring of aquatic habitats. In a single water sample, several species can be detected simultaneously by targeting short but highly variable portions of a gene with general PCR primers and next generation sequencing. Reads are contrasted with genetic libraries to identify potentially occurring species and infer the community composition of the study area. eDNA metabarcoding has been tested in a range of marine environments where it generally shows a good correlation with visual approaches and has even outperformed them. In the deep sea, this method has mostly targeted fish and meiobenthic species. Although it shows great potential in the detection of cold-water scleractinian corals, it is yet to be tested on a wider taxonomic range of habitat-forming species. This project aims to evaluate the potential of eDNA tools to identify VMEs in the Eastern Atlantic deep-sea benthos. We will develop an eDNA metabarcoding protocol to detect VME-indicator species from water samples and test its performance against camera-based surveys in well-studied areas. After calibration, we will apply this protocol to water samples from the Portuguese continental shelf and slope, which have not been visually explored but where VME-indicator species are frequently collected as bycatch from bottom fisheries. We hope to provide a fast, low-cost detection tool that facilitates the conservation and management of these crucial deep-sea habitats.

Keywords

Vulnerable marine ecosystem, environmental DNA, deep-sea, cold water coral, sponge **Contact:** Alejandra Mejia, CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, 4450-208 Matosinhos, Portugal, <u>amejia@ciimar.up.pt</u>

<u>CM 468</u>: Testing the efficiency of environmental DNA metabarcoding for detecting marine fish species using a public aquarium

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DNA-based tools, namely (e)DNA metabarcoding, have been increasingly adopted as an expedite tool to detect fish species. However, its proper application still requires some validation and optimization, particularly the volume of filtered water and the choice of markers and primers that should be used to capture fish biodiversity as thoroughly as possible. In this study we examined the influence of water volume (0.5, 1.0, 2.5, 5.0 and 10L) and mitochondrial marker genes and primers (COI, 12S rRNA, 16S rRNA) on the detection of the fish diversity present in a confined water body with a known species list. The largest Portuguese public aquarium, the "Oceanário de Lisboa", does not use water directly from natural sources, constituting a suitable controlled system to test the above-mentioned parameters. Here we report only data pertaining COI, for which we have completed the analyses so far. We employed two cocktails (A and B), specifically designed to target fish DNA, and the primer-pair mICOIintF/LoboR1, which has been shown to successfully recover marine metazoan diversity through DNA metabarcoding; all of them targeting internal segments of the COI barcode region. All the fish species present in the aquarium (61 in total, including 45 Actinopterygii and 16 Elasmobranchi) had reliable COI reference sequences in genetic databases. Preliminary results show that all COI primers combined detected 39 of the 61 fish species present in the tank (64%), however only 10% were simultaneously detected by all of them. The primer-pair mICOIintF/LoboR1 had the best performance, being capable to detect 36 of the resident species, whereas 22 were detected for cocktail A and 11 for cocktail B. Regarding the volume of water, we observed that increasing volumes from 0.5 to 5L resulted in a pronounced increase of the number of species detected, after which detection power started to saturate. Other fish species, that were also detected, and not listed for the aquarium, are likely to be food contamination or closely related sister species to the resident ones. The latter cases can be attributed to taxonomic uncertainty of either the species listed for the aquarium, or the identifications provided in reference libraries. Our research highlights the importance of using fair volumes of water and multiple COI primers to increase fish species detection, even in a small and controlled environment, such as an aquarium. Nevertheless, more research is needed and, when available, our data on 12S and 16S will also allow comparison among genetic markers.

Keywords: DNA metabarcoding, environmental DNA, biomonitoring, fish detection, controlled system

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<u>CM 558</u>: From blue sky to blue economy: Application of eDNA based methods for assessing mixed species samples in fisheries

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The application of Environmental DNA "eDNA" has revolutionized aquatic species monitoring, allowing information on the presence of multiple species to be generated simultaneously from species-specific DNA traces within water samples, at relatively high resolution, low cost and without requiring taxonomical expertise. Despite the many promising features of eDNA analysis, studies in natural open ecosystems do not come without challenges in the form of false positives and negatives as well as weak or missing correlations between species abundance/biomass and eDNA copy number.

With regards to such challenges, fisheries samples, such as mixed species catches and fish products, present a promising opportunity for testing technical and analytical aspects of aquatic eDNA analysis, and at the same time provide valuable data used for stock assessment, product content documentation, and control and enforcement. As mixed fisheries samples are from confined settings (hauls, catches), eDNA based results can be measured against corroborated species lists and known weight proportions. Catches moreover readily allow for the production of mock samples, thereby providing means for proper calibration and standardization of methods for both qualitative and quantitative analysis.

Here we evaluate the prospects and challenges for developing eDNA from a research tool into a validated routine method for quantitative assessment of catch content in mixed species fisheries, to the benefit of the fishing and processing industry and for control and enforcement. We point to a series of critical issues to address, ranging from design of sampling schemes, over DNA analysis protocols and DNA/weight calibration, to precision and reproducibility, before implementation into an industrial setting can be operationalized. We provide practical illustrative examples of the different challenges and how they may be overcome, based on our work on estimating catch composition in demersal and large pelagic fish catches, with the aim to document bycatch and unwanted catches in compliance with the EU landing obligation.

Keywords: Aquatic eDNA, mixed fisheries samples, bycatch, unwanted catch, landing obligation

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