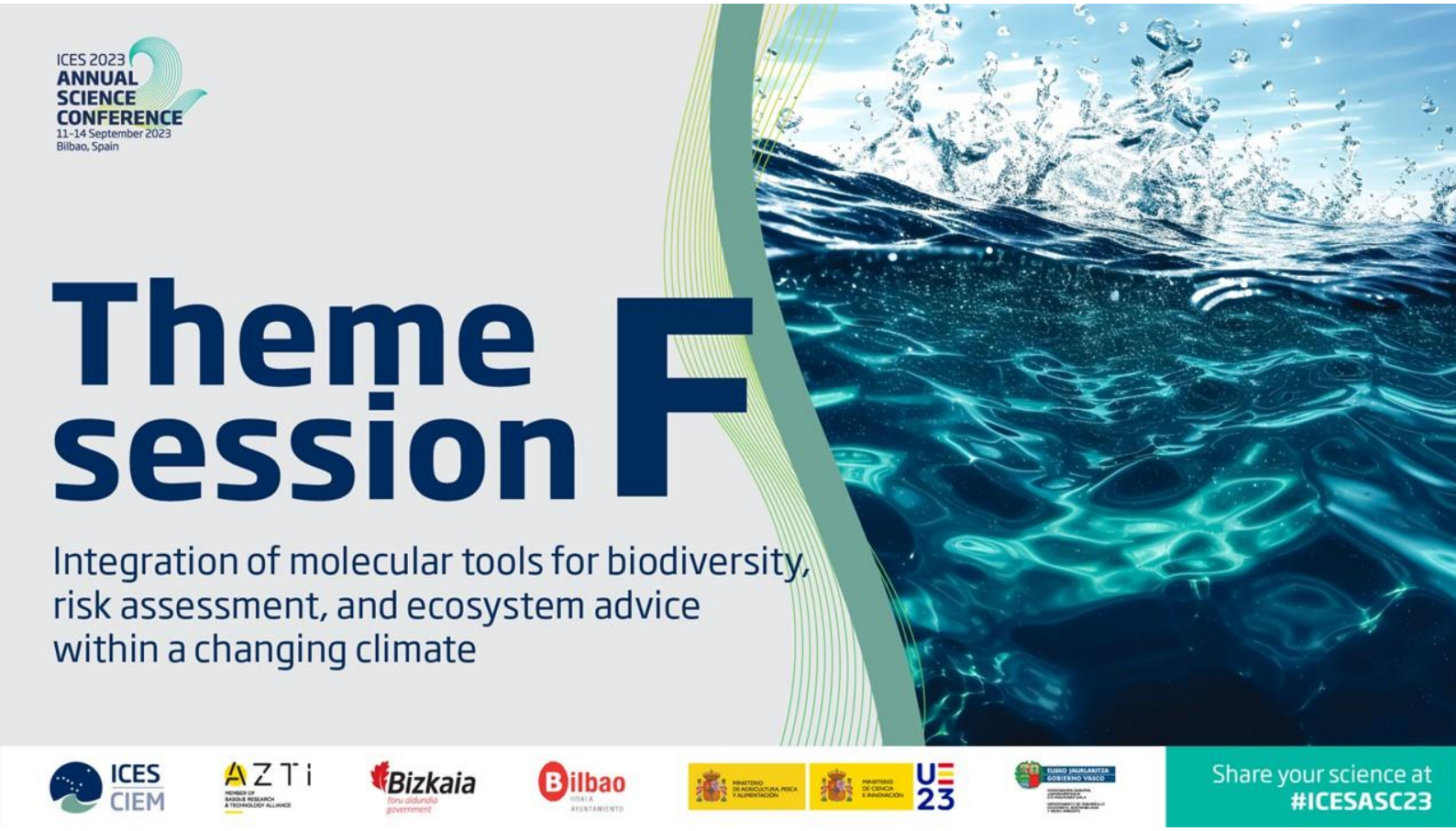

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Theme session Report

Theme Session F – Integration of molecular tools for biodiversity, risk assessment, ecosystem advice within a changing climate

Conveners: Dave Clarke (Ireland), Cynthia McKenzie (Canada), Rowena Stern (UK)

Introduction

Theme session F attracted 90 people attending in person with 44 people attending online via WHOVA app. This session was proposed to determine and assess the field of mature genetic methods deployed for identifying taxa in marine environments, with the session identified a number of areas where issues remain, that have prevented their use for implemented routine monitoring. The speakers and poster session covered a wide variety of habitats, geographical areas from Arctic to Mediterranean, and species diversity covering benthic, plankton, fish, and microbial communities. The talks were grouped into three main themes aligned with the session: molecular tools for investigating ecosystem processes in various environments, molecular tools for detection and monitoring, and molecular tools for bioassessments and advice including HABs, AIS, plastics, and climate change. Throughout the session, a number of polls were conducted by the convenors via WHOVA app for the audience to engage with, as well as a number of dedicated discussion segments to allow for full audience participation. There were 13 talks and 13 poster sessions, with Marina Parrondo (CSIC-IIM) winning the best ICES ASC 2023 poster award submitted for this session, Theme Session F. Many of the presentations were from early career scientists.

Amplicon sequencing or metabarcoding is the most mature genetic method to identify taxa from mixed environmental samples using a standard genetic marker(s). Most speakers presented genetic tools to conduct ecological assessments and seasonality of communities, their relationships in Marine Protected Areas, and appearance of non-indigenous species due to environmental changes. Talks on genetic methods as a tool to better understand multiple pressures and ecological interpretations were presented and discussed, with specific attention given to emerging rapid quantitative detection systems. This included a miniaturized HT-qPCR quantitative probe assay which can screen multiple shell fish species presented by Dennis van der Pouw Kraan (Atlantic Technological University), that would be a beneficial tool for conservation and monitoring. Laura Givens, Duke University, used third-generation Oxford nanopore technology to compare taxa associated with oyster aquaculture farms and natural oyster reefs, finding both habitats harbored similar taxa although their relative abundance were different. Oxford nanopore technologies does not require a bias-introducing PCR amplification step and offers improved relative quantification methods.

A good example of combined, multi-parameter studies was presented by Katja Metfies, which demonstrated a full year of monitoring marine plankton in inaccessible Arctic regions to track plankton community changes with sea-ice decline that supports the whole arctic community. This was the biggest arctic comprehensive monitoring programme that brought together decadal, annual and year-round MoSAiC project drifting observations. Combining these different-scaled observations and autonomous sampling, provided enhanced information on plankton communities from ice and water sample communities and the effects of polar day and night on plankton, revealing the fundamental role of ice to sustain primary productivity impacting carbon-flux and carbon export in Arctic waters. Rafaele Siano (IFREMER) presented the first European environmental DNA (eDNA) observatories in France to study microbiome and microbiological risks using metagenomics, metatranscriptomics and

metabarcoding. There are a number of current operational networks and projects engaged in integration and standardization of molecular-based observations.

During the session discussions, challenges were identified where bias was introduced by PCR amplification. Our session poll showed all 17 respondents experiencing biased datasets, and a suggested solution was to select for single copy markers and to prescreen the dataset with in-silico tools. Many talks used multiple markers to resolve taxonomic resolution or quantitative bias due to highly conserved or multiple copy markers. The use of more than one marker was recommended by most presenters. The importance of standardized methods and reference taxonomic databases was raised by many speakers. Taxonomic expertise is central to the development of comparative tools and reference databases. Most studies showed that genetic approaches showed rich biodiversity that was complementary with image studies, often picking up cryptic species missed by optical methods. The need to identify threatened species and create reference databases was being addressed by comprehensive metabarcoding studies. Nair Vilas Arrondo (IIM-CSIC) presented results of the creation of a reference database for 418 vulnerable invertebrate benthic species from deep-water seabed of Flemish Cap of the Northwest Atlantic Ocean. Ann Bucklin's presentation (University of Connecticut) showed the potential of eDNA for tracing sources and spread of zooplankton including, warmer water decapod species and potential non-native species from retrospective metabarcoding of archival samples collected by EcoMON surveys (NOAA) for 18 years, providing opportunities to develop predictive tools. Metazoogene Atlas (https://metazoogene.org/mzgdb/) was a unique mapping tool to investigate spatial and temporal trends in plankton. Presentations by Clio Hall, University of Helsinki, and Ana Sofia Lavrador (CBMA) showed metabarcoding reveals high taxonomic richness that provided complementary ecological information to microscopy datasets such as turnover and temporal connectivity. This level of detail allowed for community level differences to detect plastic fishing net pollution in seabed communities by network analysis (Alice Sbrana,University of Rome). The studies developed a wide-array of reference databases, most of them public and some regional, which raises the question of cross-standardization, maintenance and accessibility and FAIR practices for datasets.

Several presenters discussed challenges in accurate quantification of taxa. Nicole Caputo (Atlantic Technological University) and Nair Vilas Arrondo noted the effect of DNA yield, natural inhibitors on detection and quantification of taxa in benthic invertebrate and pelagic microbial species where internal controls were recommended. Droplet digital PCR allowed for direct and more accurate DNA quantification to detect species to allow for more sensitive detection (method reduces potential inhibition effects). Finally, two quantitative probe-based presentations using on-PCR based Oxford nanopore technologies and microfluidic based probe-based systems offer ways to scale up quantification of species.

One of our Theme session F topics, and a topic which was observed throughout ICES ASC 2023 was on providing science advisory tools for policy, regulation and management. Anders Lanzen presented applying genetic-based policy tools directly to address the Marine Strategy Framework Directive for microbenthos taxonomic communities. A comparison of existing tools, AMBI and microgAMBI, that evaluate multiple pressures on biodiversity was comparable with machine learning random forest methods in multiple environments.

Molecular studies of marine environments are still in its infancy, in comparison to traditional taxonomy methods, but offers complementary information to existing data. Presenters in Theme Session F still cite quantification gaps in taxonomic identification and standardization as current issues. However, there has been rapid progress since the last molecular study survey conducted by WGPME (Stern et

al. 2018), with better quantification tools, autonomy, and use of AI tools. There is a change of focus from taxa discovery to use of molecular tools for ecological interpretations and wider applications to conservation and policy for better insights. Our poll shows 8 out of 12 attendees think DNA tools are advanced enough for use in management and one study has demonstrated its applicability to policy.

Conclusions

Molecular approaches are tools that add enormous value to ecological studies and can be applied to habitat management goals. We recommend cross-disciplinary ToRs in relevant Working Groups, relating to data validation and ecological interpretation.

With changing technologies, many presenters were keeping sample archives as a valuable source for genetically acquired taxonomic or quantitative information. Molecular tools are still specialized and inaccessible routinely, so a unified platform for simple analysis tools would be useful. Importance was placed on accuracy, quality and representation in reference databases. We recommend further discussions and joint initiatives with database managers or centres to coordinate regional data and studies with basin-scale or global efforts according to FAIR principles, for standardization to assist in data re-use. These FAIR principles have been championed by the UN Decade for Ocean Science for Sustainable Development. The development of eDNA observatory networks and long-term genetic monitoring has shown multiple benefits especially in the context of other measurements. Advances in standardization are expected in the next five years, but assessments on their application with other datasets are needed. The field is moving towards use of multi-sensor autonomous platforms, requiring collaboration across multiple fields but potentially incurring data security issues and information processing tools. We recommend funding agencies promote inter- and transdisciplinary early career researcher training and specialized funding programs requiring co-development of projects with engineers, database managers, machine learning, cyber-security experts, social scientists. ICES Expert Groups can be instrumental in such collaborations.

Our recommendations

Molecular data offers a rich source of taxonomic data complementary to other oceanographic data to inform ecological insights, policy and management advice at different temporal-spatial scales. Three supporting themes emerge to enhance and integrate molecular data with those of other long-term time-series: taxonomy, reference databases, inter- and transdisciplinary research and tools.

1. Molecular tools are used in an increasing inter-disciplinary manner, although most researchers are trained in biological sciences. Training aimed at early career researchers (ECRs) should reflect that spanning taxonomy, quantification, machine learning and bioinformatics tools and database management are required. This will tackle declining knowledge and expertise of taxonomy, awareness of tools and best practices for developing and sharing reference databases. At ICES, this can be assisted through ECR interaction with Expert Groups and promoting resources of large-scale projects such as TARA oceans and TREC (Traversing European Coastlines - European Molecular Biology Laboratory) to aid in training and awareness.
2. Awareness of common standards and tools to use multiple databases are needed. This could be hosted on the ICES website and/or ICES expert groups across different disciplines to enhance inclusivity. Specific training programmes in this discipline can also be advertised through these suggested forums. A best practice document is recommended to be developed to guide new users.

3. With the development of autonomous and miniaturised genetic detection platforms, often incorporating multiple sensors, we recommend inter-disciplinary ASC theme sessions with software development, engineering, and mathematics to encourage sharing resources to improve tools, accessibility and analysis methods.
4. ICES taxonomic identification tools are a key resource but not well known or advertised. Many members have written guides. They should be promoted on multiple ICES platforms including Expert Group sites.
5. Every Expert Group should be encouraged to have a data expert member for developing database resources, in line ICES code of ethics and FAIR principles.
6. ICES databases including the plankton database, the HAEDAT harmful algal event database and AquaNIS the non-indigenous species database are powerful resources for mapping distribution and seasonality alongside other datasets and long-term monitoring datasets. Molecular datasets have been developed such as MetaZooGene Atlas compiles information from Genbank and Barcode of Life, with other similar existing databases. We recommend these datasets and tools are made available as a link on the ICES data portal, with a potential roadmap to integrate genetic data into the ICES portal to complement existing datasets.
7. This session revealed that the use of genetic tools for policy, marine management applications is only emerging but would be highly beneficial in determining ecosystem health status. Methods to evaluate ecosystem state for Marine Strategy Framework Directive are constantly being developed. Advertising case studies and papers within Expert Groups can assist in promoting their use and further research into applications. Interdisciplinary ToRs within Expert Groups should be promoted to utilise new methods for research or tool development.

Contents

CM 68: DNA metabarcoding of zooplankton species diversity and climate-driven range shifts based on time-series ecosystem monitoring of the NW Atlantic continental shelf

Ann Bucklin[1], Paola G. Batta-Lona[1], Vicki You[1], David S. Johnson[2], Peter H. Wiebe[3], David E. Richardson[4], Todd D. O'Brien[5]

The NW Atlantic continental shelf ecosystem has been surveyed for fisheries assessments and pelagic diversity for many decades. Ecosystem Monitoring (EcoMon) Surveys by NOAA Northeast Fisheries Science Center (NEFSC) have included collection and preservation of zooplankton samples for genetic analysis from 2005 to the present. A selection of samples from this time-series archive has been analyzed using DNA metabarcoding of short regions of two mitochondrial genes: Cytochrome Oxidase I (COI) and 16S rRNA. Time-series analysis has allowed quantitative description of variation in zooplankton species diversity over time (seasons, years, decades) and space (ocean regions). Identification of species based on metabarcoding used the MetaZooGene Atlas and Database https://metazoogene.org/mzgdb/, which provides reference DNA sequences for marine species by taxonomic group and ocean region. Metabarcoding using both COI and 16S gene regions was used to detect and identify rare or unexpected species, resulting from latitudinal shifts in key species over time and/or appearance of non-indigenous or potentially invasive species in new ocean regions. A particular question was the climate-driven arrival of decapod species in the Plum Island Ecosystem adjacent to the Gulf of Maine, NW Atlantic, which has been a focus of long-term ecosystem research since 2016. This study seeks to provide information and insights for considerations of inclusion and integration of DNA metabarcoding for time-series monitoring and management of marine ecosystems.

Keywords: biodiversity, zooplankton, metabarcoding, ecosystem monitoring, range shifts, invasive species

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CM 72: Detection of eDNA functional indicators using digital PCR (dPCR): Comparison with existing methods for biomonitoring environmental pressures in estuaries

Christian Gostout[1,2], Ion Abad[1], Anders Lanzén[1,3]

Biomonitoring programs measure various parameters in indicator organisms over space and time to detect environmental changes. Today, most sediment biomonitoring uses morphological identification of taxa to indicate ecosystem function or status despite the existence of molecular techniques that detect indicators using environmental DNA (eDNA), which increase the efficiency and accuracy of biomonitoring, while decreasing the cost. Among these techniques, digital PCR (dPCR) assays optimize the sensitivity and quantifiability of molecular biomonitoring. To leverage the power of dPCR with an assay for genes that serve as indicators of ecosystem function, rather than taxa, should allow very direct and accurate indication of changes in ecosystem function that may result from environmental pressures. Functional indicator gene assays using dPCR have yet to be well explored, thus we propose a method for designing this type of assay using metagenomics. In this method, metagenomics data reveal genes with potential as functional indicators through differential expression, and a dPCR assay then assesses the ability of these genes to indicate environmental pressures.

From annual monitoring of over 50 estuarine and coastal sites in the Basque Monitoring Network (BMN), AZTI and the Basque Water Agency URA have collected a dataset that tracks ecological status and physicochemical parameters related to environmental pressures, for over 25 years. Data from a recent shotgun metagenomics study using selected BMN sediment samples provided a list of functional genes with potential as bioindicators. The identified genes were selected based on having significant abundance thresholds related to environmental impacts according to TITAN (Threshold Indicator Taxa Analysis) and association with key ecosystem processes. Here, we evaluate whether metagenomics can be used to design dPCR assays for functional indicators that can reliably predict environmental pressure indices, hypothesizing that dPCR analysis of microbial functional indicators yields comparable gene abundance data to metagenomics, and maps accurately to physicochemical and morphotaxonomic based environmental pressure indices. To this end, we designed a set of custom primer pairs for dPCR assays for the identified potential functional indicators. We used the specific assays to measure the targeted gene abundances in eDNA extracted from a set of samples from the BMN. For samples with existing metagenomic data, we also compared relative gene abundance data with the dPCR assay. The project provides an objective assessment of the potential of dPCR functional indicator assays as a tool that could be useful for routine estuary biomonitoring.

Keywords: Biomonitoring, Benthos, Estuary, eDNA, Indicators, dPCR, Metagenomics

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CM 115: Time Series and Network Theory application for benthic microbial community metagenomes: From Community structure to community functioning

Ion L. Abad-Recio [1], Leire Garate[1], Ramiro Logares[2], Thorsten Stoeck[3], Anders Lanzén[1,4]

Coastal and estuarine ecosystems are complex habitats threatened by several anthropogenic pressures, including draining, urban and industrial pollution, and climate change. Microorganisms (including protists and fungi) play important roles in these ecosystems with potential for biomonitoring and bioremediation. Thanks to environmental genomics, we can investigate the functional and structural biodiversity of these microbial communities. In this study, we used metagenomics, i.e., the sequencing of all genomic environmental DNA, to profile the functional potential of microbenthic communities from two time series from the Bidasoa and Oiartzun estuaries in the south-eastern Bay of Biscay. These sites are subjected to different levels of anthropogenic pressures, with Oiartzun showing hypoxic conditions due to nutrient enrichment and Bidasoa being less impacted. Based on this metagenomic data, we built association (co-occurrence) networks of metabolic KEGG orthologous genes (including genes coding for the equivalent metabolic or cellular function). These networks were used to identify key metabolic functions, based on the number of co-occurrences and potential interactions, to try to better understand the key ecosystem processes and how they may be affected by different stressors. The metagenomic data gathered is also being used to recover Metagenome Assembled Genomes (MAGs) of uncultivated species, and linking them to metabarcoding-based association networks, to assess if different metabolic processes are carried out by different taxa, thus linking community structure and function in these diverse and complex environments.

While functional alpha diversity was similar in both estuaries, it was slightly higher in Bidasoa, and with less temporal variation. There was also greater similarity in the functional composition within the same estuary, compared to samples from different estuaries at the same date. The topology of KO cluster networks also differed between the estuaries, revealing different key orthologous genes. This study exemplifies how network theory applied to several types of environmental genomics data from time series can link microbial community function, structure and ecological status.

Keywords: metagenomes, network theory, environmental genomics, ecology, community structure, community function

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CM 126: DNA metabarcoding for large-scale studies and monitoring of fish trophic interactions

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Implementation of ecosystem-based approaches to fisheries management requires accurate knowledge of the trophic relationships between marine organisms. In the context of rapid changes in marine ecosystems due to climate change, it also requires understanding of the spatial and temporal variation of these relationships. This is usually inferred through visual stomach content analyses for prey identification and quantification, which is time-consuming, requires taxonomic expertise and has limited potential to identify degraded remains. An alternative method is DNA metabarcoding, the simultaneous identification of all taxa in a sample by amplification and sequencing of a short genomic region. Applying this to stomach contents allows the identification of much higher prey diversity, including degraded prey, and provides a more accurate taxonomic classification, without the need for extensive taxonomic expertise. It is also much more cost-effective than visual approaches, making the method especially suitable to obtain comprehensive temporal and spatial data. However, technical challenges need to be addressed for DNA metabarcoding to be routinely implemented, such as the potentially complicated sampling logistics, the detection of a high proportion of host DNA, and the limitations in translating read abundance into reliable abundance estimations. Here, we present a DNA metabarcoding protocol for assessing the diet of five commercially important fish species (four pelagic and one demersal) that prevents the amplification of host DNA through the use of blocking primers, while speeding up the analysis process, avoiding the dissection and stomach contents extraction steps. The method was tested in mock samples with different proportions of predator and presumed prey DNA and has proven effective. Applying the method to real stomachs, we were also able to identify differences in the diet of the targeted species due to fish ecology (demersal *vs* pelagic) or prey availability. Additionally, by applying our protocol to mackerel stomachs previously analysed by visual inspection, we revealed a notable importance of gelatinous organisms in the mackerel diet, which was overlooked by the visual approach. Our work reinforces the potential of DNA metabarcoding for the study and monitoring of fish trophic interactions in marine ecosystems and supports its suitability in large-scale sampling programs aimed at obtaining comprehensive temporal and spatial data. Such information, coupled with the existing knowledge obtained by visual inspection will be critical for the implementation of ecosystem-based approaches to fisheries management.

Keywords: DNA metabarcoding, trophic interactions, fish diet, stomach content, ecosystem-based fisheries management

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CM 158: Spatio-temporal dynamics of Arctic eukaryotic microbes from days to decades and across habitats

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In 2009, we started regular environmental DNA (eDNA) based biodiversity studies of eukaryotic microbial communities in the area of the Arctic long-term ecological research site (LTER) HAUSGARTEN in Fram Strait. We anticipate to characterize the role of eukaryotic microbial biodiversity in maintaining Arctic marine ecosystem processes and functionality under the impact of global change in a nested approach across different time-scales ranging from days to decades, across different habitats, and across different spatial scales. This includes regular observations and process-studies embedded into the interdisciplinary observational program implemented as part of the pelagic and benthic long-term observations at LTER HAUSGARTEN, including year-round observations of the marine (eco)-system as part of the FRAM Observatory. Here, biological observations are contextualized with measurements physical and chemical properties (temperature, salinity, nutrients, sea-ice coverage etc.) in the ambient environment. These annual and year-round biodiversity observations will help to identify and interpret community shifts in response to Arctic environmental change, eventually leading to improved ecosystem models and future scenarios, considering linkages between marine biodiversity and change in the physical- chemical environment. Our work in Fram Strait is complemented by observational data from dedicated cruises to the Central Arctic Ocean basins (CAO), such as the MOSAiC drift experiment, enabling a pan-Arctic perspective on the role of eukaryotic microbial biodiversity in Arctic ecosystems. This talk highlights the results of more than ten years of eDNA based assessment of Arctic marine eukaryotic biodiversity in Fram Strait and the CAO, and method-developments enabling microbial biodiversity surveys across different habitats, and adequate temporal and spatial scales.

Keywords: Arctic, long-term observation, eDNA, eukaryotic microbes, biodiversity, ecosystem functionality, environmental change

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CM 163: Exploring biodiversity in an ecosystem impacted by seafloor plastics is made easier by eDNA metabarcoding

Alice Sbrana[1], Davide Cicala, Giulia Maiello, Tommaso Russo

Seafloor plastic pollution is a growing environmental concern that has potential impacts on marine biodiversity and ecosystem functioning. Plastic waste that enters the ocean can become trapped on the seafloor, where it can persist for decades or even centuries. This pollution can cause physical harm to marine organisms, such as entanglement or ingestion, and can also disrupt seafloor ecosystems by altering the availability of nutrients and changing the physical and chemical properties of the environment. Conventional methods for measuring biodiversity based on the taxonomical identification of specimens often involve scientific surveys, traps, or are fisheries dependent. These, however, have many limitations at sea, and they are often very expensive and time-consuming. eDNA techniques are gaining ground and becoming increasingly common in biodiversity studies, showing promise as a tool for monitoring the impacts of anthropogenic pressures. However, these techniques have not been applied to study the impact of seafloor plastic pollution on biodiversity. For those reasons, to investigate the potential impacts of plastic pollution on marine communities, we generated environmental DNA (eDNA) metabarcoding-based biodiversity lists. Fish and invertebrate species were associated with plastic debris to create a co-occurrence network and investigate the possible impact of plastic pollution on marine communities' composition and distribution. eDNA samples were collected from 24 sampling sites with various degrees of plastic pollution in the North and Central Tyrrhenian Sea (Mediterranean Sea). We gathered eDNA from commercial fishing vessels trawl nets, which are deeply in contact with the seabed, in order to more easily identify the benthic component that might be involved in seafloor plastics. Our preliminary results show that plastic pollution is associated with changes in species composition and diversity, with some species more strongly associated with plastic debris than others. These results suggest that plastic pollution may have important consequences for the marine communities in the Tyrrhenian Sea. The study demonstrates the potential of eDNA co-occurrence networks to provide insight into the ecological impact of seafloor plastic pollution on marine communities. Further research is needed to examine the long-term impacts of plastic pollution on marine ecosystems. The potential relevance of this study is to support the integration of eDNA-based approach into the data collection, and especially scientific surveys, to improve the monitoring of marine ecosystems and the assessment of pollution-related impacts.

Keywords: molecular ecology, anthropogenic impacts, biodiversity, seafloor plastics

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CM 199: Exploring the potential for oyster aquaculture to remediate biodiversity loss in oyster reef habitats using non-destructive environmental DNA sampling

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Oyster populations have been a target for supplementation and restoration for decades due to their strong historical role in the ecology and economy of coastal areas. The rapid growth of aquaculture and its' associated change in habitat structure may mimic benefits provided by historical oyster reefs and improve biodiversity provisioning in unstructured areas. If the positive impacts of aquaculture structure on provisioning oyster reef ecosystem services was understood, then there would be a more complete understanding of the state of these coastal ecosystems for ecological management. Addressing the often-limited resources of restoration managers to incorporate molecular methods into biodiversity studies, this study introduces a method to utilize third generation Nanopore sequencing and aquatic environmental DNA (eDNA) analysis to non-invasively record biological communities associated with oyster aquaculture at a broad taxonomic scale. Over the course of nine months, water samples were collected biweekly from six bivalve aquaculture and oyster reef sites and processed for DNA extraction and amplification via the 18S, 12S, and COI markers. After sequencing, a total of 84 taxonomic families were captured at aquaculture sites and 69 families captured at oyster reefs. While each group had unique taxa associated with it, much of the biological community utilizing bivalve aquaculture did overlap with that of oyster reefs, including groups of fishes, ascidians, annelids and marine worms, and bryozoans. Classes unique to aquaculture included groups of waterfowl, anthozoans, and echinoderms. Here we show that a diverse biological community utilizes the structure provided by oyster aquaculture, many members of which are also found in present day oyster reefs. These findings indicate that thoughtful incorporation of aquaculture into ecosystem management plans could be used to relieve pressure from wild fisheries and contribute to providing ecosystem services.

Keywords: aquaculture, eDNA, biodiversity monitoring, third generation sequencing

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CM 225: Seasonal variation of non-indigenous invertebrate species in recreational marinas in the north of Portugal using DNA metabarcoding: impact of sample type

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DNA metabarcoding has been widely used in biodiversity assessments as a complement to traditional morphology-based techniques. This technique holds great potential in the early detection and surveillance of non-indigenous species (NIS) in aquatic ecosystems. As most introductions in coastal ecosystems occur by transport in ships; ports and marinas are priority hubs for the early detection of NIS using DNA metabarcoding. This can be strongly affected by the selected type of sample due to life history traits, such as habitat preferences and life cycles. The aim of this study was to survey marine invertebrate NIS in two marinas in the north of Portugal (Viana do Castelo-VC and Porto-L), in three seasons (Spring-T1, Autumn-T2, Winter-T3), using DNA metabarcoding and employing five types of samples: marinas' hard substrates, artificial substrates (sponges and acrylic plates), water for environmental DNA (eDNA), and zooplankton. Two molecular markers were used: the mitochondrial cytochrome c oxidase gene (COI) and the small subunit ribosomal RNA gene (18S). A total of 375 species and 16 NIS were detected in this study. The global number of species and NIS observed was slightly higher in L (278 species / 13 NIS), compared with VC (242 species / 11 NIS). The highest number of species was retrieved in Spring (163) in VC and in the Autumn in L (175), and in both cases the higher number of NIS was recorded in Spring and Summer (9 and 10 NIS for VC and L respectively). Regarding sample type, eDNA samples recorded the highest number of species in all seasons (59 to 97) in VC, but the highest number of NIS was retrieved by different substrates: hard substrates, eDNA and zooplankton(T1), eDNA(T2) and eDNA and zooplankton(T3). For L, a different pattern was found; the highest number of species was detected in the hard substrates (T1), zooplankton (T2) and eDNA (T3); regarding NIS, the highest numbers were always observed in zooplankton. Only 6-10% of indigenous species and 0-50% of NIS were detected by all sample types and the highest percentage of shared species was found between eDNA and zooplankton. Overall, most NIS detected in this study were Crustacea and Ascidiacea, while the indigenous communities were dominated by Annelida and Crustacea. Results show the great potential of NIS detection using DNA metabarcoding but reveal the need to sample different seasons and using several types of samples to guarantee a more comprehensive surveillance of NIS in these environments.

Keywords: DNA metabarcoding, COI, 18S, marine invertebrates, recreational marinas

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CM 226: Estuarine microbenthos metabarcoding for ecosystem status assessment – the Basque coast and beyond

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Estuarine ecosystems worldwide are threatened by several anthropogenic stressors. While environmental management has improved the situation in many places, other estuaries have crossed tipping points or are risking to do so, with consequences like reduced water quality, harmful algal blooms, collapsed coastal fisheries and reduced protection from floods and storms. Bioindicator taxa, commonly benthic macroinvertebrates, are important tools for routine monitoring of these ecosystems. Using biotic indices (BIs), the responses of individual bioindicator taxa are integrated and used in monitoring programs to assess the ecological consequences of past and present disturbances. However, the need for morphology-based classification leads to bottlenecks and biases due to limited access to taxonomic expertise, cryptic species assemblages, damaged specimens and early life stages. Using environmental DNA (eDNA) metabarcoding from microbenthic communities offers a viable solution to many of these limitations, since microorganisms can serve as indicators sensitive to a wide range of ecological status, while their smaller body size makes it possible to recover representative eDNA samples from smaller sediment volumes. However, insufficient taxonomic reference data and limited knowledge of the ecological roles and responses of individual microbial taxa requires the development of new assessment tools, based on *de novo* inference. We have evaluated two such approaches to environmental quality assessment in estuaries along the Basque coast (Bay of Biscay), based on 16S metabarcoding, namely the machine learning method Random Forests (RF), and the identification of individual indicator taxa and BI development using Threshold Indicator Taxa Analysis and quantile regression splines (TITAN/QRS). The universality of these approaches was later evaluated using public 16S datasets. In spite of the complexity of the studied ecosystems, both *de novo* inference methods performed comparably well to macroinvertebrate-based methods and correlated strongly with multiple physicochemical stressors during the last five years, formalised as pressure indices. In some cases, different types of impact could also be distinguished. The novel RF classifier and TITAN/QRS-derived BI derived from the datasets from Basque estuaries could also predict impacts with significant confidence in a selection of coastal and estuarine sites from other continents. We are currently collating a larger dataset from new and existing samples worldwide to improve and evaluate the performance of these methods further. Our results show that monitoring based on eDNA metabarcoding of microbenthic communities has a strong potential for biomonitoring, and could ultimately improve the ability to follow, understand, manage and regulate negative environmental impacts.

Keywords: microbial ecology, biomonitoring, indicator species, estuaries, benthos, community structure

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CM 239: Evaluation of environmental DNA capture and extraction methods for Harmful Algal Blooms biomonitoring

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Harmful Algal Blooms (HABs) are natural phenomena that cause global ecological, economical and human health issues. Worldwide, there is a significant interest in the development and implementation of state-of-the-art methodologies in the management of shellfish production areas associated with high risk of exposure to biotoxins. In Ireland, HAB events have been reported since early 1970's, causing mortalities of littoral and sub-littoral organisms. The national monitoring programme for phytoplankton species identification by microscopy at the Marine Institute, Ireland, commenced in the 1980's as the shellfish aquaculture sector expanded throughout the country. From 2007, the phytoplankton laboratory introduced molecular methods for the identification of organisms which are difficult to identify by light microscopy. In the last decade, environmental DNA (eDNA) analysis has rapidly developed, and the technique has become widely used for detecting aquatic organisms in a variety of habitats. Molecular methods are particularly promising when multiple species need to be detected and quantified, even in very low abundances. In particular, High-Throughput Sequencing (HTS), quantitative PCR (qPCR) and digital PCR (dPCR) are extremely sensitive methods that have been applied in recent years to identify and estimate species in natural samples. Nevertheless, molecular applications are strongly influenced by the DNA extraction method used especially in the challenging context of eDNA where stochasticity is a relevant factor that can affect the final recovery. The aim of this study was to compare different methods and analytical approaches to infer an accurate, cost-effective and rapid detection approach to improve biomonitoring resolution. Sampling was conducted in summer 2022 during a survey onboard the Marine Institute's Research Vessel "Tom Crean" with two different capture techniques (plankton net 5µm mesh and Niskin bottles attached to a CTD rosette) in five locations of Irish waters (North-East Atlantic). Mock communities of mixed strains were used for protocol validation. For all samples, four DNA extraction methods were used and compared in order to evaluate the (i) DNA yield, (ii) inhibition removal, and (iii) suitability for different downstream target-detection technologies. Ultimately, sensitivity and specificity of cross-methodologies for real-time identification and quantification of toxic-producing species was assessed in view of integration into the Irish national phytoplankton monitoring program.

Keywords: Harmful Algal Blooms, HABs, environmental DNA, eDNA, DNA extraction, efficiency, molecular methods, qPCR, biomonitoring, Ireland

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CM 250: Development and validation of molecular markers for early detection of *Alexandrium* spp. in the west coast of Ireland

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Over the last four decades, there has been a significant increase in Harmful Algal Bloom (HAB) reports worldwide. It is believed that global changes have been the main factors driving their incremented abundance and distribution in previously unaffected areas, where they can constitute a threat to anthropogenic activities and ecosystem biodiversity. Specifically, several genera of dinoflagellates are responsible for marine shellfish biotoxin events affecting the economy and human health. Species of the genus *Alexandrium* produce Paralytic Shellfish Toxins (PSTs) causing, through the consumption of contaminated filter-feeding molluscs such as mussels and oysters, the human illness known as Paralytic Shellfish Poisoning (PSP). Since PST analysis in bivalve species commenced in Ireland in the late 1990's, multiple PSP events have been recorded. From 2019, the important shellfish production area of Castlemaine Harbour, located on the Irish west coast, has suffered several weeks' closures on an annual basis due to mussels and oysters exceeding the PST regulatory limit as laid down in EU legislation. Addressing the concern arising from these recent events, the present study explores the application of molecular tools to characterize *Alexandrium* diversity, as well as to establish a sensitive routine monitoring protocol for detecting and quantifying low amounts of target DNA, complementing traditional microscopic techniques, and going beyond their limitations. In the summer of 2022, an intensive sampling campaign was carried out in Castlemaine Harbour. Water samples (surface and net hauls), sediments, and shellfish were collected weekly from four sites over a seven-week period covering the onset and duration of the observed PST event. The *Alexandrium* cells in surface water samples were morphologically identified to genus level and enumerated under a light microscope. Single vegetative cells were isolated from net hauls to establish monoclonal cell cultures. These were subsequently identified to species level through DNA barcoding. Following a review of the most relevant molecular markers targeting *Alexandrium* spp. strains within the North Atlantic areas, selected quantitative-PCR (qPCR) assays were screened *in silico* on sequences published in GenBank. Results will be presented on the screening of field samples collected in Castlemaine Harbour by means of a range of previously published and novel species-specific qPCR assays for the early warning detection and risk assessment of *Alexandrium* spp. cells in environmental samples.

Keywords: Harmful Algal Blooms, HABs, *Alexandrium*, quantitative-PCR, DNA barcoding, Ireland, early warning detection, risk assessment, shellfish production, monitoring protocol

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CM 266: Development and validation of a HT-qPCR screening panel for efficient high-resolution bioassessment of ecological and economically important shellfish species in Irish coastal waters

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Shellfish play essential roles in aquatic ecosystems and represent a significant seafood source. The growth of shellfish aquaculture industry is steady but limited by production, which often relies on recruitment of planktonic larvae. To understand the dynamics of spatially distributed stocks, improved knowledge of larvae differentiation, dispersal, and demographic connectivity is required. Monitoring shellfish larvae often relies on microscopy which is time-consuming and can lead to mis- or simplified identification due to morphological similarity in their early life stage. Recent technological advances have enhanced the ability to identify species in mixed environmental samples by means of DNA-based methodologies, showing great promise in corroborating existing bioassessment programs. While high-throughput-sequencing is the focus of modern biomonitoring, High-Throughput quantitative Polymerase Chain Reaction (HT-qPCR) on microfluidic platforms have received little attention in this context. The Biomark™ HD (Standard BioTools Inc.) system, uses microfluidics technology to process DNA-samples at nanoliter-scale volumes. This system enables the quantification of up to 96 different DNA targets, in up to 96 samples in as little as four hours. The goal of this study was to develop a panel of DNA-based markers for monitoring shellfish larvae. Following four phases; i) setup/preparation, ii) *in silico* design, iii) *in vitro* validation, and iv) evaluation, a HT-qPCR panel of molecular assays was developed to detect 25 ecologically and economically important shellfish species in heterogeneous environmental samples. The newly developed panel was then tested on 24 zooplankton samples collected from Howth Head in Dublin, Ireland, that were also subjected to taxon identification by means of microscopic analysis. The panel successfully detected the presence of, blue mussel, queen scallop, common cockle, razor, soft-shell and surf clams, velvet and green shore crabs. Correlation between larval counts and copy numbers proved that by corroborating microscopy with the new HT-qPCR panel, the taxonomic resolution substantially increased. The pipeline hereby described is adaptable to develop further panels of markers targeting a wide range of aquatic species, allowing for cost-effective and high-resolution bioassessment, which can effectively serve the management of natural resources and aid sustainable growth of the seafood sector.

Keywords: HT-qPCR, bioassessment, shellfish, DNA

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CM 268: Detecting two marine non-indigenous species from the French coast using eDNA and molecular approaches

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The RAPSODI project focuses on two non-indigenous species (NIS) that could threaten shellfish farming on the Atlantic and Mediterranean coasts of France. The first of which is the veined rapa whelk *Rapana venosa.* Since one individual was reported in the Pertuis Breton (off the coast of the île de Ré) area of the Bay of Biscay in November 2019, there have been dozens of reports in the area. The second NIS is a newly described polycladida flatworm, *Idiostylochus tortuosus* gen. nov., sp. nov. *(*Gutiérrez et al., 2023*),* already observed for two years in Arcachon Bay. High abundances of this worm may have been associated with mussel mortalities. This species was found feeding on farmed oysters and mussels in Arcachon Bay

We conducted surveys targeting the two species within three zones (along the French Atlantic coast: Hossegor lake and Arcachon Basin, the Pertuis Charentais Sea, and along Mediterranean coast: Thau lagoon). A few specimens of *R.venosa* were collected around Hossegor Lake and Arcachon Basin which suggests that this predatory gastropod is now settling in this area as well. Since 2020, its demographic expansion is evidenced in the Pertuis Charentais Sea by a spectacular increase in the number of accidental catches in the trawls and trammel nets of professional fishermen. Regarding flat worm detection, numerous specimens were observed in Arcachon and Thau lagoon oyster beds in 2022, which have resulted in numerous samples for taxa determination (sequencing). Recent sequencing data confirmed that *I. tortuosus* is present in the Thau lagoon (2020) and also confirmed its presence in the Pertuis Charentais Sea (2021).

The objectives of RAPSODI project are (1) to raise awareness among local maritime stakeholders about these NIS, (2) develop and validate protocols to detect these two NIS with molecular tools from environmental DNA (eDNA) samples, and (3) analyse samples collected *in situ*.

Better estimating the dynamics of these NIS is a prerequisite for preparing management measures. The joint use of eDNA and targeted quantitative PCR (qPCR) will help assess the spatio-temporal distribution of these NIS. At this time, there is no species-specific qPCR for the two targeted NIS. Here we present the protocol for the development of two molecular tools designed for future monitoring of the two NIS, based on seawater eDNA: one qPCR to detect *Rapana venosa* COI mtDNA, and one qPCR to detect *Idiostylochus tortuosus* 16S mtDNA.

Keywords: molecular approaches, eDNA, qPCR, *Rapana venosa, Idiostylochus tortuosus*

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CM 269: Differences between microbial communities and their ecological associations in clean and polluted estuaries from the Basque Country

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Estuarine and coastal ecosystems are fundamentally important for global ecosystems functioning and for human activities and wellbeing. Since millions of people depend on the numerous resources they offer (e.g., fisheries, transportation, and recreational activities), these ecosystems are exposed to a high anthropogenic pressure. To better monitor and regulate such pressures, it is critical to improve our understanding of the functioning of these ecosystems. Aiming to identify bioindicators, with a focus on "keystone" taxa, related to anthropogenic pressures, we studied estuaries with different degrees of disturbance across the coast of the Basque Country (Spain), by deriving eDNA metabarcoding data from microbenthos samples along a time series. Thus, we reconstructed ecological association networks, and compared the structure of these networks to impacts. Here, we defined keystone taxa as those that presented the highest degree of connectivity. Connector taxa were defined by manual inspection, selecting those that were critical for maintaining the integrity of community structure, by connecting different network modules together. Preliminary results of two estuaries, one clean and one polluted, showed different microbial composition and dominance: bacteria from the family Woeseiaceae dominated in the clean estuary, as well as eukaryotes from the Seriata order and those classified as *Navicula.* In contrast, in the polluted estuary the microbial community was mainly dominated by bacteria belonging to the genus *Sulfurovum* and eukaryotes from the Capitellida order. Moreover, the keystone and connector taxa identified differed from the compositional profile. While in the clean site the main keystone belonged to Erysipelotrichaceae, in the polluted estuary it belonged to family Porphyromonadaceae. The connector taxa in the clean estuary belonged to Ectocarpales, and to Desulfobacterales in the polluted estuary. These results highlight the power of network analyses providing additional ecological insights and helping in the identification of impact indicators of a microbial community.

Keywords: Microbial communities, estuaries, ecological association networks, keystones

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CM 383: Improving assessment of diadromous fishes distribution in the North-East Atlantic using eDNA analyses

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Diadromous fishes are ecologically, evolutionarily, and economically valuable migratory species, for which life cycle relies on the connectivity between freshwater and seawater habitats. Due to anthropogenic-derived changes in biotic and abiotic factors, many populations of diadromous species are in decline. In Europe, the current status of emblematic diadromous species i.e., sea lamprey *Petromyzon marinus* and European shads, (*Alosa alosa* and *A. fallax*) are not well understood across their native range. This is mainly due to the difficulty of monitoring these highly migratory species using conventional capture-based methods, which are typically costly, time-consuming, and invasive, and particularly challenging to implement when the current population abundances reach low levels. To address this knowledge gap, we conducted a broad-scale survey to provide an overview of the distribution of sea lamprey and European shads across a network of 46 river basins in Spain, France, United Kingdom, and Ireland using water environmental DNA (eDNA) analysis, which allows for detection and quantification of aquatic organisms without seeing or sampling them. Applied to water samples, our species-specific quantitative PCR assay did not detect sea lamprey in most of the river basins where they were expected, particularly in France and Ireland, opening the door to further studies, particularly in those rivers where sea lamprey was known to exist. In contrast, European shads were detected in most of the rivers where they were expected, based on available data and expert knowledge, but also in locations where they were thought to be absent. Our eDNA assay provides a cost-effective and non-invasive method for lamprey and shad monitoring and can serve as an early warning system for the ecological status of rivers. Furthermore, guidelines on how to interpret eDNA results for diadromous species are proposed. Better knowledge on fish distributions can be used to identify conservation priorities and guide management decisions in the near future.

Keywords: eDNA, fish monitoring, species-specific assay, diadromy, conservation, migration

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CM 389: Plankton community response to climate-driven salinity change and warming: A mesocosm experiment comparing morphology-based identification and metabarcoding

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Climate change models predict global seawater salinity and temperature changes; in the Baltic Sea, salinity is expected to decrease by ~2 PSU and temperature to increase by 2-4C by the end of the century. These changes include shifts in plankton communities, which may have negative implications for the entire food web due to the crucial role of local planktonic assemblages in the functioning of marine ecosystems. Current understanding of how salinity change will impact plankton communities has focused mainly on the salinisation of freshwater environments, with little understanding of changes in marine systems as well as of the potential synergistic effects with other stressors, such as warming. Here, we investigate the effect of variations in salinity coupled with warming on plankton community composition under different climate change scenarios for the Baltic Sea. Projections for future salinity change and warming were used to set-up an indoor mesocosm experiment in the Gulf of Finland. Each mesocosm was inoculated with natural plankton using a mixture of both marine and freshwater communities, mimicking the natural influx of freshwater species from rivers into the Baltic Sea. We identified plankton communities combining traditional morphology and environmental DNA (eDNA) metabarcoding of the mitochondrial cytochrome oxidase I (COI) and 18S rRNA genes. Our results suggest shifts in plankton community composition is dependent on both salinity and temperature treatments; for instance, copepods were dominant in higher salinity conditions and rotifers in low salinity ones. Additionally, changes in plankton community assemblage in the different mesocosm treatments were more clearly captured by metabarcoding than by morphology-based methods. Our results asses the sensitivity of planktonic community assemblages to variations in seawater salinity and temperature in the Baltic Sea across all trophic levels of the food web. We also highlight the potential of eDNA metabarcoding to monitor plankton community responses to climate change.

Keywords: plankton, climate change, warming, salinity, biodiversity, mesocosm, eDNA metabarcoding

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CM 398: Genetics as a tool for sustainable fishing and protection of vulnerable marine ecosystems - VME

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Bottom trawling activities can alter significantly deep-water ecosystems, by degrading the seabed integrity and causing the depauperation of benthic biodiversity. Deep-sea environments are often characterized by fragile benthic biodiversity hotspots, known as vulnerable marine ecosystems (VMEs). These ecosystems show structural and functional fragility and include organisms that are unique or rare, with slow growth, large longevity and/or limited dispersion, which makes their recovery difficult when their habitats are altered. VMEs may be negatively affected by fishing, pollution and climate change, including rising water temperatures and ocean acidification. Many aggregations of deep-water corals and sponges are classified by the FAO as VMEs, since they host in their habitat a great biodiversity of epibenthic fauna that is fundamental for the general maintenance at the ecosystem level. The conservation and protection of these habitats requires detailed information on the distribution of VMEs but these are generally poorly described. The general objective of this study is the development of a non-invasive tool to detect and delineate the spatial distribution of VMEs, to ultimately protect them against fishing and develop strategies to prevent their damage. Our case study is focused in the Flemish Cap, Northwest Atlantic, where an annual research survey provides information on the ecosystem and the vulnerable benthic species living in the area. A total of 418 invertebrate samples, collected during the survey in 2022, were characterized using both, classical and molecular taxonomy based on two mitochondrial markers (COI and 16S rDNA). The resulting molecular barcodes are unique to each species inhabiting the ecosystem and served to create a complete and curated reference database to characterize VMEs in this region. The newly created database will help in the immediate future to recognize VME sites through non-invasive tools based on environmental DNA coupled with metabarcoding, providing a cost-effective, fast, and accurate tool to assess the presence, distribution and community composition of vulnerable ecosystems.

Keywords: barcoding, environmental DNA, marine invertebrate, metabarcoding, vulnerable marine ecosystems

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CM 418: Monitoring the variability of microplankton communities' structure in the Alboran Sea with high throughput sequencing

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Monitoring of plankton communities' structure is a key tool in the evaluation of the marine environmental status of the eutrophication, pelagic habitats, trophic webs and invasive species, *sensu* EU Marine Strategy Framework Directive. For this purpose, we characterized the variability of the microplankton communities' structure across the North Alboran Sea (SW Mediterranean Sea), by means of metabarcoding of the V4 region of the 18S rRNA gene. Water samples (surface and DCM) were collected quarterly, following a gradient from the coast to open sea, from the Strait of Gibraltar (West) to the Almeria-Oran front (East). We observed a differentiated seasonal cycle, as well as coastal and offshore gradients in the composition of the microplankton communities. The data are discussed in relation to the time series data obtained by means of microscopy since 2010.

Keywords: metabarcoding, microplankton, MSDF, V4 18S rRNA, SW Mediterranean

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CM 425: Coastal microbiomes in estuarine ecosystems of France: the eDNA network ROME

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An eDNA-based monitoring network named ROME (*Réseau d'Observatoires de Microbiologie Environmentale intégrée,* rome.ifremer.fr) piloted by Ifremer is deployed in France since 2020 in 4 estuarine ecosystems that integrate oyster farms: Veys Bay in Normandy, Brest Bay in Brittany, Marennes Oleron Basin in New Aquitania, and Thau Lagoon in Occitania. This network of observatories aims at evaluating the influence of river inputs on the structuration of estuarine microbiomes and the potential emergence of new microbiological risks for human, aquaculture and ecosystem health. The virome (RNA-metaT), bacteriome and protistome (eDNA-metaB) are analyzed from common environmental DNA/RNA samples extracted from both fortnightly water samples and monthly adult oyster tissue samples. Coastal microbiome biodiversity is described including pathogens for human and aquatic invertebrates and harmful microalgae. River influence and coastal microbiome resilience are investigated by comparing coastline and off-shore sampling stations submitted to decreasing run-off impact. Oyster batches are used as integrator of the microbial site biodiversity and to study holobiontic associations, including parasitism. Preliminary data have shown that different microbial communities occur across the in-off shore gradients, differently among sites, and depending on local river inputs. Some taxa of terrestrial origins might explain this difference and could be identified as bioindicator of run-off impact. Known bivalve parasite genera were detected by metabarcoding including taxa causing important diseases.

Keywords: eDNA, coastal monitoring, rivers, bioindicators, ecosystem health, HABs, pathogens, oysters

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CM 430: Integration of new methods for evaluating marine protected area connectivity and efficiency

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Natura 2000 policies aim at establishing a network of marine protected areas (MPAs). The effectiveness of such networks relies on connectivity, i.e., the functional linkage exchange between individual MPAs. However, connectivity assessments are not yet standard elements of marine conservation planning. To fill this knowledge gap, we initiated a case study in the Natura 2000 site 'Borkum Reef Ground' in the German Exclusive Economic Zone (EEZ), and particularly around a restoration site for the European flat oyster (*Ostrea edulis*). We aim to address the following questions: (i) Is there a spillover from the restored habitats through the spread of planktonic life stages into surrounding habitats, which could be detected though metabarcoding of plankton samples? (ii) Do specific patterns of biodiversity develop in the vicinity of a restored oyster reef, i.e., does it develop refugia and habitat for other species? (iii) Can an increased local biodiversity be detected through traces of environmental DNA (eDNA)? We conducted ship-based zooplankton and eDNA sampling at the oyster restoration site in June – August 2022, sampling in direct vicinity of the reef through divers. Reference sampling was conducted in another Natura 2000 site 'Sylt Outer Reef' where no oyster restoration is done. DNA was extracted from zooplankton and eDNA samples, followed by screening for oyster presence using quantitative PCR (qPCR) and metabarcoding. We detected *O. edulis* and over 200 other species covering a wide range of metazoan phyla. The most species-rich phyla were Arthropoda, Mollusca, Chordata, Cnidaria and Annelida. On the basis of this case study, we present a concept of assessing connectivity and biodiversity in a marine restoration measure.

Keywords: eDNA, metabarcoding, biodiversity, connectivity, restoration, Ostrea edulis, zooplankton, qPCR, larval dispersal

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CM 445: Characterization of VMEs with DNA: mind the gap

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Vulnerable marine ecosystems (VMEs) are characterized by being highly vulnerable to disturbances and an impaired ability to recover that takes a long time and is not always possible. Some of these vulnerable marine habitats include seamounts, hydrothermal vents or cold-water coral reefs. Cold water corals are of significant ecological value and provide a variety of ecosystem services, such as providing habitat for a wide variety of organisms, many of which are of commercial interest. In addition, they are long-lived and have low growth rates, long reproductive cycles and low recruitment rates. Because of their nature and the importance of their ecosystems as biodiversity hotspots, there is an international call to protect these vulnerable marine ecosystems from trawling, the most destructive form of deep-sea fishing, with dramatic consequences for benthic communities. It is therefore of vital importance to develop non-destructive methods to describe and map the seabed in these areas. One of the most promising methodologies is the detection of environmental DNA, a non-invasive tool that only requires the collection of water. This method relies entirely on DNA databases that are neither complete nor accurate, especially for invertebrate species living in poorly described habitats. To improve existing public databases and the biodiversity characterization of the Flemish Cap seabed (NAFO), marine invertebrates were sampled during the 2022 research surveys. These invertebrates were identified both taxonomically and by barcoding using two mitochondrial markers (COI and 16S rRNA). Our preliminary results show difficulties in the genetic identification to species level of the Octocorallia subclass using these widely used barcoding markers and furthermore, that COI amplification is often challenging in echinoderms. These could result in an underestimate of the biodiversity due to the lack of sequence divergence that implies a lack of discrimination power within these groups of animals, as well as the potential occurrence of false negatives due to the primer-biased amplification. More joint efforts by taxonomists and geneticists are needed to elucidate and understand the barriers between deep-sea coral species and develop non-invasive tools to monitor these environments.

Keywords: cold water corals, Cnidaria, COI, 16S rRNA, barcoding, cryptic

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CM 454: Trawl-associated opportunistic eDNA sampling probe for large scale fish community assessment

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With the increasing threats to biodiversity and ecosystem functions, the need to improve marine resource monitoring is imperative for effective ecosystem management. The routine implementation of molecular techniques, such as environmental DNA (eDNA) metabarcoding, is bound to play a primary role in assessing rapid changes in biodiversity and community structure in every habitat. Here, we adopted a metabarcoding-based approach through the opportunistic use of a recently developed simple eDNA sampling tool (the 'metaprobe') during a statutory Mediterranean trawl survey program (MEDITS). We collected samples from 21 sites in three different areas along the Italian coast: North Adriatic Sea, North-Central Tyrrhenian Sea, and Sardinian Sea. The passive absorption of DNA by sterile gauze rolls in the metaprobe placed inside the fishing net combined with the use of an elasmobranch- (Elas02) and a teleost-specific (Tele02) 12S metabarcode, allowed the identification of most of the fish species caught by the trawl net, and produced an accurate reconstruction of the vertebrate community composition of the examined sites, reflecting differences in species assemblages linked with both geography and depth range. Environmental DNA data also returned a biodiversity 'bonus' mostly consisting of pelagic taxa not captured by bottom trawlers, including rare and elusive species, such as endangered elasmobranchs (e.g., the shortfin mako (*Isurus oxyrinchus*), the sandy skate (*Leucoraja circularis*), and the blue shark (*Prionace glauca*)), which are of particular importance for the improvement of conservation plans. Our results corroborated the idea that this simple, non-interfering and inexpensive sampling tool can considerably increase the extent and granularity of marine ecological data. We argue that the metaprobe eDNA approach is ready for integration with existing survey programs, thereby playing a key role in routinely generating large data for evidence-based marine management.

Keywords: biodiversity, marine fish, Mediterranean Sea, environmental DNA

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CM 532: A meta-analysis of potential biomarkers linked to the consumption of microplastics in marine fish

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Microplastics present a persistent threat to the marine environment. Despite mounting evidence that microplastics pose a threat to marine species, quantifying the physiological response of species to microplastics remains a significant challenge. In this study, we present a meta-analysis of potential biomarkers linked to the ingestion of microplastics by marine fish species caught in the wild and in carefully monitored laboratory settings. A total of 25 published studies, 20 laboratory studies and 5 field studies met the requirements and were included in the meta-analysis. For both study types, the measured oxidative stress biomarkers included in the meta-analysis were glutathione-s-transferase (GST) and malondialdehyde (MDA) levels to assess detoxification enzyme activity, glutathione reductase (GRd) that regenerates an essential non-enzymatic antioxidant glutathione, superoxide dismutase (SOD) and catalase (CAT) as a measure of antioxidant enzyme activities and acetylcholinesterase (AChE) to assess neurotoxicity. Preliminary results of the meta-analysis showed an overall response of AChE in brain tissue to MPs exposed to microplastics in laboratory conditions and a weak response in SOD in blood cells in fish species in wild conditions. Overall, initial outcomes from this study highlight the need for further research on marine species to determine the toxic effects of environmental microplastic exposure to marine species in the marine environment.

Keywords: meta-analysis, biomarkers, microplastics, fish, ingestion

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CM 590: Comparison between COI metabarcoding and microscopy for zooplankton monitoring of the Adriatic biodiversity

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Most metabarcoding studies focus on limited temporal sampling, which can result in missing seasonally present species. To assess the efficacy of COI metabarcoding as a monitoring method and compare its results to classical methods of formalin sample analysis under the loupe, we sampled a 100 m deep station in the South Adriatic and a 20 m deep station in the North Adriatic in duplicate samples using a 200-µm plankton net, from bottom to surface, monthly from March 2021 to February 2022. We used the mlCOIintF-jgHCO2198 primer pair and an MZG COI referent database enriched with zooplankton sequences produced from our own barcoding efforts for zooplankton community assessment using COI metabarcoding, including for most common zooplankton taxa not yet present in GenBank or BOLD, such as *Muggiaea kochii.*

Our results indicate that the calanoid copepod composition obtained by metabarcoding largely matched those obtained by microscopy, but metabarcoding produced a larger number of species for some genera, such as *Paracalanus*. The metabarcoding approach also recovered the diversity of chaetognaths, pteropods, and heteropods well, but it largely failed to detect pelagic tunicates, as it did not produce any Salpid sequences and only recorded two doliolid species, one of which is a newly described species, *Dolioletta advena*. For Appendicularia, metabarcoding only detected *Oikopleura longicauda*, while for Cnidaria, COI metabarcoding was mostly successful in reproducing community composition, but it consistently produced large numbers of *Nectadamas diomedeae* sequences, a species not previously recorded in the Mediterranean according to WoRMS or in our formalin samples.

COI metabarcoding was largely able to reproduce the temporal and spatial variability of zooplankton, and it is a good substitute for classical methods for many taxa. However, given the limitations of the metabarcoding approach, we recommend its use in combination with classical methods to obtain a more comprehensive understanding of the biodiversity of zooplankton in the Adriatic Sea.

Keywords: Adriatic Sea, metabarcoding, monitoring, COI, zooplankton

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CM 644: Comparison of the three metabarcoding genes (18S, 28S and COI) for the Adriatic Sea zooplankton biodiversity monitoring

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The monitoring of zooplankton in the Adriatic Sea has long been relied on traditional methods of biodiversity description using stereomicroscopy, but the development and more common use of metabarcoding in biodiversity studies significantly improved community completeness and high-throughput sample processing in zooplankton monitoring. The choice of metabarcoding genes represents an important step for the successful amplification of taxonomically diverse zooplankton samples. During a one-year period (March 2021 – February 2022), monthly zooplankton sampling was carried out in the northern and southern Adriatic Sea and net samples were processed for biodiversity assessments using metabarcoding. To achieve successful detection of diverse zooplankton taxa, three barcodes (18S, 28S and COI) were used. Detailed community compositions of the three barcodes were compared to evaluate taxa coverage and potential of each barcode in targeting organisms of specific interest for the monitoring studies (e.g., non-indigenous species). The highest number of different invertebrate taxa (267) was identified with 18S while 28S and COI identified 144 and 201 invertebrate taxa, respectively. Holoplankton and meroplankton representatives of the 9 major invertebrate phyla (Annelida, Arthropoda, Bryozoa, Chaetognatha, Chordata, Cnidaria, Ctenophora, Echinodermata and Mollusca) were identified with all three barcodes, while 18S and 28S enabled identification of the phylum Nemertea representatives as well. Phylum Arthropoda dominated all three metabarcoding datasets (18S, 28S and COI) in reads number with majority of all Arthropoda taxa classified to Copepoda. The 18S and COI datasets showed a much higher richness of copepod taxa (50 and 66 taxa, respectively) compared to 28S (9 taxa). Only 15% of the identified copepod taxa were shared between 18S and COI, while others were barcode specific. Genus *Paracalanus* was the most abundant copepod taxa in datasets of all three barcodes. 28S barcode was characterised with lower taxa richness for all the detected phyla compared to the other two barcodes. Overall, multigene metabarcoding confirmed significant differences between zooplankton communities of the northern and southern Adriatic station, and temporal patterns for each region were also present. Our results confirm that robustness and extensive approach of metabarcoding have the potential to complement traditional zooplankton identification methodologies in monitoring.

Keywords: zooplankton, metabarcoding, Adriatic Sea, monitoring

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