AquaEcomics

Exploring Aquatic Ecology through Omics 2025

Evian-les-Bains, France
March 17 → 20

CARRTEL
CENTRE ALPIN DE RECHERCHE SUR LES RÉSEAUX TROPHIQUES ET ÉCOSYSTÈMES LIMNIQUES

UNIVERSITÉ SAVOIE MONT BLANC

INRAE

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WELCOME WORD

Dear Participants,

Welcome to the AquaEcOmics meeting, a flagship event for the international scientific community dedicated to advancing omics methods in aquatic ecology. Cutting-edge omics approaches continue to revolutionize aquatic sciences and open new avenues in ecological research. Over three conference days, we will explore a diverse array of omics applications through seven thematic sessions, fostering discussions that will shape the future of our field.

We are honored to bring together more than 200 experts, practitioners, and policymakers from more than 25 countries, showcasing the global commitment to integrating omics technologies into aquatic research. Your contributions are essential to the success of AquaEcOmics, and we sincerely thank each of you for your participation—whether in person here in Évian-les-Bains or remotely. Your engagement and enthusiasm for knowledge-sharing will make this event truly impactful.

We hope you will leave this conference enriched with new insights, inspiration, and good memories of your time in Évian-les-Bains.

Welcome, and let's make this a remarkable event together.

Dr. Isabelle Domaizon, Director of INRAE CARRTEL, Thonon-les-Bains, France

SCIENTIFIC AND LOCAL ORGANIZING COMMITTEE

Luisa Orsini (SC)

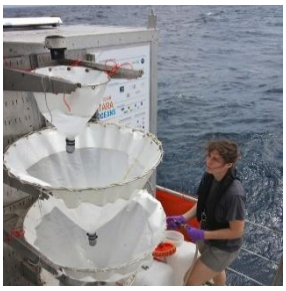
Lois Maignien (SC)

Kristian Meissner (SC)

Bettina Thalinger (SC)

Pedro Beja (SC)

Ramiro Logares (SC)



Lucie Bittner (SC)

Naiara Rodriguez-Ezpeleta (SC)

Florian Leese (SC)

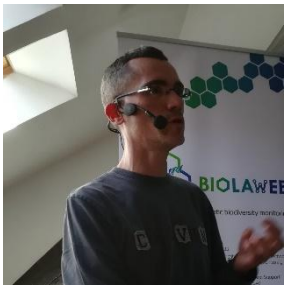
Isabelle Domaizon (LOC)

Frédéric Rimet (LOC)

Jonas Bylemans (LOC)

Nicolas Tromas (LOC)

Clarisse Lemonnier (LOC)



Benjamin Alric (LOC)

Benoit Paix (LOC)

Valentin Vasselon (LOC)

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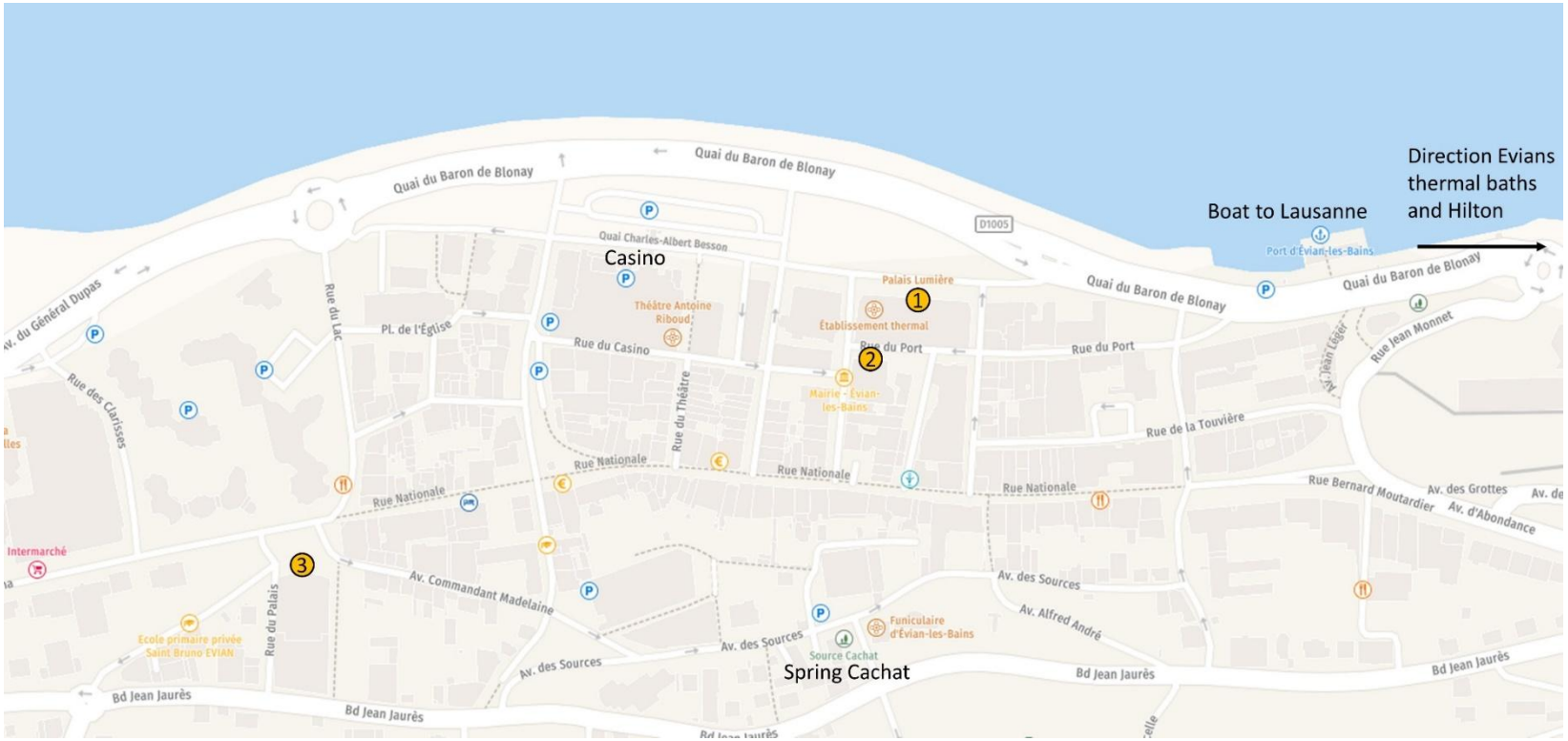
SCIMABIO interface

CONFERENCE VENUE



1. Palais Lumière: Main conference venue
2. Espace Brunnarius: Welcome drink & Lunch
3. Palais des festivités: Poster session & Conference dinner

PROGRAM AT A GLANCE

SESSION TOPICS

Session 1	Omics in aquatic ecosystems through participatory science
Session 2	Leveraging omics data to go beyond detections and towards eco-evolutionary insights
Session 3	Understanding Aquatic Ecosystems in an Era of Environmental Change: From Omics Monitoring to Forecasting
Session 4	Knowledge and technological and gaps in omics: what are they and how can they be filled?
Session 5	Biodiversity Monitoring and Reporting: a stakeholders perspective
Session 6	Understanding biotic interactions through the prism of Omics techniques
Session 7	Integrating omics in macroecology to elucidate ecosystem patterns and processes

SCIENTIFIC PROGRAM

Time	Monday 17.03.2025	Tuesday 18.03.2025		Wednesday 19.03.2025		Thursday 20.03.2025		Friday 21.03.2025		
		Auditorium	Parallel room	Auditorium	Parallel room	Auditorium	Parallel room			
8:30		Registration		Registration		Registration				
8:45										
9:00		Opening Word		Keynote lecture **Florian Altermatt**		Keynote lecture **Silvia Acinas**		Table Ronde		CEN Standardisation Technical Committee meeting
9:15		Presentation of Sponsors								
9:30		Keynote Lecture **Karoline Faust**								
9:45				Coffee Break		Coffee Break				
10:00										
10:15		Coffee Break		Session 7 part 2	Session 3 Validation of Omics Tools	Session 5 Implementation & Standardisation	Session 3 Anthropogenic Impacts part 1		Physalia Course	
10:30										
10:45		Session 1	Session 4 Sampling & Analyses							
11:00										
-										
12:30										
12:45		Lunch		Lunch		Lunch				
13:00										
13:15										
13:30										
13:45										
14:00										
14:15		Session 2 Functional Diversity	Session 6 Macro- & Micro-organismal Interactions	Session 3 Bioindicators	Session 2 Quantification & Intraspecific Diversity	Session 3 Anthropogenic Impacts part 2	Session 4 Reference Databases			
-										
16:00										
16:15		Coffee Break								
16:30										
16:45		Session 6 Micro-organismal Networks	Session 7 part 1	Coffee Break		Coffee Break				
17:00	Registration - Palais Lumière									
17:15						Session 4 Technical Advancements	Session 5 Biotic & Abiotic Drivers of Biodiversity			
17:30				Poster Session - Palais des Festivités						
17:45										
18:00		Wine & Cheese - Palais Lumière								
18:15										
18:30	Welcome Drink - Espace Brunnarius									
18:45										
19:00				Conference Dinner - Palais des Festivités		Closing Ceremony				
19:15										
19:30										
19:45										
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DETAILED PROGRAM

MONDAY (17.03.2025)

The conference registration desk will open on Monday 17th of March. Participants can register from 17h00 until 19h00 at the main conference venue (Palais Lumière). From 18h30 onwards participants can enjoy a welcome drink and a buffet in Espace Brunnarius located directly behind the main conference venue.

TUESDAY (18.03.2025)

KEYNOTE SPEAKER

Karoline Faust is an Associate Professor at KU Leuven who heads the Lab of Microbial Systems Biology, which focuses on investigating microbial community dynamics in silico and in vitro. In particular, her team is studying interactions between human gut bacteria and the resulting dynamics with synthetic communities in controlled conditions. In addition, the team develops new tools for the analysis of microbiome data, specifically the analysis of microbial networks. The group's work is thus situated at the intersection of bioinformatics, systems biology, and microbial ecology.

Microbetag: a reverse-ecology tool for microbial network interpretation

Ecological interactions are important drivers of microbial community composition and dynamics but are difficult to resolve experimentally. Microbial association networks inferred from abundance data do not predict microbial interactions reliably and are therefore difficult to interpret. In this context, it is helpful to work with genomes that are already known or assembled from metagenomic sequencing data, since they allow applying reverse ecology methods to predict traits and ecological interactions from genes. Here, I will present a new tool, microbetag, that makes use of such methods to help interpret microbial networks.

SCIENTIFIC PROGRAM

Tuesday 18.03.2025		
Time	**Auditorium**	**Parallel room**
8:00	Registration	
8:15		
9:00	**Opening Word**	
9:15	Presentation of Sponsors	
9:30	Keynote Lecture **Karoline Faust**	
9:45		
10:00		
10:15	Coffee Break	
10:30		
	-Session 1- Moderators: Bettina Thalinger & Kristy Deiner	-Session 4- Moderators: Jennifer Harris & François Keck
10:45	**O001. Fidji Sandré** Passive eDNA sampling for enhanced species detection	**O009. Leo Barbut** Using Lagrangian transport model in support of eDNA monitoring in Belgian part of the North Sea
11:00	**O002. Ryo Iwamoto** QuickConc: a novel cationic-assisted eDNA capture method for enhanced biodiversity monitoring	**O010. Vincent Prié** Field sampling of eDNA under complex hydrological conditions
11:15	**O003. Sofie Derycke** eDNA collection by the Belgian fishing fleet: a new gateway towards high resolution marine biodiversity data collection	**O011. Lana Grizancic** Net or Niskin: sampling method most suitable for the northern Adriatic phytoplankton monitoring
11:30	**O004. Lauren Rodriguez** From whale watching to marine biodiversity monitoring: enhancing eDNA sampling with citizen science	**O012. Manuel Lopes-Lima** Unlocking Africa's biodiversity: advancing surveys with cutting-edge eDNA techniques
11:45	**O005. Valérie S. Langlois** Towards revolutionizing conservation efforts in Canada	**O013. Joana Veríssimo** Navigating methodological trade-offs in eDNA biodiversity monitoring: insights from a Mediterranean watershed
12:00	**O006. Bettina Thalinger** Studying bird and fish biodiversity in Canada by engaging local communities in environmental DNA based monitoring	**O014. Philipp M. Rehsen** Improving biomass and abundance assessment of aquatic indicator taxa by combining semi-automated imaging and DNA metabarcoding
12:15	**O007. Yuki Minegishi** ANEMONE Global: establishment of a global network of eDNA-based aquatic biodiversity monitoring.	**O015. Bastien Macé** Finding NeMO: a bayesian adventure in detecting elusive species with environmental DNA
12:30	**O008. Kirsty Deiner** Are lake accumulators of biodiversity information from environmental DNA?	**O016. Gledis Guri** Predicting trawl catches using environmental DNA
12:45	Lunch -Espace Brunnarius-	
-		
14:00		
	-Session 2- Moderators: Silvia Acinas & Nicolas Tromas	-Session 6- Moderators: Fabrice Not & Benjamin Marie
14:15	**O018. Arthur Monjot** Metatranscriptomes-based sequence similarity networks uncover genetic signatures within parasitic freshwater microbial eukaryotes	**O025. Léa Combes** Diversity and long-term temporal dynamics of Microsporidia in Lake Aydat using a paleogenomic approach
14:30	**O019. Stéphane Vuilleumier** Bacterial players of the chloromethane cycle in aquatic environments	**O026. Kamil Hupalo** Having IMPACT on monitoring aquatic diversity: first experimental results evaluating eDNA as an integrative tool for studying parasites

Tuesday 18.03.2025		
Time	**Auditorium**	**Parallel room**
14:45	**O020. Theodor Sperlea** Detecting semi-annual oscillations in the biotic community of aquatic habitats worldwide using metabarcoding and machine learning	**O027. Sébastien Duperron** Impact of cyanobacterial blooms on fish gut microbiota: from ecotoxicology to biological invasions?
15:00	**O022. Nathalie Joli** Transcriptomic and epigenomic insights into gene and transposable element regulation in a polar diatom during prolonged darkness and re-illumination	**O028. Benoît Paix** Ecology and evolution of chemical interactions between the brown algae Dictyotales and their epibacterial communities
15:15	**O023. Marie-Lee Castonguay** Influence of pollutants, temperature and density on the production and persistence of environmental DNA and RNA (eDNA and eRNA) in aquatic environments	**O029. Samuel Orgeas-Gobin** Saving the best for the last: late apoptosis as a mechanism to preserve symbiosis in the thiotrophic bivalve *Lucinoma borealis*?
15:30	**O024. Teun Everts** An omics-based spatial prioritization framework to counter widespread aquatic invaders	**O030. Erwan Quéméré** Diving deep into kelp forest food webs using dietary DNA
15:45	**O017. Pierre Foucault** Temporal dynamics of microbiomes and life strategies in peri-urban lakes	
16:00		
16:15 16:30	Coffee Break	
	-Session 6- Moderators: Karoline Faust & Ramiro Logares	-Session 7- Moderators: Tristan Cordier & Rosa Trobajo
16:45	**O031. Rafael I. Ponce-Toledo** Modeling the evolution of microbial networks	**O035. Rimet Frédéric** Biogeography of microalgae in freshwaters: new contributions from metabarcoding?
17:00	**O032. Binta Diémé** Cross-feeding interaction within Microcystis phycosphere: new perspectives from the combination of metagenomics and metametabolomics approaches	**O036. Paula Mendoza** Exploring diatom diversity and biogeography in Canary Island watercourses: insights from DNA Metabarcoding
17:15	**O033. Pascal I. Hablützel** Leveraging metagenomics and metatranscriptomics to gain novel insights in the ecological dynamics of microeukaryotic plankton communities	**O037. Briand Jean-françois** Geographic genetic divergence in tychoplanktonic taxa dominating diatom communities in the marine plastisphere
17:30	**O034. Shan Pushpajom Thomas** Co-occurrence networks reveal interactions between aquatic prokaryotes and protists	**O038. Emma Jamon** Divergent Prokaryotic communities across mangrove sediment bioregions: insights from 16S rDNA metabarcoding data
17:45		**O039. Maurine Vilcot** Revealing a diversity continuum in tropical fishes: simultaneous eDNA assessment of populations and communities
18:00 - 19:15	Wine & Cheese -Palais Lumière-	

WEDNESDAY (19.03.2025)

KEYNOTE SPEAKER

Prof. Florian Altermatt supervises a team of about 30 people, who address fundamental and applied questions in ecology, with a focus on spatial ecology, biodiversity and ecosystem processes. His work aims to understand how species occur in space and time, how they interact, and how processes such as invasions, dispersal or global change affect natural communities. He uses both experimental and comparative approaches, integrate novel tools such as eDNA, and parallel it with theoretical models. He uses a variety of study systems suited to address broad-ranging questions in ecology. His group is part of the Department of Evolutionary Biology and Environmental Studies at University of Zurich and the Department of Aquatic Ecology at Eawag.

Utilising aquatic environmental DNA to address local to global biodiversity targets

Adequate data on aquatic biodiversity are a necessary prerequisite for biodiversity science. Assessing and attributing the state and change of biodiversity is also essential for bending the curve of biodiversity loss and guiding necessary policy action. Since the mid 2010s, analysis of environmental DNA (eDNA) has become established as a novel and highly powerful approach to assess the state and functioning of aquatic ecosystems and is becoming increasingly implemented by stakeholders, yet its potential is not yet fully tapped. I will present current state in aquatic eDNA research with a particular focus on the policy-relevance of eDNA and its utility in contributing towards the Global Biodiversity Framework (GBF). I will then summarise key technological developments in eDNA science to assess organismal diversity across the full tree of life, to establish ecological indicator analyses in aquatic systems, and its potential for spatial and temporal upscaling as a key reference for local to global biodiversity action. Technological advances in laboratory and sequencing techniques have enabled rapid uptake of the method, yet current challenges remain in the need for adequate reference databases, commonly agreed quality standards (including FAIR principles), and overcoming methodological constraints in retrofitting novel eDNA-based approaches to existing biodiversity monitoring approaches. I will outline the next steps needed to effectively implement eDNA for decision making and addressing global biodiversity targets.

SCIENTIFIC PROGRAM

Wednesday 19.03.2025		
Time	**Auditorium**	**Parallel room**
8:00	Registration	
8:15		
9:00	Keynote Lecture **Florian Altermatt**	
9:15		
9:30		
9:45	Coffee Break	
10:00		
	-Session 7- Moderators: Johan Pansu & Cécile Lepère	-Session 3- Moderators: Pedro Beja & Isabelle Domaizon
10:15	**O040. Tristan Cordier** Combining modern surface-to-seafloor eDNA datasets to unlock the potential of sedimentary ancient DNA.	**O050. Coci Manuela** New omics observatory for marine biodiversity in the Adriatic: a case study
10:30	**O041. Benjamin Marie** More than just disorder - metabolite diversity of Microcystis strains shows tight correspondence to genotype and may contribute to ecotype specificities	**O051. Bassam Abubaker** Environmental drivers of diatom diversity: insights from DNA metabarcoding
10:45	**O042. Rosa Trobajo** Diatom metabarcoding for good ecological status assessment and beyond	**O052. Lena Brouwir** Methodology for creating a freshwater bioindicator using periphyton genomics
11:00	**O043. Antonija Kulaš** Diatoms genetic diversity across different climate zones	**O053. Thomas Reinhart** How do eDNA monitoring methods compare to traditional bryophyte surveys in rivers?
11:15	**O044. Anastasija Zaiko** Kelp in the climate equation: leveraging 'omics' approaches to unveil the role of giant kelp habitats as a blue carbon sinks	**O054. Nieves López Rodríguez** From marine to freshwater: using fish eDNA to assess community dynamics across salinity gradients
11:30	**O046. Andrea Burfeid Castellanos** Changed succession patterns in the Arctic Sea observed through metabarcoding	**O055. Pedro Beja** Metabarcoding across the tree of life reveals conservation significance and biodiversity patterns in a tropical river (Corubal, Guinea Bissau)
11:45	**O047. Sergio González-Motos** Gene synchrony and rhythmicity in neighbouring marine microbiomes generates insights on functional redundancy	**O056. Yvonne Schadewell** Harnessing the power of eDNA biodiversity assessment to enhance subsurface water flow pathway reconstruction
12:00	**O048. Ramiro Logares** Integrating short- and long-read metagenomics to reveal local and global macroecological patterns in marine microbial populations	**O057. Hannah Rau** New perspectives on the community composition of groundwater ecosystems using eDNA metabarcoding
12:15	**O049. Mechthild Schmitt-Jansen** A comparative metagenomics approach reveals a common functional potential of plastisphere microbiomes across oceans	
12:30		
12:45	Lunch -Espace Brunnarius-	
-		
14:00		

Wednesday 19.03.2025		
Time	**Auditorium**	**Parallel room**
	-Session 3- Moderators: Luisa Orsini & Raffaele Siano	-Session 2- Moderators: Florian Altermatt & Aurélie Bonin
14:15	**O058. Till-Hendrik Macher** Fit for purpose? Evaluating benthic invertebrate DNA metabarcoding for ecological status class assessment in streams under the Water Framework Directive	**O068. Jonas Bylemans** From anarchy to clarity, data pre-processing and statistical choices influence quantitative environmental DNA (eDNA) analyses.
14:30	**O059. Cristiana Cravo-Laureau** Bioindicators of littoral and retro-littoral wetlands ecosystem functioning	**O069. Mohamed Yosri Zanni** Modeling the interactions between decay and dispersion of eDNA in the Bay of Biscay
14:45	**O060. Jono Warren** Microbial biofilms as indicators of environmental change in English rivers.	**O070. Céline Condachou** Linking metabarcoding quantitative information to fish environmental DNA concentration in Neotropical rivers
15:00	**O061. Sara Beier** Artificial intelligence assisted modelling reveals that species properties rather than species diversity determine community responses to environmental change	**O071. Marie-Pier Brochu** Three-year monitoring of lake sturgeon (*Acipenser fulvescens*) occurrence in a spawning ground using environmental DNA
15:15	**O062. Benjamin Alric** Graph theory at the service of assessing the ecological status of lake ecosystems based on phytoplankton communities	**O072. Micaela Hellström** Reproductive and migratory patterns in fish revealed by MBC analyses of monthly samples.
15:30	**O063. Leire Garate** Sediment microbial communities and their association networks differed depending on their disturbance level	**O073. Emilie A. Didaskalou** Unlocking demography: developing an eDNA-based toolkit to measure sex ratios.
15:45	**O064. Erik Zschaubitz** Machine learning-driven analysis of metabarcoding data to identify anthropogenic trace substances in aquatic ecosystems	**O074. Tamas Malkocs** Species-level detection and population genetic inference of small cetaceans from environmental samples, using mitochondrial and RADseq-derived markers
16:00	**O065. Anders Lanzén** Estuarine microbenthos as ecosystem health indicators, from the Basque estuaries to the whole Bay of Biscay, the world, and beyond	**O075. Marie-Thérése Werner** Population genetic analyses of a key invertebrate species using mitochondrial and nuclear DNA markers
16:15	**O066. Luisa Orsini** Biodiversity time machine: a holistic approach to monitoring and forecasting freshwater ecosystems	**O076. Daniel Zumel** Hybrid horizons: detecting hybridization in natural populations using a novel eDNA toolkit
16:30	**O067. Niamh Eastwood** Exploring the landscape-level drivers of lake biodiversity using data-driven analysis of environmental DNA	**O077. Els De Keyzer** Repeated river–lake introgression in the adaptive radiation of Sailfin silverside fishes in Lake Matano, Sulawesi
16:45	Coffee Break	
17:00		
17:15		
17:30	Poster Session -Palais des Festivités-	
17:45		
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18:45		
19:00	Conference Dinner -Palais des Festivités-	
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POSTER PRESENTATIONS

No.	Presenter	Poster title
P01	Tristan Lefébure	Monitoring biodiversity of an alpine watershed using eDNA metabarcoding and ecological surveys: a collaborative work between students, scientists and citizens
P02	Vid Švara	ProTecteDNA: portable solutions for eDNA-based biodiversity monitoring in protected areas
P03	Abigaël Chieux	On-site species detection based on eDNA: example of the Natterjack toad (*Epidalea calamita*)
P04	Chiara Mercier	The noose is tightening on parasites: how environmental nucleic acids (RNA/DNA) help us identify the different forms of freshwater parasites
P05	Verena Trenkel	eRNAmaris - the use of environmental RNA to improve fish stock assessments in marine systems
P06	My Dung Jusselme	Wastewater treatment with an oxidizing agent: efficiency on pathogens and antimicrobial resistance
P07	Emilie Delpuech	eDNA as a tool for precision biodiversity reporting: aligning science and stakeholder needs
P08	Jessie-Lee Langel	Can eDNA be a decision support for sea turtle nesting monitoring?
P09	Marine Vautier	Three converging eDNA approaches to track the reproductive dynamics of two invasive dreissenid mussels in lakes
P10	Yeseren Kayacan	Diurnal and intratidal variation in microbial community structure and gene expression in a mudflat biofilm
P11	Sébastien Duperron	The culture collection of cyanobacteria at the MNHN (National Museum of Natural History)
P12	Paula Gauvin	Evaluating littoral zone restoration in lake through a multi-taxa eDNA ecological assessment
P13	Fidji Sandré	Tracking aquatic biodiversity with eDNA: a study in quebec's mining regions
P14	Manon Daudinet	Identifying macrophytes using environmental DNA metabarcoding for biomonitoring and ecosystem management
P15	Clara Dignan	Using OMICS methods to evaluate and monitor marine microbial responses to human in a management perspective - the MICROSURV project
P16	Fanny Charrier	Decyphering the tolerance of leaf litter biofilms to the biofungicide Kasugamycin through the combination of metametabolomics with structural and functional descriptors
P17	Jennifer Harris	Microbial diversity baseline linked to hydrogeological conditions and anthropic pressures in the Beauce Aquifer, France.
P18	Judith Piontek	The potential of eDNA metabarcoding for monitoring demersal fish communities in marine proteced areas of the Baltic Sea
P19	Sulivan Jouanneau	Evolution of genetic and phenotypic diversity in a marine microbial community exposed to pollutants: a microcosm study.
P20	Lin Zi	Developing high throughput metametabolomics in freshwater Periphyton to enhance chemical risk assessment

No.	Presenter	Poster title
P21	Charlotte Van Driessche	Precision in river monitoring: key eDNA sampling sites unlock comprehensive fish biodiversity insights
P22	Lemonnier Clarisse	Comparison of shot-gun sequencing and metabarcoding to assess alpine lakes phytoplankton diversity
P23	Dimitra-Ioli Skouroliakou	Enhancing plankton monitoring: a comparison of short and long-read eDNA metabarcoding for characterizing plankton communities in the Belgian part of the North Sea
P24	Enora Geslain	Refining eDNA taxonomic assignments with a phylogenetic approach
P25	Joana Veríssimo	Evaluating the impact of sampling strategies and bioinformatics on ethanol-based DNA metabarcoding
P26	Paul Hamilton	Building the diatom (Heterokontophyta) DNA library with rare species using traditional sequencing
P27	Nika Tivadar	Preliminary comparison of microscopy and molecular methods for determining cyanobacterial composition of a frequently blooming pond
P28	Théo Deremarque	Using environmental DNA to track the spread of invasive host-parasite complexes: a case study of the invasive freshwater fish *Pseudorasbora parva* and the cryptic fungal parasite *Sphaerothecum destruens*
P29	Eloïse Duval	eDNA monitoring at a large scale to spot at-risk salmonid populations regarding an emerging infectious disease
P30	Gael Denys	The eDNA as an interesting tool to protect endangered endemic fish species against restocking: an example with French graylings (Teleostei, Salmonidae)
P31	Laura Jamet	Does the Aquitanian pike (Teleostei, Esocidae) breed during the closed angling season? Elements of answer according to the eDNA approach.
P32	Nieves López Rodríguez	Can eDNA metabarcoding be used to develop a biological quality index for disconnected pools in temporary rivers?
P33	Sébastien Autret	Assessment of the cyanobacteria risk, considering the invasive bryozoan *Pectinatella magnifica*
P34	Martel Alexis	Guilt by association? The role of cyanobacteria-associated bacteria in harmful algal blooms.
P35	Mélissa Eon	Chemical landscape of invasive aquatic plant exometabolomes
P36	Ivaylo Sirakov	How the feeding regimes impact the waste metabolomes in aquaponics
P37	Stéphan Jacquet	Quantifying an example of invasion dynamics and interaction with endemic species: the case of the red blood mysid *Hemimysis anomala* in Lake Geneva
P38	Briand Jean-françois	Relative effect of physico-chemical parameters and contaminants on the diversity of diatom communities along the French Mediterranean coast
P39	Amélie Malherbe	Combination of metabolomics and machine learning to unravel environmental drivers of spatial heterogeneity of microbial metabolome assemblage in aquatic periphyton: The COMBO project
P40	Florian Leese	TrendDNA: Studying long-term biodiversity change using environmental DNA contained in the German Specimen Bank

THURSDAY (20.03.2025)

KEYNOTE SPEAKER

Dr. Silvia Acinas is a researcher at the Department of Marine Biology and Oceanography at the ICM (Institut de Ciències del Mar), CSIC in Barcelona. Her research focuses on linking microbial genetic diversity with functional capacities of ecologically relevant marine microbial taxa using metagenomics, metranscriptomics, single cell genomics, and isolation. These approaches are used to gain insights into the evolutionary mechanisms underlying diversification processes. She now focuses on polar marine ecosystems (Arctic and Antarctic), which are threatened by climate change but also blue-biotechnology research.

Unlocking the Ocean Microbiome: From Ecology to Blue Bioprospecting

The planet's greatest diversity lies within the invisible world of planktonic microorganisms. With an estimated 10^{29} prokaryotic cells and at least 10^{11} microbial species (Locey and Lennon, 2016), these organisms harbour immense genetic and metabolic potential and enable the degradation of numerous natural and human-introduced (allochthonous) substances, highlighting their adaptability and potential for innovation. Advances in marine biodiversity research have increasingly revealed microorganisms with molecules and genes of significant commercial and scientific value. Our research group investigates marine microbes' ecology, functional diversity and genetic capacities of marine microbes for Blue Bioprospecting. This presentation highlights several studies based on large-scale metagenomic surveys (Tara Oceans and Malaspina) providing as well examples of blue bioprospecting related to (1) bacteria from the ocean and marine sediments capable of degrading methylmercury, a neurotoxin, and (2) the discovery of novel CRISPR-Cas9 systems from the deep ocean.

SCIENTIFIC PROGRAM

Thursday 20.03.2025		
Time	**Auditorium**	**Parallel room**
8:00	Registration	
8:15		
9:00	Keynote Lecture **Silvia Acinas**	
9:15		
9:30		
9:45	Coffee Break	
10:00		
	-Session 5- Moderators: Kristian Meissner & Florian Leese	-Session 3- Moderators: Anastasija Zaiko & Frederic Rimet
10:15	**O078. Tiina Laamanen** Technology readiness level of biodiversity monitoring with molecular methods - where are we on the road to routine implementation?	**O087. Martijn Callens** Local accumulation of organic matter in marine sand extraction areas drives changes in sediment prokaryotic communities with potential consequences for nitrogen cycling
10:30	**O079. Christina Pavloudi** Preliminary results from the European Marine Omics Biodiversity Observation Network (EMO BON): long-term genomic monitoring and FAIR stakeholder reporting	**O088. Valentin Ambroise** Impact of 10 years of deforestation and population growth on biodiversity seen through the lens of metabarcoding
10:45	**O080. Sandra Garcés-Pastor** Taxonomy-free approach to diatom indicators based on three eukaryotic markers	**O089. Rein Brys** The power, challenges and integration of omics-based ecological insights as a cornerstone for invasive species management in aquatic environments
11:00	**O081. Kálmán Tapolczai** A novel framework for phytoplankton biomonitoring: trait assignment of 23S rRNA sequences	**O090. Ana Baricevic** Omics in the service of characterising the eukaryotic plankton community in the Adriatic Sea
11:15	**O082. Laurine Viollaz** Environmental DNA approach to assess phytoplankton communities in lake environments	**O091. Eeva Eronen-Rasimus** Under-ice methanotrophy may offset Baltic sea ice methane fluxes
11:30	**O083. Jelger Herder** Exploring the potential of eDNA-metabarcoding as an alternative to conventional fish monitoring under the Water Framework Directive	**O092. Anastasija Zaiko** Understanding emerging ecosystems: tracing biodiversity and ecological change in a periglacial Arctic lagoon using eDNA
11:45	**O084. Valentin Vasselon** The fellowship of the ring test confronting a method to rule them all: testing the transferability and comparability of diatom DNA metabarcoding protocols for biomonitoring	**O093. Flora Mottet** Using multi-omics to illuminate responses of sediment microbial communities to hydrological changes in lotic systems and their consequences on carbon cycling
12:00		**O094. Kiemel Katrin** Uncovering effects of environmental change through eDNA metabarcoding: a long-term perspective
12:15	**O086. Olivier Monnier** The carbon footprint of diatom molecular research	**O095. Nicolas Tromas** Predicting cyanotoxins concentrations in lakes and reservoirs using microbial community information
12:30		
12:45	Lunch -Espace Brunnarius-	
-		
14:00		

Thursday 20.03.2025		
Time	**Auditorium**	**Parallel room**
	-Session 3- Moderators: Nicolas Creusot & Helène Agogué	-Session 4- Moderators: Karolina Bacela & Till-Hendrik Macher
14:15	**O096. Nicolas Creusot** Multiomics approach to characterize the link between the structural biodiversity and the microbial activity along the natural dynamic of freshwater periphytic biofilms and its response to the chemical stress: main outcomes from the MICROBIOMIQ project	**O106. François Keck** Navigating the seven challenges of taxonomic reference databases
14:30	**O097. Tayebeh Soltanighias** Occurrence of antibiotic-resistant bacteria in household plumbing system	**O107. Arne J. Beermann** dbDNA - A phylogeny- and expert identifier-driven grading system for reliable taxonomic annotation of (meta)barcoding data
14:45	**O098. Chakresh Kumar** Metagenomic insights into the functional microbial diversity of the lower stretch of the river Ganga: mapping antibiotic and metal resistance genes	**O108. Ljubica Vlaović** Challenges in the investigation of the diatom community of saline habitats: a case study of Plava Banja (Serbia)
15:00	**O099. Pedro A. Inostroza** Multi-compartment impact of micropollutants and particularly antibiotics on bacterial communities using environmental DNA at river basin-level	**O109. Kristina Petrović** eDNA-based assessment of phytoplankton community structure and dynamics in a saline lake in Serbia: comparison with microscopy-based method
15:15	**O100. Gaëtan Burgaud** A comprehensive pan-European exploration of fungal plastisphere dynamics along river-to-sea continuums	**O110. Nergiz Dukan** MSFD and impact monitoring with eDNA: Insights from case studies in the Belgian part of the North Sea and Avlékété beach, Benin
15:30	**O101. Nina Röder** Genome-wide responses to chronic Bti and copper exposure in a laboratory culture of *Chironomus riparius*	**O111. Liliana Ballesteros-Mejia** Laying the foundations of a national genetic baseline for species identification in support of biodiversity research and public policies in France.
15:45	**O102. Lise Klunder** Making the invisible visible: molecular methods to trace the success of the restoration of river Emscher	**O112. Vincent Haÿ** A molecular reference database of mitochondrial genomes for freshwater fish in France.
16:00	**O103. Réalis-Doyelle Emilie** From ice to heat: variation in phenotypic and transcriptomic response of Arctic Charr populations from contrasted environments	**O113. David Mann** Metabarcoding "by-catch" is precious
16:15	**O104. Mahshid Oladi** Environmental DNA-based profiling of benthic microbial communities along a crude oil spill gradient in a coral reef in the Persian Gulf	**O114. Daniel Wewer** Overcoming limitations in eDNA metabarcoding with nanopore sequencing of whole mitogenomes
16:30	**O105. Lou-Anne Jannel** Aquatic biodiversity on Reunion Island: responses of biological communities to environmental and anthropogenic pressures using environmental DNA	
16:45	Coffee Break	
17:00		

Thursday 20.03.2025		
Time	**Auditorium**	**Parallel room**
	-Session 4- Moderators: Mechthild Schmitt-Jansen & Erwan Quéméré	-Session 5- Moderators: Sofie Derycke & Owen Wangensteen
17:15	**O115. Jim Birch** FIDO: a new type of autonomous aquatic sampling instrument for 'omics studies	**O122. Baudry Thomas** eDNA optimization for regular monitorings of white-clawed crayfish (*Austropotamobius pallipes* species complex): from lab testings to large-scale field validation
17:30	**O116. Armando Espinosa Prieto** Leveraging hybridisation capture for detecting rare events and as a PCR-free metabarcoding approach for vegetation surveys	**O123. Marina Chauvet** Seeking for microbial bioindicators of river run-off inputs: new insights from eDNA data sets
17:45	**O117. Hiroki Yamananaka** Enhancing eDNA metabarcoding accuracy: mitigating PCR bias with droplet PCR	**O124. Haderlé Rachel** Environmental DNA metabarcoding for the assessment of vertebrate biodiversity along the estuarine gradient of the Rance river (Brittany, France)
18:00	**O118. Patrick Łypaczewski** Unrestricted metabarcoding with Nanopore long-read sequencing	**O125. Héloïse Verdier** eDNA outperforms traditional methods for detecting organic pollution in a non-perennial river
18:15	**O119. Fabrice Not** Aquatic diversity of microbial eukaryotes assess by Oxford Nanopore Technology and Illumina sequencing	**O126. Ole Bjørn Brodnicke** Innovating biodiversity monitoring: translating eDNA research and democratizing data to empower stakeholders and the public
18:30	**O120. Alba M. Losa** Addressing workflow challenges of ONT sequencing approaches for riverine microbial communities: long-read vs short-read sequencing	**O127. Daniël van Berkel** eDNA-based assessment of species diversity and its temporal development in a Dutch offshore wind farm
18:45	**O121. Olivier Collard** Nanopore eDNA long-read metagenomics: an holistic window for eDNA analysis	**O128. Auriel Sumner-Hempel** Monitoring Elasmobranch diversity using eDNA in offshore wind farms
19:00		
19:15	**Closing Ceremony** -Auditorium-	
19:30		
19:45		

ABSTRACTS

ORAL PRESENTATIONS

O001. Passive eDNA sampling for enhanced species detection

Fidji Sandré (INRS ETE, Québec), Tuan Anh To (INRS ETE, Québec), Julie Couillard (INRS ETE, Québec), Valérie S. Langlois (INRS ETE, Québec)

Biodiversity is essential for the proper functioning of ecosystems. However, in Canada, it is particularly threatened in regions where mining activities fragment habitats and degrade aquatic ecosystems. Rigorous biodiversity assessment is therefore crucial to guide conservation strategies, but it requires extensive data collection, which traditional methods, such as direct observation and trapping, often fail to provide effectively. In this context, environmental DNA (eDNA) has emerged as an innovative and promising tool. This technique involves analyzing DNA traces released into the environment by organisms through cells, excreta, scales, enabling the detection of even the rarest species in a non-invasive, faster, and more cost-effective manner than traditional methods. In this study, eDNA was used to assess the presence of several fish species around two mining sites in the James Bay area. Sampling campaigns were conducted in 2022 and 2023, and the collected eDNA was analyzed using qPCR with species-specific detection kits developed as part of the pan-Canadian iTrackDNA project. The results revealed the presence of walleye and brook trout, as well as yellow perch and Northern pike, both considered invasive species. Notably, the absence of sauger and the endangered lake sturgeon around the mining sites was also observed. Although mining activities did not seem to impact water quality, they may influence hydrology and alter habitats. Organisms are also subject to competitive pressures, and eDNA may help reveal certain ecological dynamics. It provides a concrete solution for better biodiversity management in environments subject to intense human pressures and can contribute to the protection of threatened species and to a deeper understanding of rapidly changing ecosystems.

O002. QuickConc: a novel cationic-assisted eDNA capture method for enhanced biodiversity monitoring

Iwamoto, Ryo (AdvanSentinel Inc., Japan), Kuroita, Tomohiro (AdvanSentinel inc., Japan), Wu, Qianqian (Kobe University, Japan), Minamoto, Toshifumi (Kobe University, Japan)

Environmental DNA (eDNA) analysis is a non-invasive tool for monitoring biodiversity and species distribution. However, capturing eDNA from water samples can be challenging due to the presence of various PCR inhibitors and the difficulty of concentrating DNA from large water volumes. Traditional methods, such as glass fiber filtration and Sterivex, have limitations, particularly in turbid environments or when dealing with large volumes of water. We introduce QuickConc, a novel, power-free eDNA concentration method that utilizes benzalkonium chloride (BAC) along with dispersed glass fibers to enhance nucleic acid capture. The QuickConc method was tested in various aquatic environments, including rivers, sea, and pond, and its performance was evaluated in comparison with traditional glass fiber filter and Sterivex. QuickConc consistently yielded higher total eDNA concentrations, recovering 2 to 3 times more eDNA than the other methods. Species-specific qPCR analyses

revealed that QuickConc significantly increased the detection of fish species across all environments, with up to 10 times higher copy numbers detected. Metabarcoding results further demonstrated the effectiveness of QuickConc in detecting a broader range of species, particularly in river water samples, compared to traditional methods. QuickConc's user-friendly, power-free design offers a promising new approach for eDNA concentration, making it particularly suitable for fieldwork in remote or resource-limited settings. Additionally, its ability to efficiently handle both clear and turbid water samples provides greater flexibility for ecological monitoring and conservation efforts.

O003. eDNA collection by the Belgian fishing fleet: a new gateway towards high resolution marine biodiversity data collection

Derycke Sofie (ILVO, Belgium), Cornelis Isolde (ILVO, Belgium), Carneiro Antonio (INESCTEC, Portugal), Marques Pedro (INESCTEC, Portugal), Martins Alfredo (INESCTEC, Portugal), De Pape Daan (DP Technics, Belgium), Vlietinck Dany (NV Rederij Nathalie, Belgium), Polet Hans (ILVO, Belgium), Hostens Kris (ILVO, Belgium)

Marine organisms release DNA molecules into the environment through tissues, excrements or slime. This environmental DNA (eDNA) allows to detect fishes by simply collecting seawater. As such, eDNA has tremendous potential for environmental and fisheries-related research, especially when eDNA collection can be automated. As the Belgian fishing fleet covers an extensive geographic range and is out at sea throughout most of the year, a close collaboration between researchers and fishermen sets the scene for marine data gathering at unprecedented spatial and temporal resolutions. We developed an automatic eDNA multi-sampler that is placed on the fishing vessel, which can filter subsurface seawater at specified geographic locations. The eDNA on the sterivex filter is preserved with a fixative, after which the tubing system is flushed with bleach to prevent cross-contamination between samples. The sampler can be preprogrammed and controlled remotely in real time without intervention of fishermen. The successful deployment of the sampler on a fishing vessel for almost three months provides the entry to rollout eDNA sampling towards the whole fishing fleet. Secondly, we developed an easy and standardized protocol with low-cost passive eDNA samplers to study fish diversity in data poor regions. Fishermen attach these so-called metaprobes to their fishing nets and beam trawl chains. The metaprobes are stored at room temperature in ethanol or silica beads. So far, eDNA collected by metaprobes inside the net and preserved in ethanol gave the most accurate reflection of the catch composition. Finetuning of the preservation with silica beads is ongoing to improve sample handling and transportation. The participation of the fishing fleet in eDNA sample collection invokes the data collection framework of the future, where standardized protocols and easy-to-use samplers ensure reliable data collection in the marine environment at scales scientists alone can never achieve.

O004. From whale watching to marine biodiversity monitoring: enhancing eDNA sampling with citizen science

Rodriguez Lauren (Univ. Innsbruck, Austria), De Bonis Lorenzo (Univ. College Cork, Ireland), McKee Jack (Univ. College Cork, Ireland), McKenna James (Institute of Marine Research, Norway), Urvois Teddy (INRAE, France), Barbaccia Eleonora (Politecnico di Milano, Italy), García Ovide Belén (Univ. Iceland Research Centre in Husavik, Iceland), Villa Enrico (CW

Azores, Pico Island, Portugal), Rogan Emer (Univ. College Cork, Ireland), Dillane Eileen (Univ. College Cork, Ireland), Westgaard Jon-Ivar (Institute of Marine Research, Norway), Hjellnes Helene (Institute of Marine Research, Norway), Quéméré Erwan (INRAE, France), Jung Armelle (Des Requins et Des Hommes, France), Azzellino Arianna (Politecnico di Milano, Italy), Lanfredi Caterina (Tethys Research Institute, Italy), Rasmussen Marianne (Univ. Iceland Research Center in Husavik, Iceland), Traugott Michael (Univ. Innsbruck, Austria), Thalinger Bettina (Univ. Innsbruck, Austria)

Environmental DNA (eDNA) - the genetic material shed by organisms into their environment - provides a non-invasive, user-friendly approach to studying marine biodiversity. Yet, challenges such as inconsistent sampling techniques and variability in molecular methods across laboratories still impede large-scale eDNA studies performed together with citizen scientists. To address these issues, the eWHALE project (a multinational Biodiversa+ initiative), focused on improving both eDNA field sampling and laboratory processing for marine megafauna monitoring via whale watching and citizen science in the North-East Atlantic and Mediterranean Sea. In 2023, water samples were collected on board whale watching boats near seven marine mammal species and two shark species. A variety of sampling conditions were tested, including (a) sampling location (flukeprint or breach site), (b) timing of sampling after the animal's presence, (c) filtered water volume and (d) type of eDNA filter plus handling experience by non-experts. To ensure consistency in DNA extraction protocols across four participating laboratories, a ring test was conducted before sample analysis. Each lab processed a subset of samples and measured both total and species-specific DNA concentration. The ring test revealed underperformance in one lab's extraction process, leading to adjustments of their protocol before continuing with further analysis. Field sampling results showed no significant differences in DNA quantities between flukeprint and breach site samples, and sampling immediately after the target species' presence improved detection success. Larger water volumes (10 liters) filtered using Smith-Root self-preserving filters also enhanced DNA yield. These findings are the basis for ongoing marine megafauna eDNA sampling supported by citizen scientists, providing a foundation for participatory marine conservation efforts.

O005. Towards revolutionizing conservation efforts in Canada

Valérie S. Langlois (INRS, QC, Canada), Jérôme Dupras (Univ. Québec en Outaouais, QC, Canada) and Caren C. Helbing (Univ. Victoria, BC, Canada)

Timely and accurate assessment of the presence of at-risk or invasive species is critical for effective responses to climate change and human impacts. At-risk species are often difficult to find while invasive species are often well established before their infiltration is detected using conventional surveying methods. However, all organisms release genetic material such as DNA into their surroundings, leaving traces of themselves that can be detected using environmental DNA (eDNA) methods. These approaches are powerful tools in the conservation toolbox as they are transforming how risk assessments, and the evaluation of mitigation and remediation effectiveness are done. Despite this, poorly performing tools hinder broad adoption of eDNA-based detection methods, due in part to their associated high false negatives and false positives that can impair effective management decision-making. iTrackDNA is a multi-year, large scale applied research project that is addressing these concerns with researchers and End-Users across North America and sectors. It is building End-

User capacity through innovative, accessible, socially responsible genomics-based analytical eDNA tools for effective decision-making by: 1) supporting the creation of a targeted eDNA detection national standard; 2) building eDNA kits to detect 100 priority invertebrates, fish, amphibians, birds, reptiles, and mammals in Canadian coastal and inland ecosystems; 3) applying 10 eRNA kits for determining animal biosurveillance, biosanitation, and bioremediation effectiveness; 4) generating decision support software for modeling regional biodiversity changes integrating Indigenous Ecological Knowledge; 5) developing an eDNA training, certification, and inter-lab validation framework for consultants, researchers, regulators, and managers; and 6) producing a guidance document on eDNA-based methods integration into management, policy & regulations. Recent progress will be presented to highlight iTrackDNA activities to build and augment the eDNA community of practice through national eDNA standards adoption and transformative testing to confidently enable eDNA applications in coastal and inland ecological surveys and biosurveillance to support conservation efforts.

O006. Studying bird and fish biodiversity in Canada by engaging local communities in environmental DNA based monitoring

Bettina Thalinger (Universität Innsbruck, Austria; University of Guelph, Canada), Larissa Holman (Ottawa Riverkeeper, Canada), Shanna MacDonald (University of Guelph, Canada), Rachel Empey (University of Guelph, Canada), Megan Cowperthwaite (University of Guelph, Canada), Katerina Coveny (University of Guelph, Canada), Dirk Steinke (University of Guelph, Canada)

Canadian waters harbour a high bird and fish diversity, which is potentially threatened by climate change, invasive species and anthropogenic influences. Knowledge on species distribution and movement is thus crucial for protection and management measures. By combining the sensitivity and efficiency of environmental DNA (eDNA) based methods with local knowledge, we sought to obtain detailed information on bird migrations and fish species distributions, and gap the bridge between scientists, NGOs, local communities, and policy makers. Bird eDNA samples were collected weekly by volunteers during fall migration at 23 Important Bird Areas across Canada and analyzed with newly developed avian metabarcoding primers. Fish eDNA samples were obtained from the Ottawa river between Lake Timiscaming and Montreal together with local volunteer teams. We aimed to a) incorporate a citizen science friendly sampling protocol, b) establish a robust strategy for the detection of bird and fish assemblages via eDNA metabarcoding, and c) provide a sound interpretation of the obtained results in relation to conventional monitoring data. Our results show that ninety-five percent of eDNA-based bird detections were highly plausible, and more than half were confirmed by visual observations. Significant changes in local bird community composition could be detected via eDNA during the migration period. For fish species, a substantial overlap between eDNA-based data and conventional monitoring results was confirmed. Furthermore, our results emphasize the superior sensitivity of the molecular approach, the importance of field and laboratory replicates, and the incorporation of local hydrology in data analysis. These case studies exemplify that citizen science is the ideal basis for future large-scale eDNA sampling campaigns. Additionally, this approach connects different stakeholder groups, generates robust data for protection and management decisions, and increases the local support for such measures.

O007. ANEMONE Global: establishment of a global network of eDNA-based aquatic biodiversity monitoring

Imane Sioud (Tohoku Univ., Japan), **Yuki Minegishi** (Univ. of Tokyo, Japan), Tadashi Kajita (Univ. of the Ryukyus, Japan), Yukinobu Isowa (Univ. of the Ryukyus, Japan), Michio Kondoh (Tohoku Univ., Japan).

All-Nippon Environmental DNA Monitoring Network (ANEMONE), established in 2019 in Japan, has been conducting nationwide aquatic biodiversity surveys using environmental DNA (eDNA) techniques, engaging researchers, local governments, and NPOs. This initiative has now scaled up into a global program, expanding biodiversity monitoring across international waters and fostering collaboration among diverse participants to standardize methods and enhance data collection efforts. In late 2024, the first international survey was conducted, involving 18 groups from 12 countries across the Indo-Pacific region. This initial phase focused on fish, yielding valuable insights into these ecologically significant organisms. Results from this survey are expected soon, setting the stage for broader and more comprehensive biodiversity assessments in the future. The phased approach establishes regular sampling sites and regional hubs that support local monitoring while ensuring consistency in data collection. Currently, samples are sent to Japan for centralized analysis, which guarantees methodological uniformity, with plans for future decentralization of processing capabilities. This international network facilitates large-scale data collection and species detection, even in remote or less-studied areas, enhancing our understanding of aquatic biodiversity. Community engagement is essential for the project's success. Through workshops, citizen science programs, and educational outreach, the initiative fosters an inclusive environment that expands its reach and impact. By promoting the open sharing of data, transparency is enhanced, encouraging wider use by researchers, policymakers, and conservation organizations globally. As the program continues to grow, its collaborative framework and expanding reach will contribute to a deeper understanding of global biodiversity patterns, offering crucial insights for addressing conservation challenges amid climate change and biodiversity loss.

O008. Are lake accumulators of biodiversity information from environmental DNA?

Catia Lucio Pereira (ETH Zurich, Department of Environmental Systems Science, Zurich, Switzerland), LeDNA collaborative (more than 300 contributors), **Kristy Deiner** (ETH Zurich, Department of Environmental Systems Science, Zurich, Switzerland)

The global loss and redistribution of biodiversity is a hallmark of the Anthropocene. Our challenge is to generate information about how altered biodiversity influences ecosystems and use this information to change our impact on the biosphere. To meet this challenge, we must know where species are, how their distributions change in time and why. However, current methods for determining species distributions are expensive, time intensive and hard to do for multiple species and large geographic regions- rendering global trend analysis near infeasible. We therefore need a paradigm shift. We hypothesize that transported eDNA allows for sampling multiple species on large spatial scales by testing in over 380 lake from 55 countries in a global collaborative sampling campaign that happened on or near May 22, 2023, International Day for Biodiversity. We will present the results of test which lakes in the world are biodiversity information accumulators and whether eDNA measures the global

latitudinal gradient of species diversity. If lakes accumulate eDNA from their catchments, sampling them will provide the paradigm shift needed to vastly change the cost, speed and geographic scale with which species can be surveyed through time to understand what effect their change has on the biosphere.

O009. Using Lagrangian transport model in support of eDNA monitoring in Belgian part of the North Sea

Leo Barbut (RBINS, Belgium), Nergiz Dukan (ILVO, Belgium), Sofie Derycke (ILVO, Belgium), Ludovic Lepers (RBINS, Belgium), Genevieve Lacroix (RBINS, Belgium)

The North Sea faces significant anthropogenic pressures, particularly from the installation of offshore energy infrastructures. Traditional methods for monitoring around offshore wind farms are challenging. Environmental DNA (eDNA) could be an interesting alternative, but its potential is limited by gaps in understanding its dispersal and transport in marine environments. This study addresses these gaps by coupling Lagrangian dispersal model with eDNA analysis to predict the likely origin of sampled water across 16 stations in the Belgian part of the North Sea. By incorporating decay rates into particle tracking model, we assess the seasonal dispersal and transport patterns of eDNA over two years. These predictions enable us to determine the probable sources of water samples, enabling the design of optimal sampling schemes for eDNA-based monitoring. Our findings represent a first step toward automating Lagrangian dispersal models for more effective eDNA monitoring in offshore ecosystems.

O010. Field sampling of eDNA under complex hydrological conditions

Vincent Prié (SPYGEN, France), Alice Valentini (SPYGEN, France), Eva Thierry (Office Français de la Biodiversité, France), Emilie Breugnot (Office Français de la Biodiversité, France), Gaëlle Jardin (Office Français de la Biodiversité, France), Thibault Vigneron (Office Français de la Biodiversité, France), Anthony De-Burghrave (Office Français de la Biodiversité, France), Nicolas Roset (Office Français de la Biodiversité, France), Pascal Irz (Office Français de la Biodiversité, France), Nicolas Poulet (Office Français de la Biodiversité, France)

Regardless of laboratory protocols, field sampling remains the key to a good eDNA survey. In aquatic environments, sampling must integrate the different environmental conditions, which may be a sum of microenvironments, especially when targeting invertebrates or sessile species such as freshwater mussels. The waters of small streams are generally well mixed due to the steep slope and coarse granulometry, which compensates for advection phenomena, but this is not the case for large rivers with slow and laminar flow. Large rivers can have complex hydrodynamics, with tributaries, oxbows, braids, associated wetlands, etc. In this study, we tested two hypotheses: (i) a downstream sampling point integrates the information collected in the different upstream environments; (ii) the different samples collected along a bank-to-bank transect provide homogeneous information. These hypotheses were tested on two species groups, fish and mussels, as they are good indicators of different environmental conditions and host some threatened, rare species together with common and invasive species, and mussels are sessile whereas fish are mobile, which may affect the dispersal of eDNA in the water column. The results show that (i) the communities detected at each sampling point are very heterogeneous and the downstream sampling point does not integrate all the information collected in the different environments upstream, detecting on

average 80% of the fish species and only 65% of the bivalves present upstream; and (ii) at the scale of a transverse transect (left bank/centre/right bank), samples taken independently detect on average 83% of the fish species inventoried on the transect and 69% of the bivalves. This study shows that to optimise the detection of all biodiversity in complex aquatic environments, especially the rarest species, (i) the sampling strategy must take into account the diversity of environments (habitats and hydrological regimes) present at each study site, and eDNA samples must be taken from each of these environments, and (ii) at the scale of a transect (right bank/centre/left bank), it is recommended to collect multiple samples, ideally with a large volume of water per sample.

O011. Net or Niskin: sampling method most suitable for the northern Adriatic phytoplankton monitoring

Lana Grizancic (Centre for Marine Research, Ruđer Bošković Institute, Croatia), Ana Baricevic (Centre for Marine Research, Ruđer Bošković Institute, Croatia), Mirta Smodlaka Tankovic (Centre for Marine Research, Ruđer Bošković Institute, Croatia), Ivan Vlasicek (Centre for Marine Research, Ruđer Bošković Institute, Croatia), Mia Knjaz (Centre for Marine Research, Ruđer Bošković Institute, Croatia), Ivan Podolsak (Centre for Marine Research, Ruđer Bošković Institute, Croatia), Tjasa Kogovsek (Centre for Marine Research, Ruđer Bošković Institute, Croatia), Martin Pfannkuchen (Centre for Marine Research, Ruđer Bošković Institute, Croatia), Daniela Maric Pfannkuchen (Centre for Marine Research, Ruđer Bošković Institute, Croatia)

The northern Adriatic Sea, a semi-enclosed basin and the northernmost region of the Mediterranean, is marked by steep ecological gradients in its physical and chemical properties that are driving significant bioecological changes. For this coastal area, heavily impacted by human activities, phytoplankton structure information, serve as a crucial bioindicator of water quality - for monitoring ecosystem state and functioning. Recently, metabarcoding has been proposed as an effective technique for assessing overall phytoplankton biodiversity in marine environmental studies. To optimize metabarcoding data in our study of northern Adriatic phytoplankton, various sampling methods were employed to identify the most effective approach for capturing the area's phytoplankton diversity. Phytoplankton net tows and Niskin bottle sampling are among the most widely accepted techniques and were used in this research. Vertical net tows collect the entire community present in the water column that is larger than the net's pore size (50 μm), while Niskin bottles capture the total phytoplankton community across size ranges but are limited to specific depths (5 m and 20 m). Both methods were utilized in this study to evaluate their effectiveness in representing the complete phytoplankton community, with the aim of identifying the best approach for future metabarcoding studies and standardizing methods for monitoring this dynamic region. The metabarcoding dataset, comprising of monthly samples over two years from two adjacent long-term monitoring stations in northern Adriatic, was analyzed to highlight differences between these sampling methods. The results provide insights into the potential of each method for future use in phytoplankton monitoring in the northern Adriatic using molecular techniques such as metabarcoding.

O012. Unlocking Africa's biodiversity: advancing surveys with cutting-edge eDNA techniques

Manuel Lopes-Lima (BIOPOLIS/CIBIO), Filipa M.S. Martins (BIOPOLIS/CIBIO), Vasco Fernandes (BIOPOLIS/CIBIO), Fábio Amaral (BIOPOLIS/CIBIO), Joana Veríssimo (BIOPOLIS/CIBIO), Vincent Prié (BIOPOLIS/CIBIO), Pedro Beja (BIOPOLIS/CIBIO)

Sub-Saharan Africa is expected to witness the fastest population growth of any region in the world throughout the 21st century. This demographic trend will increasingly strain the region's abundant biodiversity. To tackle this challenge and achieve the goals outlined in the Kunming-Montreal Global Biodiversity Framework, implementing a comprehensive biodiversity monitoring and assessment program is crucial. In this light, our research team is focused on refining and optimizing environmental DNA (eDNA) techniques to improve biodiversity surveys in African rivers. The aim is to establish reliable protocols that enhance the detection of aquatic species, including rare or elusive ones, by tailoring eDNA methods to the unique environmental conditions of Africa's river systems. This work includes field trials across multiple African countries, such as Guinea-Bissau in West Africa, Equatorial Guinea, Angola, and Namibia. We are assessing various factors that could influence the effectiveness of eDNA monitoring, such as sampling methods, materials used, laboratory protocols, and bioinformatics workflows. Here, we present the initial findings of our work in Africa and offer optimized protocols for utilizing eDNA techniques to survey the biodiversity of its river ecosystems. This research marks an important advance in non-invasive biodiversity assessment, aiding in the sustainable management and conservation of Africa's aquatic ecosystems.

O013. Navigating methodological trade-offs in eDNA biodiversity monitoring: insights from a Mediterranean watershed

Joana Veríssimo (Biopolis, Portugal), Manuel Lopes-Lima (Biopolis, Portugal), Fábio Amaral (Biopolis, Portugal), Cátia Chaves (Biopolis, Portugal), Vasco Fernandes (Biopolis, Portugal), Mutaleni Kemanja (Namibia University of Science and Technology, Namibia), Amílcar Teixeira (Centro de Investigação de Montanha, Bragança), Filipa MS Martins (Biopolis, Portugal), Pedro Beja (Biopolis, Portugal)

Environmental DNA (eDNA) technologies promise significant advances in biodiversity monitoring, yet their application requires extensive optimisation and standardisation. Recent research demonstrated that increased sampling and analytical efforts are needed to improve biodiversity estimates, though fully optimising study designs is often hindered by resource constraints. Consequently, researchers must carefully navigate methodological trade-offs to design effective monitoring studies. We conducted a water eDNA survey of vertebrates in a Mediterranean watershed to identify key methodological factors influencing species richness and composition estimates. We examined the impacts of using high- versus low-capacity filtration capsules, varying levels of biological and technical replication, and the pooling of PCR replicates before indexing. The primary sources of variation identified were capsule filtration capacity and site replication across the watershed. While biological replication within sites and PCR replication also improved biodiversity estimates, their effects were comparatively smaller. Pooling PCR replicates before indexing performed much poorly than analysing them independently. Methodological impacts were stronger on terrestrial than on

aquatic species. Based on these results, we recommend that priority should be given to high-capacity filtration and sampling across multiple sites. Site-level replication deserves lower priority, especially when filtering large water volumes. PCR replication is crucial for detecting rare species but should be balanced with increased site sampling and eventually site-level replication. Avoiding the pooling of PCR replicates is important to enhance sensitivity for rare species. Overall, we stress the importance of balancing methodological choices with resource constraints and monitoring goals, and we emphasize the need for research assessing methodological trade-offs in different study systems.

O014. Improving biomass and abundance assessment of aquatic indicator taxa by combining semi-automated imaging and DNA metabarcoding

Philipp M. Rehsen (University of Duisburg-Essen, Aquatic Ecosystem Research, Essen, Germany; Centre for Water and Environmental Research (ZWU), Essen, Germany), Mikko Impiö (Finnish Environment Institute (SYKE), Helsinki, Finland), Florian Leese (University of Duisburg-Essen, Aquatic Ecosystem Research, Essen, Germany; Centre for Water and Environmental Research (ZWU), Essen, Germany), Arne J. Beermann (University of Duisburg-Essen, Aquatic Ecosystem Research, Essen, Germany; Centre for Water and Environmental Research (ZWU), Essen, Germany).

Understanding biodiversity change requires a comprehensive understanding of not only the species inhabiting an ecosystem but also their biomass and abundance, as well as the multiple stressors affecting these. However, assessing biodiversity with high taxonomic resolution and precise biomass information is a time-consuming process and thus rarely - if at all - applied in any biomonitoring program. While new DNA-based approaches like DNA barcoding offer precise species identification, they lack information on specimen size and biomass. In contrast, high-throughput imaging techniques enable rapid measurements of a specimen's size and morphological features but may lack taxonomic resolution. In this study, we combined DNA metabarcoding with semi-automated imaging and deep neural networks. We collected a dataset of 683 specimens from 14 EPT species (biological quality elements (BQEs) of the orders Ephemeroptera, Plecoptera and Trichoptera) in a multiple stressor field experiment. Each specimen was imaged, weighed, and barcoded using the COI barcode gene. Using the semi-automated imaging device BIODISCOVER, we captured 143,830 images of these specimens from two perpendicular cameras. We trained convolutional neural networks (CNNs) with these pictures for species identification and biomass estimation and evaluated their performance. In addition, we investigated whether pre-training CNNs on identifying species improves the performance of estimating the biomass of aquatic biological quality indicator taxa, thus potentially reducing the need for extensive labeled data in future studies. Our findings demonstrate that combining DNA metabarcoding with automated imaging and deep neural networks can create fast and efficient biodiversity assessment data. This approach has the potential to significantly enhance research and application, possibly improving biodiversity protection efforts by providing accurate and rapid assessments of species composition and biomass.

O015. Finding NeMO: a bayesian adventure in detecting elusive species with environmental DNA

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Biodiversity monitoring using environmental DNA (eDNA) metabarcoding has expanded rapidly in recent years, especially in aquatic ecosystems. This non-invasive technique is now widely embraced by ecologists and stakeholders alike. However, eDNA sampling, like all methods that deal with presence/absence data, has inherent limitations, particularly regarding non-detection. Despite its critical role in accurately determining species presence or absence prior to deriving biodiversity metrics, detection uncertainty is rarely quantified in eDNA studies. Occupancy modelling – a statistical approach to estimate species' occurrence and detection probabilities – offers a powerful solution but remains underutilized in this context. To bridge this gap, we developed the NeMO R package designed to fit multi-species occupancy models within a Bayesian framework specifically addressing the nested structure of eDNA protocols, which typically involve multiple field and PCR replicates. Our package is adaptable to different protocol designs, with options to incorporate sequencing read counts and PCR replicate pooling. It also allows users to test how environmental variables may influence species' presence and detection. Crucially, the package helps to determine the minimum number of field replicates, PCR replicates, and sequencing depth required to confidently confirm species absence. We illustrate the practical application of this approach using a comprehensive dataset from a previous study investigating fish biodiversity along the Rhône River. Our findings underscore the importance of quantifying detection uncertainties, with significant implications for stakeholders managing elusive species and for ecologists computing traditional biodiversity metrics, such as α and β-diversity. This package is designed for broad usability, empowering users to precisely assess detection uncertainties and optimize resource allocation in their protocols, especially when targeting elusive species.

O016. Predicting trawl catches using environmental DNA

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Quantifying the biomass, or number of individuals, diversity, and distribution of marine species is a critical aspect of understanding and managing marine ecosystems. In recent years, there has been growing interest in using environmental DNA (eDNA) for marine ecosystem management and biodiversity assessment. However, the main challenge hindering eDNA

applicability has been the inability to infer absolute species abundances from multispecies analysis (eDNA metabarcoding). In this study, we demonstrate a way forward by estimating the abundance of commercially important fish species in a Norwegian fjord using a joint Bayesian statistical model of traditional trawl-catch data and molecular data derived from eDNA. Using this model, we accurately predict out-of-sample trawl catches using eDNA alone. Moreover, our model provides empirical estimates for key processes linking marine eDNA concentration to the fish population abundance estimated from trawl observations, including trawl catchability, DNA shedding, degradation, dilution, transport, recovery rate, and isolation efficiency. These processes, including amplification efficiencies correcting for Polymerase Chain Reaction (PCR) bias, are species-specific and enable the translation of eDNA metabarcoding data into abundances. These findings have broad implications for the use of eDNA in marine ecosystem management and conservation efforts.

O017. Temporal dynamics of microbiomes and life strategies in peri-urban lakes

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Although lakes represent less than 3% of the earth's surface, they play a crucial role in biodiversity and nutrient cycles, are strongly influenced by human activities, and thus considered as climate change indicators. Within these ecosystems, urban and peri-urban ecosystems are suffering strong and specific threats, such as heat island effect, flash floods, pollution, noise, and soil contamination, while they deliver major ecosystem services. In this study, we surveyed 8 peri-urban lakes (Île-de-France, France), ranging from mesotrophic to hypereutrophic states, during one year. We investigated whether the dynamic of lake's microbiomes have similar metabolic trajectories according to their trophic status, using omic approaches. We hypothesize that the fingerprint of trophic status on the microbial dynamics will overcome the seasonal patterns. Functional potential at the community level was highly stable throughout the year and the lakes, compared to the taxonomic composition. But, beside their specific gene-content composition, lakes also displayed distinct functional dynamics. Seasonal dynamics were strongly highlighted for the degradation of carbohydrates or ABC transporter genes. We focused on the relationship between MAG size and a set of functional traits linked to nutrients acquisition, mobility, growth and local environmental adaptation. Small-size MAGs were the most abundant at low Chla values, while larger-size MAGs peaked only with higher Chla values. Finally, lakes' trajectory lengths appeared to be mainly driven by Chla concentration ranges. The results of this study provide important insights into the effects of eutrophication and the functional resilience of urban lake microbiomes. Peri-urban environments, such as the Paris region, offer ideal settings to understand lake functioning, within a close vicinity but a large range of trophic status.

O018. Metatranscriptomes-based sequence similarity networks uncover genetic signatures within parasitic freshwater microbial eukaryotes

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Microbial eukaryotes play a crucial role in biochemical cycles and aquatic trophic food webs. Their taxonomic and functional diversity are increasingly well described due to recent advances in sequencing technologies. However, the vast amount of data produced by omics approaches require data-driven methodologies to make predictions about these microorganisms' role within ecosystems. Using metatranscriptomics data, we employed a sequence similarity network-based approach to explore the metabolic specificities of microbial eukaryotes with different trophic modes in a freshwater ecosystem (Lake Pavin, France). A total of 2 165 106 proteins were clustered in connected components enabling to analyze a large number of sequences without any references in public databases. This approach improved the number of proteins considered by 42%. Our study revealed a significant number of shared protein families among mixotrophic and phototrophic microorganisms as well as mixotrophic and heterotrophic microorganisms, highlighting the versatility of mixotrophic metabolisms. Genetic similarities in proteins of saprotrophs and parasites also suggests that fungi-like organisms from Lake Pavin, such as Chytridiomycota and Oomycetes, exhibit a wide range of lifestyles, influenced by their degree of dependence on a host. While we observed a relative functional redundancy of primary metabolisms, nearly 130 000 proteins families appeared to be trophic mode-specific. We noted a particular specificity in obligate parasite-related protein clusters, underscoring a high degree of specialization. Although no universal marker for parasitism was identified, candidate genes can be proposed at a fine taxonomic scale. We notably provide several protein families that could serve as keys to understanding host-parasites interactions representing pathogenicity factors. All these protein families could offer valuable insights for developing antiparasitic treatments in health and economic contexts.

O019. Bacterial players of the chloromethane cycle in aquatic environments

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Chloromethane (CH_3Cl), a gas widely used in industry, is mainly produced naturally, and in oceans and by plants in particular. CH_3Cl is toxic to living organisms, and its photolytic degradation is responsible for 15% of atmospheric ozone depletion by halogenated compounds. Certain methylotrophic microorganisms can use CH_3Cl as the sole carbon and energy source, and contribute to define the observed steady-state concentrations of CH_3Cl in the environment. The global CH_3Cl cycle remains to be assessed in detail. We investigate the role of microorganisms in the consumption and also the production of CH_3Cl using omics approaches on environmental samples, enrichment cultures, and isolated strains. We will

present our recent results on the identification and characterization of novel chloromethane bacterial systems for chloromethane utilization from aquatic environments, and their occurrence and dynamics in estuarine sediments and laboratory enrichment cultures derived from them under different regimes of chloromethane exposure.

O020. Detecting semi-annual oscillations in the biotic community of aquatic habitats worldwide using metabarcoding and machine learning

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Recurring patterns in global biosphere dynamics are closely tied to daily and seasonal oscillations in abiotic parameters that are, in turn, driven by Earth's obliquity, rotation, and revolution around the Sun. While circadian rhythms and annual oscillations in biotic systems are well studied, examples of global and persistent subannual cycles in biotic systems are very rare. Here, we develop a machine learning approach to analyze DNA metabarcoding time series data and, subsequently, detect a biotic semi-annual oscillation (SAO) in aquatic biotic communities globally and across taxonomic domains. We show that this dynamic is linked to the abiotic SAO in solar radiation and suppressed by a lack of nutrients or sunlight. Our results suggest a central role for photoautotrophs in the aetiology of biotic SAOs but also show that they are community-level phenomena that cannot be attributed to single species. Furthermore, the biotic SAOs may act as a cue for irregular phenomena such as phytoplankton blooms. Based on these observations, the whole Earth system should be taken into consideration when attempting to understand local ecological dynamics. The results presented here are a showcase for the use of a combination of machine learning and eDNA metabarcoding in uncovering novel insights into fundamental ecological processes.

O021. Limiting similarity in temporary streams based on diatom eDNA

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This study investigates the effects of water intermittency and nutrient load on benthic diatom assemblages in Cyprus' freshwater ecosystems, where water scarcity is an increasing issue due to climate change and anthropogenic pressures. In Mediterranean climates, hot, dry summers and uneven precipitation patterns lead to intermittent watercourses, which are becoming more prevalent as formerly permanent streams experience disruptions from dam construction, water abstraction, land-use changes, and rising temperatures. Despite their

ecological significance, intermittent watercourses remain understudied and inadequately represented in conservation and management frameworks. Benthic diatoms, with their rapid response to environmental changes, serve as effective bioindicators for freshwater ecosystems. This study evaluates their assemblage structure and composition concerning water level supply (intermittent vs. permanent) and nutrient load (pristine vs. polluted). Our approach combines environmental DNA (eDNA) metabarcoding and trait-based methods, specifically Community-Weighted Mean (CWM) and Null model approaches, to detect both trait convergence resulting from environmental filtering and trait divergence resulting from limiting similarity to provide a comprehensive view of diatom community dynamics. Integrating eDNA metabarcoding allows for a more refined, high-throughput analysis of diatom species diversity and distribution, capturing community shifts that might otherwise go undetected through morphology alone. Trait-based analyses, combined with eDNA data, provide insights into how specific diatom traits respond to environmental stressors, with CWM highlighting ecosystem processes by weighting traits by species abundance. This combined approach enables a more sensitive assessment of diatom responses to intermittent water availability and nutrient stress, underscoring their utility as indicators in Mediterranean freshwater management. We explore three hypotheses: (H1) Permanent and intermittent streams exhibit distinct taxonomic and trait-based diatom compositions influenced differently by nutrient levels; (H2) Water scarcity exerts a stronger influence than nutrient load, with nutrient-driven compositional changes more pronounced in permanent streams; (H3) Biotic interactions and environmental filtering shape community structures in both stream types, with a divergence-convergence shift in trait categories under water scarcity conditions. The findings underscore benthic diatoms' potential in indicating water scarcity and nutrient conditions, emphasizing the necessity for multi-stressor assessments in intermittent watercourses. With Cyprus facing escalating droughts, this research highlights critical ecological responses to environmental pressures and advocates for integrating intermittent stream conservation in water management policies, enhancing strategies for sustaining Mediterranean freshwater biodiversity.

O022. Transcriptomic and epigenomic insights into gene and transposable element regulation in a polar diatom during prolonged darkness and re-illumination

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Polar diatoms are key components of Arctic and Antarctic food webs. This study investigates the survival strategies of *Fragilariopsis cylindrus* during prolonged periods of darkness in culture, simulating the polar night, and subsequent return to light. In the dark, the cells enter a quiescent state with no cell division, relying on minimal energy from respiration and slow consumption of cellular reserves. Upon re-exposure to light, the diatoms rapidly resume photosynthesis and cell division, highlighting the remarkable robustness of this species to the

challenges of the polar night. Furthermore, we linked the intense transcriptomic rearrangements that occur during transitions from light to dark and back to light to epigenomic changes, in particular with the temporal dynamics of the activating histone mark H2BUb enrichment on protein-coding genes, which is likely to facilitate survival and recovery. Notably, transposable elements showed enrichment of the repressive mark H3K27me3, which coexists with DNA methylation, suggesting an ancient mechanism of transposable element repression.

O023. Influence of pollutants, temperature and density on the production and persistence of environmental DNA and RNA (eDNA and eRNA) in aquatic environments

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For several decades, climate change and release of contaminants into the environment due to anthropogenic activities have caused major changes to biodiversity, which include direct toxicity, loss of native biodiversity, and the introduction of invasive species. There is now a strong international commitment to protect biodiversity. One innovative method to rapidly identify and monitor all species present in aquatic ecosystems is the use of environmental DNA and RNA (eDNA and eRNA). Since mRNA indicates physiological status and response to environmental contaminants, eRNA could be used to determine developmental status, stress, and other aspects of the biological status of organisms. However, it is essential to understand the fate of eDNA and eRNA in aquatic environments when it is subjected to environmental pressures. The objective of this project is to perform a series of experiments in controlled laboratory conditions to assess the influence of temperature variation, density, and contaminant exposure such as lead (Pb) and polyethylene (PE) microplastic particles on the production and degradation of both eDNA and eRNA. Landlocked salmon (*Salmo salar*) and American eel (*Anguilla rostrata*) are both aquatic species of interest in biomonitoring studies and are used as biological models for this project. The results from this study may highlight the usefulness of eDNA and eRNA tools for ecotoxicology studies and environmental risk assessments. This information is important in management decisions concerning the future of biodiversity and ecosystem health, particularly in polluted aquatic environments and with a changing climate.

O024. An omics-based spatial prioritization framework to counter widespread aquatic invaders

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Prevention and early-detection-rapid-response strategies are cornerstones in biological invasion management. However, many conservation managers are confronted with species that have invaded large areas and for which a different set of management strategies are required. In such cases, hands-on management recommendations are often lacking. Using the American bullfrog (*Lithobates catesbeianus*) invasion in Belgium as a case study, we show that prior analyses based on environmental DNA (eDNA) and population genomics may inform managers on the spatial prioritization of management efforts. First, we setup mesocosm and field experiments to assess the relationship between eDNA concentrations and number of bullfrogs. We found that eDNA concentrations were not only strongly related to bullfrog population sizes (R2adj > 0.8), but also that natural breeding sites (i.e. source populations) can be distinguished from non-breeding sites. By extending this relationship to natural systems, we show that eDNA-based analyses can reveal the spatial configuration of invasion hubs and hence infer invasion dynamics. We conducted landscape genomic analyses to locate potential barriers and facilitators to invasive spread by evaluating geneflow between these hubs. We then looked into ecological characteristics of breeding sites to identify ponds that would support breeding populations upon colonization. Permanent water bodies with abundant emergent vegetation and sparse tree cover along the shoreline were strongly associated with breeding sites. Additionally, we conducted eDNA metabarcoding analyses to quantify the ecological impact of invasive bullfrogs on amphibian communities, and found that spatiotemporal niche overlap mediates severe amphibian declines associated with this invader. Finally, all the discussed aspects are integrated into one general framework that can facilitate the prioritization of resources to spatially optimize reduction, containment or damage control strategies.

O025. Diversity and long-term temporal dynamics of Microsporidia in Lake Aydat using a paleogenomic approach

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Parasites have long been underestimated, but their central role in ecosystem functioning is now recognized. They can represent a significant portion of ecosystem diversity, ranging from 30% to 71%. The development of high-throughput sequencing has allowed for a better understanding of the diversity of aquatic microbial eukaryotic communities, including a wide variety of parasites. However, some groups of parasites, such as microsporidia (parasites of invertebrates and vertebrates, including humans), remain understudied due to their genetic divergence from other eukaryotes. Recent studies conducted in the laboratory have revealed a previously unknown diversity of microsporidia in aquatic ecosystems. These parasites could have a significant impact on aquatic food webs due to their broad host range. However, there is little information on the long-term temporal dynamics of microsporidia and their diversity in relation to lake history, its uses, and environmental variables. Thus, the objective of this project is to study the temporal dynamics of microsporidia in relation to the Anthropocene (~ from 1900) in a lake under anthropogenic influence using a paleogenomic approach. A sediment core representing 64 years of accumulation was sampled from lake Aydat (Auvergne). Metabarcoding data obtained with primers targeting microsporidia and their potential eukaryotic hosts were used to build a co-occurrence network to reveal potential host-parasite interactions. The sequencing results showed that the identified microsporidian genera were similar to those present in the water column, with *Crispospora* and *Pseudoberwaldia* being the most abundant and the only two genera retrieved in all samples. Significant variations in diversity and abundance were observed over the years, with a sudden increase in *Vittaforma*, a genus containing microsporidia infecting humans, in the 2000s. These temporal variations may be explained by the dynamics of yet unidentified eukaryotic hosts.

O026. Having IMPACT on monitoring aquatic diversity: first experimental results evaluating eDNA as an integrative tool for studying parasites

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Parasites are usually presented as biological villains due to threats posed to human health and wildlife conservation. However, most metazoan parasites have no zoonotic potential and are instead crucial to the health and ecosystem functioning, making up an overwhelming proportion of current biodiversity. Nonetheless, parasites remain the most neglected components of biodiversity monitoring and have only recently begun to be considered in conservation discussions. Monitoring of parasite biodiversity is hampered by inefficient tools for their detection, with current methods involving the sacrifice of many hosts. Thus, it is necessary to develop less invasive and non-lethal monitoring approaches. Environmental DNA (eDNA) offers a potential solution, particularly for studying aquatic parasites by allowing them to be monitored in environmental matrices without sampling the hosts. Given that eDNA is increasingly used in aquatic biodiversity monitoring worldwide, it offers a great opportunity for obtaining parasite diversity data in existing monitoring programs. Recent literature reviews show that eDNA has proven useful in the detection of single parasite species. However, there is no consensus on the optimal sampling conditions (i.e., filter pore size and water volume) for detecting certain parasite groups. Moreover, there are very few studies targeting multiple parasite species within and across defined parasite groups at once. Here, we will present the first results from an indoor experiment conducted within the framework of the newly acquired project IMPACT where multiple filter types were tested for the simultaneous detection of multiple fish parasite groups using eDNA.

O027. Impact of cyanobacterial blooms on fish gut microbiota: from ecotoxicology to biological invasions?

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Fish are sentinels of freshwater ecosystem health, and cyanobacterial blooms are the most common stress to which they are exposed. Aside from their direct impact on fish health, notably through oxygen depletion and production of cyanotoxins, exposure to cyanobacteria has the potential to modify gut microbiota composition and holobionts functions. To test this, our group conducted lab experiments by exposing medaka fish (*Oryzias latipes*) to pure toxins, metabolite extracts and cell cultures of the microcystin-producing cyanobacterium *Microcystis aeruginosa*. Holobionts responses were investigated using metabarcoding, metagenomics and non-targeted metabolomics approaches that revealed a direct causal link between exposure and major changes in fish gut microbiota and metabolism. Building on this foundation, field studies were conducted to test whether such a link was also observed in natural environments. For this, specimens of perch (*Perca fluviatilis*) and pumpkinseed (*Lepomis gibbosus*) were collected from six lakes representing a gradient of exposure to cyanobacteria, all located near Paris, France. Results indicate that gut communities from these two species were distinct and varied according to chlorophyll a levels, validating the results from lab experiments. Interestingly, gut community as well as metabolite compositions were less affected by highest Chla concentrations in the pumpkinseed compared to the perch, the former showing less signs of dysbiosis. This indicates a better ability to tolerate and acclimatize to a broader range of environmental conditions. The pumpkinseed is an invasive species, and our results suggest that increased resistance of gut

microbiota and functions could be a yet-underestimated asset allowing some invasive species to outcompete native ones. This study emphasizes the relevance of animal-associated microbiota to ecotoxicology, as well as its potential contribution to the study of biological invasions.

O028. Ecology and evolution of chemical interactions between the brown algae Dictyotales and their epibacterial communities

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Acting together as a functional unit named "holobiont", seaweed and their associated microbial communities are characterized by complex interactions poorly understood within their ecosystems. In this context, the seaweed holobiont *Taonia atomaria* and its epibacterial community were studied as a new model of seaweed holobiont within the Dictyotales order. Using a multi-omics approach combining metabarcoding and metabolomics, a large number of environmental parameters were investigated to understand their effects on the functioning of the whole holobiont under global change. Results from spatiotemporal monitoring and analyses along the thallus revealed the importance of several metabolites involved in selecting specific colonizing bacterial communities on the algal surface. Among them, dimethylsulfoniopropionate (DMSP) produced by the seaweed under higher irradiance, explains the simultaneous growth of *Roseobacter* taxa specialized in the catabolism of this metabolite. Other compounds such as sesquiterpenes and diterpenes were identified as a major chemical family specific to *T. atomaria*. These surface metabolites known to be involved in active chemical defense against specific bacterial strains, were specifically produced at the surface of the meristematic parts. The production of DMSP and sesquiterpenes was then investigated within the whole order of the Dictyotales (31 species from 11 genera) through a large biogeographical metabolomics study. Results suggest that DMSP is produced by all brown algae from the order, but preferentially from those located within temperate regions, highlighting the importance of this compound as a cryo- and osmo-protectant. The origin of the production of sesquiterpenes and diterpenes and their chemical precursor, the geranygeranylglycerol was identified within the phylogeny and found specific to a monophyletic clade. Interestingly, the others genera (lacking of these chemical families within the order), were instead specialized in the production of phenolic compounds which can also act as a distinct chemical defense. This observation suggests a "terpene-phenol" trade-off for the production of chemical defenses. In a context of global change, these studies brought new insights to better understand how brown algae can interact and evolve with their microbiome under heavy anthropic pressures.

O029. Saving the best for the last: late apoptosis as a mechanism to preserve symbiosis in the thiotrophic bivalve *Lucinoma borealis*?

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Lucinidae are symbiotic bivalves that host sulfur-oxidizing chemosynthetic bacteria in their gills. The species *Lucinoma borealis* lives buried in sediments of seagrass beds and a population is present near Roscoff (Brittany, France). The symbiotic bacteria are autotrophs oxidizing H_2S to produce energy, and transfer organic carbon produced to their host. We

aimed to highlight the molecular and metabolic processes in the host that are affected by sulfide deprivation. The experimental design involved collecting samples of Lucinidae and placing them for 4 months in H_2S-depleted aquariums. Activity and symbiotic load were measured using qPCR, along with the analysis of genes involved in the apoptosis process. In combination with TEM microscopy, metabarcoding, and metabolomics analysis, the goal was to use independent methods to study the effect of H_2S depletion on the holobiont's response. Results show that *L. borealis* survives under laboratory conditions without their natural symbiotic environmental loads. Despite the absence of H_2S, symbionts were still present and active after 60 days of depuration. More surprisingly, apoptosis of symbiont-containing cells was triggered only after 60 days of depuration. Delayed apoptosis suggests a strategy to preserve symbionts for as long as possible. Using TEM microscopy, we did not observe direct apoptotic elements, but noted its consequences on the gill structure. Metabarcoding indicated that *L. borealis* was not infected by any pathogens or opportunistic bacteria, and that symbionts remained present even after 4 months without H_2S substrates. However, symbiont loads decreased, and resulted in a significant reduction in metabolome diversity within a few days. All independent methods employed in this study suggest that *L. borealis* maintains its valuable symbionts as long as possible. It was previously hypothesized that Lucinidae are unable to acquire new symbionts as adults. Thus, we hypothesize that delaying apoptosis and symbiont loss is a mechanism that allows *L. borealis* to resist periods of low sulfide while maintaining its capacity to quickly restore a fully functional symbiosis when conditions become favorable again.

O030. Diving deep into kelp forest food webs using dietary DNA

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Kelp forests are iconic coastal ecosystems, harbouring highly diverse communities of algae, meiofauna, and macrofauna, valued for their ecological, economic, and cultural significance. These ecosystems confront multiple threats including pollution, overfishing, seaweed harvesting, over-grazing events, and temperature anomalies linked to climate change. These have led to large-scale episodes of deforestation worldwide, often accompanied by disruptions in ecosystem functioning. Understanding the intricate interaction network within these communities is critical for predicting the cascading effects of such pressures. Kelp forests have been the subject of numerous studies on their level of primary production, their genetic characteristics and the associated communities. However, precise knowledge regarding their structure and trophic functioning remains lacking. For example, traditional methods such as stable isotope analyses provide insights on dominant trophic pathways, but they do not provide adequate taxonomic resolution to assign species-specific interactions. In this study, we used a multi-marker metabarcoding approach to reconstruct food webs within kelp forests in two protected areas in Britany: the Iroise Marine Natural Park and the natural reserve of the Seven Islands. We examined the diet (both animal prey and algae) of twenty keystone species including seals, cormorants, fish and invertebrates such as urchins, starfish, and crabs. Our findings revealed a remarkable diversity of prey with the vast majority identified at the species level. We also uncovered cryptic dietary niche partitioning in fish that likely contributes to stabilizing species coexistence. Additionally, dietary DNA metabarcoding

data provided information on the trophic dependency levels of the studied species on kelp forests, offering valuable insights for conservation efforts.

O031. Modeling the evolution of microbial networks

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Microbial networks represent the relationships and interactions between different microbial species, which can be competitive, cooperative, or neutral. Understanding these interactions is crucial considering that microbial communities impact the functioning of various ecosystems, from soil and ocean environments to human health through the gut microbiome. The change of microbial networks over time can reveal how microbial communities respond to environmental changes, shifts in host health, or nutrient availability suggesting that microbial ecosystems might be subject to evolutionary pressures. Despite ecosystems are not usually considered units of selection, recent works have expanded the evolutionary thinking and applied this framework to ecosystems suggesting that they can evolve by natural selection. Our work proposes to study the evolution of ecosystems through network modeling and exploit time series Omics data to understand the change in microbial communities and test evolution-driven hypotheses. Through network modeling, we can track how species interactions strengthen or weaken, how the structure of the community shifts and unravel the complexity of microbial ecosystems, enabling a deeper understanding of their temporal dynamics and evolutionary trajectories.

O032. Cross-feeding interaction within Microcystis phycosphere: new perspectives from the combination of metagenomics and metametabolomics approaches

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Facing global changes, cyanobacteria blooms are of increasing concern regarding their impact on environmental, animal and human-health, with potential strong economic consequences. Despite evidences about environmental factors able to promote such harmful events, there is still a paucity of knowledge about the physiological and ecological mechanisms behind those. Nevertheless, cross-feeding interactions between cyanobacteria and their microbiome

might be involved, allowing an optimal and symbiotic use of nutrients in this phycosphere. To elucidate such mechanisms, metagenomics and dedicated bioinformatics are of particular relevance due to their ability to predict metabolic network and microbial interactions, while metabolomics appears as promising to further validate such predictions. In this context, the present study aims at characterizing cross-feeding interactions between Microcystis and associated heterotrophic bacteria by taking advantage of a multi-omics approach. Metagenomics was the basis for reconstructing genomes and their associated genome-scale metabolic networks (GSMNs), with the objectives of capturing and comparing the production of microcystins, the metabolic functions of 12 Microcystis strains and the joint metabolism of the phycosphere sampled during bloom in a lake. Metabolic modelling further identified the taxa associated with Microcystis's metabolism, shedding light on the interrelation between taxonomic and functional diversity as a favoring factor of toxic blooms. Complementary to those, metabolomics depicted and compared the chemical landscape of these 12 communities. Our investigations highlighted discrepancies in the chemical composition of the 12 Microcystis communities. In particular, metabolomics showed that some metabolites were detectable in only one or few strains (e.g. Aeruginosin ln608). Even if several classes of bioactive peptides were identified (e.g. Microcystins, Microginins, Microcystbiopterins), investigations are ongoing to enlarge the annotation in order to support the validation of metabolism prediction from GSMNs. Overall, our study demonstrates the relevance of multi-omics and computation models for suggesting functional mechanisms related to the relationship between cyanobacteria and their microbiome.

O033. Leveraging metagenomics and metatranscriptomics to gain novel insights in the ecological dynamics of microeukaryotic plankton communities

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Seasonal fluctuations profoundly affect marine microeukaryotic plankton composition and metabolism as well as biotic interactions. But accurately tracking these changes has been a long-standing challenge. High-throughput sequencing of environmental samples has dramatically improved our understanding of the molecular activities of complex microbial communities in their natural environments. For instance, by enabling taxonomic profiling and differential gene expression analysis, microbiome studies have revealed intriguing associations between community structure and ecosystem functions. In this study, we present a year-long metatranscriptomic data set from the North Sea. Our data illuminate the contributions of microeukaryotic taxa to biomass production and nutrient cycling at different times of the year and allow delineation of their ecological niches. We characterized the metabolic signatures of different seasonal phases in detail, thereby revealing the metabolic versatility of dinoflagellates, the heterotrophic dietary strategy of *Phaeocystis globosa* during its late-stage blooms. While we demonstrate that sequence data analysis is an invaluable tool to understand the ecology of planktonic communities, its effectiveness in characterizing microbial ecosystem functioning at the systems level has been limited by the quality and scope of reference sequence databases. We therefore applied state of the art bioinformatics

tools to leverage publicly available genome/gene sequences for planktonic organisms to build a customized protein sequence database. Based on this, our goal is to conduct a systems-level interrogation of environmental samples, which can effectively augment the insights obtained through traditional gene-centric analysis. By further expanding on the taxonomic and functional complexity of our database we improve our ability to map the molecular traits that drive changes in the composition and functioning of marine planktonic networks through space and time.

O034. Co-occurrence networks reveal interactions between aquatic prokaryotes and protists

Shan Pushpajom Thomas (University of Toledo, USA), Trisha Spanbauer (University of Toledo, USA)

Gaining a comprehensive understanding of microbial communities and identifying keystone species requires determining the intricate interactions between species. Unlike macro-organisms, where interactions can be directly observed, the enormous diversity of microbial communities makes studying interactions within specific niches challenging. Omics tools like metabarcoding and co-occurrence network analysis of microbial populations within a niche can help overcome this issue. Microbial species interactions can be inferred from metabarcoding datasets by analyzing the presence, absence, and relative abundance of different species. Correlation analysis is used to identify relationships between species, which can include positive interactions (e.g., mutualism, commensalism) and negative interactions (e.g., competition, amensalism). These interactions can then be visualized using co-occurrence networks. For this study, we used metabarcoding data of the prokaryotic and protist community (16S and 18S rRNA genes) from Lake Erie water samples collected in 2021 and 2022, for May, June, July, August, and September from nine near-shore sites. The co-occurrence networks were constructed using SPIEC-EASI and MicroEco packages in R and visualized using Gephi (v0.10.1) software. The co-occurrence patterns are more interconnected in protist communities when compared to prokaryotic communities. Protist communities contain a larger number of modules (subcommunities), which indicate the presence of a variety of niches in aquatic environments. The bacterial community interactions are more localized inside the modules, which indicates that the modules can function independently. *Cyanobium sp*. and *Microcystis sp*. are prominent central nodes, indicating that these species are likely to be keystone species, heavily influencing the network structure and function. The results revealed intricate interactions between two major components of the aquatic food chain and show its capability to be employed as routine bio-monitoring tools to detect changes in the aquatic environments. The next step in this work is to incorporate environmental variables into the network and thereby enhance the accuracy of the predicted interactions.

O035. Biogeography of microalgae in freshwaters: new contributions from metabarcoding?

Rimet Frédéric (UMR Carrtel, USMB, INRAE, France)

Metabarcoding is a methodology used now since about 10 years for microalgae and diatoms in particular. The methodology has been developed especially to answer monitoring questions since benthic diatoms are routinely used to assess lakes and rivers ecological

quality. Therefore, it has been applied on a large range of freshwater ecosystems, from lakes to rivers and in various regions. Compared to traditional microscopy, the major interest of taxa identification and detection with metabarcoding is the comparability robustness of inventories between samples, the taxonomic precision, the ability to detect rare taxa and the easy access to the phylogenetic dimension which can bring new insights to diatom. Our objective is to find out it this mass of metabarcoding data can provide new knowledge on the ecology and biogeography of diatoms? I will show with a selection of examples how endemism and cosmopolitanism level can be highlighted between rivers and lakes from different regions and climates, and how the interconnections between ecosystems can improve communities' homogenization. We will focus on a large lake to show how benthic diatom communities can be influenced by the classically known environmental filters (physical and chemical), but also in less expected ways can be strongly influenced by mass effect from the watershed. Finally, we will discuss the cryptic diversity of species which is detected with metabarcoding and how this can improved our knowledge of the geographical distribution and ecology of diatom taxa.

O036. Exploring diatom diversity and biogeography in Canary Island watercourses: insights from DNA Metabarcoding

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Freshwater ecosystems in the Macaronesian region are characterised by a high proportion of watercourses in volcanic substrates with fluctuating flow regimes, featuring both temporary and perennial streams with a great diversity of habitats. Despite the ecological importance of ravines (locally known as barrancos) in the Canary Islands, knowledge about the freshwater diatom diversity and their distribution remains poor. As part of the CONACAN project, which focuses on freshwater biodiversity and conservation, diatom communities from three Canary Islands—La Gomera, Tenerife, and La Palma— have been studied using DNA metabarcoding for the first time. Within the archipelago, these islands are known for their high altitudes and prominent ravines. During our analysis, gaps in the metabarcoding data were identified that are crucial for revealing the genetic diversity of specific diatom groups and for understanding the biogeography of species with restricted distributions, such as *Nitzschia tenerifa* and *Nitzschia macaronesica*. These species remain underrepresented in existing genetic data, limiting the comprehension of their ecological roles and distribution patterns. To address this, sequences of these diatoms have been compared with extensive datasets currently available and several species isolations have been performed, including some of these restricted species, to obtain their reference DNA sequence. Our preliminary findings provide valuable insights into the taxonomic and genetic diversity of diatom communities and their ecology and biogeography. This will offer a foundation for the potential application and future adaptation of the Water Framework Directive (WFD) to the distinct hydrogeological and environmental conditions of the Canary Islands.

O037. Geographic genetic divergence in tychoplanktonic taxa dominating diatom communities in the marine plastisphere

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Diatoms constitute the main photosynthetic group in marine biofilms throughout the world's ocean, on natural and artificial surfaces, yet are poorly studied compared to their planktonic counterparts. Here, biofilms were collected from PVC (Polyvinyl chloride) panels immersed (i) during one year in two NW Mediterranean sites, a mesotrophic one (Toulon bay) and an oligotrophic one (Banyuls bay) and (ii) during one month in Toulon bay and two other eutrophic sites (Lorient in Southern Brittany in the Atlantic Ocean and Reunion Island in the Indian Ocean). Plastispheres were analyzed using both microscopic and molecular approaches focusing on the relationship between diatoms and others microorganisms in biofilms. Light microscopy indicated spatio-temporal differences in cell abondance and biovolumes. Metabarcoding, targeting the rbcL gene for diversity and composition, revealed that diatom richness was maximal in the early stages of biofilm and showed a clear temporal evolution of beta-diversity in the Mediterranean sea. Including prokaryotic and fungal communities, we elucidated how microorganisms interact within biofilms throughout the colonization process of the plastisphere. In Lorient, Reunion Island and Toulon, environmental parameters were shown as the stronger drivers structuring diatoms communities, considering both planktonic and biofilm lifestyles. A core biofilm community represented by few abundant species was observed across location. The occurrence of tychoplanktonic taxa emphasizes the specificity of diatoms among other microorganisms in biofilms. Finally, a complete absence of common genetic variants between Lorient, Reunion Island and Toulon suggested that dissemination through marine current over a broad geographic scale led to adaptation processes.

O038. Divergent Prokaryotic communities across mangrove sediment bioregions: insights from 16S rDNA metabarcoding data

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Microorganisms account for about 20% of the Earth's total biomass and are essential to ecosystem functioning. In mangroves, which are dominant pantropical intertidal forests, microorganisms play key roles in nutrient cycling and contribute to the health of plant hosts. However, the global biogeographical patterns of microbial communities in mangrove sediments remain poorly characterized and understood. To explore whether their global distribution is shaped by deterministic processes, we conducted a meta-analysis of 16S rRNA gene sequences from mangrove sediments across 12 countries. To ensure comparability

between sequences OTUs, a bioinformatic standardization was performed by targeting the common portion of the biomarker in all the sequences, aligning consensus in silico primers, and trimming reads downstream of the matching portion. Our results revealed significantly higher OTU richness and diversity in the Indo-West Pacific floristic area, particularly in the Indo-Malaysian bioregion, echoing patterns observed in mangrove plant macroecology. This finding is unprecedented. Global distance-decay relationships were marginally positive but became more pronounced when restricting the analysis to a specific geo-morphological mangrove type. Beta-diversity varied across studies, likely due to methodological discrepancies, stressing the need for standardized metabarcoding protocols and bioinformatic workflows. Additionally, a large portion of sequences remained unassigned, revealing an important unexplored microbial diversity. Random forest analysis indicated that some of these unassigned sequences may hold key insights into mangrove microbial biogeography. This study highlights both the limitations and the untapped potential of metabarcoding data. While such data now provide global coverage, emerging technologies may increase challenges for cross-study comparisons.

O039. Revealing a diversity continuum in tropical fishes: simultaneous eDNA assessment of populations and communities

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Evaluating biodiversity patterns is crucial to unravelling the processes that shape community and population structure. However, intraspecific and interspecific diversity have traditionally been studied separately by community ecology and population genetics, typically relying on visual surveys or extensive tissue sampling. We propose that environmental DNA (eDNA) metabarcoding can be used as a non-invasive and efficient alternative, to facilitate and standardize multi-scale sampling of diversity by capturing both species and intraspecific genetic diversity information from single seawater samples. We analysed 21 eDNA samples collected from 11 sites in the Caribbean Sea, using two mitochondrial DNA genetic markers: the 12S to assess fish species diversity and the D-loop to capture haplotypic diversity within several *Haemulon* species. Our results indicate that eDNA reliably captured community structure using a clustering approach that proxies species richness (MOTUs), and most importantly assessed intraspecific haplotypic variation via a denoising approach (ASVs). Specifically, 505 unique haplotypes were detected in *Haemulon plumierii*, demonstrating that haplotype diversity in the D-loop region can serve as a proxy for the number of individuals, i.e., species abundance. We further investigated whether spatial patterns of intra- and interspecific diversity were similar. We showed that the number of haplotypes within *H. plumierii* spatially covaried with species richness across samples. Spatial covariation was also observed between genetic and species β-diversity, both of which were primarily explained by distance between sampling sites and thus dispersal processes. This study pioneers the simultaneous evaluation of species and genetic diversity using eDNA, highlighting its potential to facilitate biodiversity monitoring and enhance our understanding of eco-evolutionary processes that shape community assembly across scales of biological organization.

O040. Combining modern surface-to-seafloor eDNA datasets to unlock the potential of sedimentary ancient DNA.

Tristan Cordier (NORCE Climate and Environment - Norwegian Research Centre AS and Bjerknes Centre for Climate Research, Bergen, Norway)

Recent ocean explorations using environmental genomics tools have revolutionized our understanding of plankton biodiversity at global scale and along the water column. These plankton genomic resources provide a fantastic opportunity to separate the plankton DNA signatures that have settled on deep seafloor sediments from indigenous benthic biodiversity. Combining modern surface-to-seafloor eDNA datasets not only allow us to specifically link plankton and benthic biodiversity to modern ocean ecosystems processes, but also unlock the potential of sedimentary ancient DNA (sedaDNA) for paleoceanography. I will present ongoing work that aims at delivering sedaDNA-based reconstructions of Late Quaternary marine ecosystems in the Arctic Ocean and Nordic Seas. I will also discuss technical challenges in sedaDNA data analysis and highlight potential solutions to mitigate those.

O041. More than just disorder - metabolite diversity of Microcystis strains shows tight correspondence to genotype and may contribute to ecotype specificities

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Microcystis is one of the most common bloom-forming cyanobacteria in freshwater ecosystems worldwide. This species remarkably produces numerous bio-active accessory metabolites, which are believed to be potentially involved with different ecological and/or physiological processes. Their genuine contribution to the evolutive success of Microcystis blooms remains undetermined. To better depict the potential relation between the local genetic diversity of blooming Microcystis populations and the respective associated chemical diversity, we conducted a joined genomic and metabolomic analysis of 65 Microcystis strains

collected from various lakes from France and European countries. Interestingly, both core- and noncore-gene phylogenetic analysis place 59 of these strains in 12 distinct genetic clades of at least 2 genomes, being widely distributed along the whole Microcystis phylogeny and presenting specific signatures of accessory metabolite biosynthesis. The chemical analysis of metabolite diversity produced by these strains, cultured under lab conditions, reveals the production of stable metabolite corteges, beyond little variations along replication, growth phases and culture conditions. Indeed, these strains belonging to 12 different genotypes correspond to 13 distinct metabotypes according to an accurate one-metabotype-for-one-genotype rule. This observation reveals that Microcystis collected from certain environments present a large set of genetic and subsequent corresponding metabotype diversity, whereas all strains originating from certain other lakes present a net genetic uniformity. Overall, our investigations reveal that the production of accessory metabolites constitute well conserved chemical traits across the different Microcystis genetic clades, suggesting these molecules may be involved in key adaptative and selective processes, that still remains unspecified."

O042. Diatom metabarcoding for good ecological status assessment and beyond

Rosa Trobajo (IRTA, Catalonia, Spain), Javier Pérez-Burillo (University of New South Wales, Australia), David Mann (RBGE, Scotland, UK)

Our metabarcoding studies began with commissioned work on Mediterranean rivers in Catalonia, to establish whether diatom metabarcoding could be used in assessments of ecological status to fulfil the requirements of the Water Framework Directive (WFD). We showed very good agreement between morphology- (LM) and DNA-based (metabarcoding) assessments of ecological status. Where there were important discrepancies affecting the WFD class assignation, we were able in several cases to identify the cause, including unacknowledged biases in the LM approach, e.g. undetected Fistulifera species, which led to inappropriately high estimates of ecological status. Adjustments to relative abundance to compensate for likely rbcL copy number differences did not, for our rivers, improve agreement between LM and DNA. As identified using the current reference dataset, diatom species varied greatly in their genetic structure in a combined Catalonia-France dataset, some being almost uniform while others showed complex patterns of variation; some variants showed clear ecological and geographical differentiation, e.g. in *Achnanthidium minutissimum* and *Fistulifera saprophila*. We then compiled available datasets from N America and Eurasia studies of rivers and lakes to examine wider distributions. We again found differences in genetic structure, some species (e.g. *Discostella spp*.) being genetically uniform while others comprised many variants, though rarely with clear evidence of geographical restriction (*Ulnaria* was an exception). Many ASVs, especially the most abundant overall, are very widely distributed and geographical patterns break down rapidly when descending the phylogenetic tree.

O043. Diatoms genetic diversity across different climate zones

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Understanding the factors that control distributions of taxa is crucial and still poorly understood in microbial ecology, especially for microalgae such as diatoms. Recent phylogenomic studies showed that many diatom species host a considerable genetic diversity, but the factors controlling this diversity are still poorly known. In this study, we analysed diatom communities, from rivers across four climate zones (Nordic, Temperate, Equatorial and Mediterranean), spanning Europe, Africa, Central and South America, using eDNA metabarcoding (rbcl barcode, a coding region for RuBisCo). Of the 3,302 ASVs (amplicon sequence variants) recorded, 1,905 were assigned to 394 diatom species. On the other hand, ASVs not assigned to species level were underlining the extent of the unknown diversity especially in equatorial climate. We examined the genetic diversity of these species in relation to their abundance, site occupancy and climate zones. We showed that cosmopolitan species which occur in different climate zones and occupy many sites, host a high genetic diversity. Conversely, endemic species restricted to a single climate zone were locally abundant and host few genetic variants. However, there are a few exceptions to this general rule. Phylogenetic analysis of selected 32 species revealed that some of them such as *Sellaphora nigrii* (De Notaris) Wetzel & Ector and *Sellaphora pupula* (Kützing) Mereschkovsky exhibited clear phylogenetic intraspecific clades restricted to particular climate zones. This intraspecific pattern dominated among analysed species. In contrast, taxa such as *Achnanthidium minutissimum* (Kützing) Czarnecki and *Nitzschia palea* (Kützing) W. Smith did not show clades clearly related to climate zones. Assembly rules within diatom species were analysed and revealed that a neutral process seems to dominate for species like *A. minutissimum* and *N. palea*, but this result remains to be confirmed. Our results highlighted that climate can be one of the driving factors shaping and influencing genetic diversity within diatom species on a large geographic scale. These findings support the idea that allopatric speciation may be a dominant process in diatom species when different climate zones are considered.

O044. Kelp in the climate equation: leveraging 'omics' approaches to unveil the role of giant kelp habitats as a blue carbon sinks

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Kelp forests are crucial to marine ecosystems, providing habitat, enhancing biodiversity, and supporting coastal resilience. Kelp forests are increasingly being recognized for their potential to contribute to carbon sequestration through acting as important natural sinks. Consequently, kelp may play a vital role in climate regulation, mitigation and adaptation. However, quantifying their carbon storage potential, especially in terms of long-term sequestration in marine sediments remains a challenge. Established methods such as stable isotope, pigments and lipid analysis are known to underperform when it comes to detection and identification of kelp-derived carbon in marine sediments. Here we present findings from a project focused on developing innovative environmental DNA (eDNA)-based tools to trace blue carbon dynamics within habitats dominated by the giant kelp *Macrocystis pyrifera*. By comparing whole genomes of globally distributed *M. pyrifera*, we augment kelp genomic resources to better understand intraspecific diversity regionally and globally. This improved

resource has enabled the development and validation of a species-specific *M. pyrifera* digital droplet PCR assay. In turn, this new assay has been used in a proof-of-concept study aimed at detecting giant kelp eDNA and associated algal species in marine sediments found in currently healthy, declining, and historical kelp forest habitats. Overall, the results will deepen our understanding of kelp forests' contribution to blue carbon frameworks, providing critical insights for climate action, conservation policies, and sustainable marine resource management. The standardized eDNA-based tools developed through this project have the potential to transform how we assess marine carbon stocks and their integral role in global carbon cycles.

O045. Changed succession patterns in the Arctic Sea observed through metabarcoding

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Climate change is impacting the Arctic Sea and the communities of organisms living there to a high degree. A good way to monitor the subsequent changes is the use of microalgal communities, that as single celled organisms can quickly respond to changes in their surroundings. Microalgae can further produce harmful algal blooms and are important components of the ecosystem, both participating in the primary production as well as within the microbial loop. Changes in temperature change the oceanography and nutrient concentrations, causing shifts of the communities living there. Microalgae of the Arctic in general and West Greenland in particular have gone understudied, which creates a spotty baseline, mostly filled by relatively recent monitoring systems such as those of the Arctic Fast station, ranging back to 2018. Here we show how we established the microalgal distributions and shifts in communities through metabarcoding and metatranscriptomics. Monitoring efforts had shown a clear succession pattern with a diatom bloom appearing in spring until silica depletion, to be then substituted by mixed communities including dinoflagellates. In the summer of 2024, we found that an increase of nutrients, have changed the succession of microalgae replenishing a bloom of diatoms in the place of the expected increase in flagellates, producing a similar peak to the original spring observations. In combination with data from the DNA-based monitoring of the Arctic Fast Station, we were able to see changes in the succession. In contrast with the previous microscopy surveys, OMICS will enable us to more precisely ascertain the origin of the microalgae to learn about the location of origin of new invaders. Through this, OMICS will enable a further resolution of the changes in the planktonic microalgae community due to Atlantic influences in the West-Greenlandic Arctic Sea. With it we hope to glimpse the ecological and biogeographical mechanisms of change.

O046. Gene synchrony and rhythmicity in neighbouring marine microbiomes generates insights on functional redundancy

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The amount of functional redundancy in microbiomes is a matter of debate. Investigating the synchrony and the rhythmicity in the temporal dynamics of microbes populating environmentally similar locations can provide new insights into functional redundancy. When comparing the dynamics of neighbouring microbiomes, high functional redundancy could be depicted by cases where metabolic functions are synchronous over time while the genes or taxa contributing to those functions are not. In turn, low functional redundancy would be exemplified by cases where both functions and genes or taxa are synchronous over time. The same behaviour is predicted for the rhythmicity in each location. Here we investigate the amount of synchrony and rhythmicity at the functional, gene, and taxon levels over 7 years in monthly metagenomes from two marine-coastal microbiomes in the Mediterranean Sea separated by ~150 km and connected by a dominant south-west marine current. We found functions, genes, and taxa showing high, low, or anti-synchrony, as well as rhythmic and arhythmic patterns. The distribution of functions, genes, and taxa displayed an average rhythmic pattern (PnMax > 8). Yet, overall, they exhibited low average synchrony (Sy~0.25), revealing a high degree of idiosyncrasy. The analysis of 45 key biogeochemical functions indicated high synchrony and rhythmicity in some functions even when the most abundant corresponding genes had low synchrony and rhythmicity, pointing to functional redundancy in crucial metabolisms. Furthermore, the taxonomic composition for the set of 45 key biogeochemical functions was found to follow a similar pattern to that of the global ocean (TARA), pointing to dynamics that may occur at a global scale.

O047. Integrating short- and long-read metagenomics to reveal local and global macroecological patterns in marine microbial populations

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Microbes are vital to ocean ecosystems, driving biogeochemical cycles, food webs, and climate regulation. Understanding microbial populations is essential for elucidating their adaptations and ecosystem roles. Despite its importance, microbial population genomics is still emerging. This study integrates short- and long-read metagenomics to explore the population genomics of marine microbial species, addressing both local and global patterns. First, we investigated the genomic differentiation of 495 abundant prokaryotic short-read metagenome-assembled genomes (MAGs) over 12 and 7 years in two neighboring stations in the Mediterranean Sea and across the global surface ocean. The Single Nucleotide Variants (SNVs) analysis indicated a stronger population differentiation at large spatial scales, potentially modulated by temperature and salinity, compared to long temporal scales. Population structure was also detected in both time series, with evidence of positive selection in diverse genes, pointing to adaptations to seasonal environmental fluctuations. Second, long-read metagenomes from the Mediterranean Sea allowed us to recover 30 MAGs of higher quality than our short-read counterparts, some of them as single-contig complete

genomes. Specific long-read MAGs featured population structure, which, in several cases, was linked to warm and/or cold waters. Adaptive genes were identified in different species, potentially representing the adaptation of diverse populations to varying environmental conditions. Our work showcases the effectiveness of combining short- and long-read metagenomics to unravel the spatiotemporal dynamics of marine microbial populations. By integrating these approaches, we may gain comprehensive insights into global microbial biodiversity and macroecological patterns, as well as the underlying mechanisms driving ecosystem dynamics.

O048. A comparative metagenomics approach reveals a common functional potential of plastisphere microbiomes across oceans

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Recent developments in functional metagenomics enables a better understanding and assessment of pollution-induced patterns in global aquatic community biodiversity and functioning. Over the last decades trillions of plastic particles have accumulated in the oceans, which are heavily overgrown with biofilms (termed plastisphere). Studies, so far, focused on the taxonomic composition of these consortia, but their functional potential remains understudied. Our aim was to examine the functional potential of open ocean plastisphere microbiomes based on genome-resolved metagenomics in comparison to the surrounding seawater plankton. To identify general principles in adaptation processes across spatial scales, we collected plastic from the North Atlantic and North Pacific Garbage patch on research cruises in summer 2019. Metagenomic DNA samples were extracted and resulting sequencing data was trimmed, assembled and binned into bacterial metagenome-assembled genomes (MAGs). The MAGs were used for Hidden Markov Model profiling utilizing public KEGG profiles of metabolic pathways. More than 1700 plastisphere MAGs have been reconstructed and compared to >450 corresponding seawater MAGs. The plastisphere displayed a distinct microbiome that is taxonomically comparable across oceans but different to the surrounding seawater. Further, the plastisphere metagenomes were characterized by larger genome sizes, more coding genes and a higher genetic potential for dealing with harsh environmental conditions of the ocean's surface in comparison to plankton, despite residing in the same environment. Our results suggest that the overriding factor for the high functional similarity of the plastisphere in both oceans is the habitat for biofilm formation. We illustrate the great potential of functional metagenomics to identify the assembly rules in microbial communities in a changing world.

O049. New omics observatory for marine biodiversity in the Adriatic: a case study

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We present an overview of the recent establishment and development of the Italian Omics Observatory Newtork of marine biodiversity, constituted by 4 long-term ecological data sites within the Italian National Biodiversity Future Centre. Specifically, we are developing an observatory at the Senigallia station, situated in the central part of the Adriatic Sea. The

station is identified by a buoy in the sea, which has been sampled monthly, beginning in February 2024. Among the primary results achieved are: 1) the integration of the Italian initiative into the global network of genomic observatories (i.e., EMOBON); 2) the adoption and implementation of Standard Operating Procedures (SOPs) for sampling the various biological components of the water column; 3) the establishment of a repository of biological samples and associated data, aimed at supporting long-term studies on Mediterranean biodiversity. Our immediate research goals are to identify biodiversity patterns within the station over time, compare these patterns across all 4 stations, and understand the factors influencing these patterns.

O050. Environmental drivers of diatom diversity: insights from DNA metabarcoding

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Environmental DNA (eDNA) metabarcoding has become an efficient method for multiple samples identification based on the genetic material occurring in the environment. The objective of the study is to do a comparative analysis between different identification approaches to study how environmental variables shape benthic algal communities in a riverine network. A total of 130 samples from Hungarian watercourses were taken in August and September 2018, and in May and June 2019. Environmental variables were measured *in situ* and in the laboratory. Periphyton samples were collected from the most common substrates. Subsamples preserved in Lugol's solution were analyzed by inverted microscopy to identify all algal groups. Other subsamples were used to prepare cleaned diatom slides from which diatom species were identified. DNA metabarcoding was performed on other subsamples using a 312 bp fragment of the rbcL gene. Microscopy on benthic algae offered a broad perspective on community composition but often limited by taxonomic resolution. Diatom microscopy enables species-level identification, even though it is labor-intensive and requires specialist expertise. DNA metabarcoding offers a fine taxonomic resolution and reveals hidden diversity within diatom communities with potentially distinct ecological preferences. This approach provides valuable insights into the influence of abiotic factors on benthic diatom communities, helping to elucidate how environmental variables shape these communities in riverine ecosystems. However, DNA metabarcoding's accuracy depends on the quality and completeness of reference databases, and while it highlights diatom diversity, an integrated approach with microscopy can offer a comprehensive view of the algal community.

O051. Methodology for creating a freshwater bioindicator using periphyton genomics

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As part of the AquaBioSens project (FEDER Wallonie 2021-2027, Water Smart Cities), freshwater biodiversity is studied to develop a bioindicator for water quality monitoring using genomic analysis of periphyton cultivated on a standardized passive sampler. This bioindicator will complement existing indices like the diatom benthic index. Periphyton was sampled from three rivers: Le Blanc Gravier (BG; plastic supports), La Vierre (Vi; stones) and La Braunlauf (Br; stones). In parallel, periphyton was grown in a lab-scale pilot (Pi; plastic

supports). DNA was extracted using the NucleoMag® DNA microbiome kit (Macherey-Nagel). Community composition was analyzed via 16S (27F/1492R) and 18S (V8-V9) metabarcoding using nanopore sequencing. Sequences were cleaned, trimmed, basecalled (Dorado v0.8.0), and relative abundances were estimated with Emu v3.4.5 and PR² v5.0.0 databases.

Twelve samples from four sites are analyzed using 16S and 18S metabarcoding. Simpson index (α-diversity) are >0.8 for 12 (16S) respectively 9 (18S) samples. The samples are composed of the phyla (18S) Stramenopiles, Chlorophyta, Streptophyta and Alveolata, and (16S) Acidobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Planctomycetes and Proteobacteria, the orders and genera (18S) Naviculates (*Navicula*, *Sellophora*), Achnanthales (*Cocconeis*, *Planothidium*), Bacillariales (*Nitzchia*), Bacilliariophyceae, Chlorellales, Prasiolales and Sphaeropleales, and (16S) Nostocales (*Dulcicalothrix*), Burkholderiales (*Rhizobacter*, *Rhodoferax*), Rhizibioales, Sphindomonadeles and Synechococcales (*Chamesiphon*). Diatoms (47% ± 6.6), and green algae (33% ± 5.3) are the most abundant eukaryotic taxa and seasonal trends are seen, particularly among the Naviculales, Achnanthales, and Chlorellales. Proteobacteria (56% ± 4,2) and cyanobacteria (16% ± 5,8) are the most abundant prokaryotic taxa, with fluctuations seen among the Nostocales. These results are the first steps towards the development of a novel genomic based bioindicator for water quality monitoring. Follow up efforts will focus on (1) expanding the current dataset, and (2) enhancing the detection of so far non-detected groups (i.e. ciliates, amoebae) by optimizing the DNA-extraction protocol.

O052. How do eDNA monitoring methods compare to traditional bryophyte surveys in rivers?

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Despite their ecological importance, mosses remain under-represented in studies and conservation policies, mainly due to the specific and highly qualified expertise required for their identification. Molecular-based monitoring may represent a new advance in our understanding of these particular taxa. We evaluated the effectiveness of eDNA metabarcoding in detecting riverine and riparian bryophytes from river water samples. Our study is the first comprehensive evaluation of hybridization capture (HC) versus PCR-based eDNA metabarcoding, using vascular plant and bryophyte-specific primers, for bryophyte monitoring and in comparison with field surveys. The bryophyte-specific PCR method (bryoPCR) identified 101 species, 16 if which were shared with the HC method, and 37 with field surveys. The capture-based method retrieved 27 species, 9 of which were unique to the method and 11 common with the field surveys. Both eDNA methods identified bryophyte species that were not recorded in the field surveys but are known from the catchment. Although bryoPCR provided more species than HC, both methods exhibit comparable taxonomic resolution at the specie level. Each method outlines a unique composition of the community when applied to the same sites. Factors such as primer specificity and amplification bias contribute to the variation observed in the species composition detected. Our results highlight the importance of exploring the methodological variability of eDNA approaches before interpreting ecological patterns. In addition, we show the importance of

identifying and developing tailor-made primers for studies on bryophytes to improve detection and the reliability of these studies. The capture-based method requires further optimization before considering it for eDNA applications.

O053. From marine to freshwater: using fish eDNA to assess community dynamics across salinity gradients

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Gambia is the smallest country in Africa; almost 20% of its surface is covered. The River Gambia is a vital water source and a biodiversity hotspot (e.g., mangroves, fish). Due to a very long and severe drought, seawater has intruded into the river drastically transforming the ecosystem. Despite the river's ecological importance, its biodiversity remains largely unexplored, limiting our ability to assess the impacts of seawater intrusion. Environmental DNA (eDNA) presents a promising tool to address this knowledge gap. It offers a cost-effective, non-invasive method to detect a wide range of species, including those that are difficult to observe and capture, thus providing valuable insights into community dynamics. This study aims to evaluate the effectiveness of eDNA for assessing fish community dynamics and the interactions between marine and freshwater species along the river's salinity gradient. In summer 2024, we collected eDNA samples from 16 sites along the river, with two one-litter replicates taken from three subsampling locations: left shore, right shore, and central channel (both upstream and downstream). The samples were analyzed using four replicates per filter with the Teleo02 primer, sequenced on a MiSeq platform, while salinity gradients were measured using conductivity. We expect to achieve consistent sequencing depths for marine, brackish, and freshwater species across the salinity gradient, as the Teleo02 primer targets a broad range of species. However, taxonomic resolution may vary, with some species only identified at the genus or family level due to incomplete reference databases. Brackish zones may exhibit the lowest alpha diversity due to a lack of truly adapted species, whereas the marine and freshwater areas should show distinct communities. This study will provide key insights into how fish communities respond to seawater intrusion and

contribute to improve our current knowledge about the aquatic biodiversity of the River Gambia, a biodiversity hotspot threatened by climate change.

O054. Metabarcoding across the tree of life reveals conservation significance and biodiversity patterns in a tropical river (Corubal, Guinea Bissau)

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Biodiversity information is needed for the ecological assessment and environmental management of tropical rivers, which are under growing pressure from climate change and multiple anthropogenic drivers. Here we use eDNA surveys to characterise aquatic biological communities in an overlooked freshwater biodiversity hotspot in West Africa (Corubal River). The survey was carried out in 2022 and 2023 in articulation with local stakeholders and involved sampling at 25 transects, covering different aquatic habitats. At each site, a large volume of water (>60L) was filtered using peristaltic pumps and two capsule filters, and each filter was preserved in a buffer. DNA extraction, amplification, purification, sequencing and bioinformatic processing followed standardised procedures, to ensure reproducibility, avoid contamination, maximising detectability and reducing false positives. Amplification involved primers targeted at vertebrates (V05), fish (teleo), freshwater bivalves (Unionida and Venerida) and all Eukaryotes (EUKA02). We detected several aquatic and semiaquatic vertebrate species of conservation concern (e.g., Hippopotamus, African Clawless Otter, Senegal Flapshell Turtle), but missed abundant aquatic reptiles such as crocodiles. We also detected a number of aquatic vertebrate species previously unrecorded in the country. Analysis of biodiversity patterns across the tree of life showed significant longitudinal patterns of community change, including variations in species richness and replacement. There were also significant lateral community variations between the main river channel, tributaries and floodplain lakes. Overall, our study reveals the power of environmental DNA to describe aquatic biodiversity patterns in poorly explored tropical rivers, providing important insights for their assessment and environmental management.

O055. Harnessing the power of eDNA biodiversity assessment to enhance subsurface water flow pathway reconstruction

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Rainfall runoff contributes to a large proportion of the discharge in streams and therefore, heavily influences stream water quality but also flood generation. Rainfall runoff generation is usually a combination of overland flow and subsurface flow processes, the latter of which being especially difficult to trace. Here, we explored the viability of environmental DNA (eDNA) for biodiversity assessment and simultaneously for subsurface water flow pathway reconstruction. The degree of similarity of community patterns indicates biological and therefore, in principle, also hydrological connectivity. We applied eDNA metabarcoding to characterise 10 drilling cores (0.7-3.2 m depth) at 3 hillslopes (10x50 m) in 4 catchment areas in Germany and Austria. In total, 1793 microbial and invertebrate species could be identified.

Analysis of alpha and beta diversity composition in the different catchments showed significant differences in spatial clustering patterns between taxonomic groups but also between geomorphological properties of the catchment. We could assign 516 indicator species across taxonomic groups in various depth layers and identify habitat-specific communities that can be used as hydrological tracers. Partitioning of beta diversity identified three-dimensional connectivity patterns that indicate barriers as well as pathways of hydrological connectivity within each hillslope. Variation between catchments reflects their geographic and geological differences. Although our results support the potential of eDNA to identify flow pathways and enhance our understanding of subsurface flow processes, we are still at the beginning of understanding the viability of eDNA as a tracer in hydrological research. Nonetheless, making use of such naturally occurring tracers can extend our understanding of hydrological phenomena and can contribute to a more accurate flood prediction.

O056. New perspectives on the community composition of groundwater ecosystems using eDNA metabarcoding

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Groundwater ecosystems are highly diverse, serve as the most important source of drinking water, and are increasingly threatened by human-induced environmental stressors, such as chemical pollution and global warming. These ecosystems host numerous faunal and microbial communities whose biological interactions are crucial for maintaining groundwater quality but are still not fully understood. Traditional methods for identifying groundwater metazoans depend on morphological identification from organisms collected at monitoring sites, which is time-consuming and rely heavily on taxonomic expertise. To enhance the assessment of groundwater biodiversity across extensive geographic regions and entire biomes, we employed two complementary sampling strategies, based on metabarcoding of the cytochrome oxidase I (COI) gene region. These strategies include (1) bulk-sample metabarcoding of organisms collected from designated monitoring sites and (2) environmental DNA (eDNA) metabarcoding from aquifer water surrounding these sites. Our findings indicate that eDNA metabarcoding offers a more representative and less biased depiction of groundwater biodiversity compared to traditional approaches. It covers the full scope of aquifer metazoan communities, including organisms that are not captured in artificial monitoring traps. Incorporating microorganisms and protists into our approach opens several novel research avenues, such as including functional analyses of groundwater food webs and assessments of groundwater and drinking water quality, based on data covering the entire aquifer biome. This comprehensive strategy aims to provide an integrated view of biodiversity within groundwater ecosystems, establishing a robust foundation for improved conservation and monitoring practices.

O057. Fit for purpose? Evaluating benthic invertebrate DNA metabarcoding for ecological status class assessment in streams under the Water Framework Directive

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The ecological state of aquatic ecosystems is systematically monitored using various bioindicators in many countries worldwide. In the European Union, freshwater biomonitoring is the central component of the EU Water Framework Directive (WFD, 2000/60/EC) and currently based on morpho-taxonomic methods. DNA metabarcoding is a novel approach to assess the ecological state fast and efficiently based on organismal DNA signatures and thereby support and upscale biomonitoring. However, compliance of metabarcoding with existing morpho-taxonomic methods must be ensured prior to official implementation. Thus, this study co-designed by research institutions and environmental agencies explored necessary key parameters and performed method intercalibration for the implementation of metabarcoding into WFD assessments of running waters. We focussed on benthic invertebrates as the most commonly used bioindicators. We analysed 170 invertebrate samples collected as part of the German federal state WFD routine stream biomonitoring, first via microscopic determination and then using metabarcoding. Our goals were to quantify overlap in i) taxonomic composition and ii) ecological status derived with both methods. For this purpose, we established data harmonisation measures to integrate invertebrate metabarcoding data into the official national WFD classification modules considering abundance and presence/absence data. Our results revealed a high (ca. 70%) overlap of bioindicator taxa found with both methods. Metabarcoding identified significantly more small invertebrate taxa and detected similar proportions of the important bioindicator 'EPT' taxa (mayflies, stoneflies, caddisflies). Despite deviations in some detected bioindicator taxa, the derived ecological status classes were highly correlated between methods, particularly after intercalibration (R^2=0.74, Spearman rho=0.86). Regardless of whether we used abundance or presence/absence data, the resulting stream type classifications showed strong agreement. Thus, our study not only demonstrates the consistency of the methods for the stream types analysed but is also the first to operationalise a path to integration of metabarcoding data into the WFD assessment modules based on formal intercalibration guidelines.

O058. Bioindicators of littoral and retro-littoral wetlands ecosystem functioning

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Littoral and retro-littoral wetlands are vital ecosystems that support diverse habitats and species while enabling various human activities. However, these environments face severe decline due to climate change and human activities, threatening the ecological services they provide. Developing ecosystem-based decision support tools is essential for managing and preserving these wetlands and their biodiversity. While bioindication tools exist for assessing water quality in rivers and estuaries, they often overlook marshes and wetlands. Moreover, current indicators primarily focus on macro-organisms, neglecting the crucial roles of microbial life, especially prokaryotes and protists, in trophic webs and biogeochemical cycles. The benthic community, comprising prokaryotes (bacteria and archaea) and eukaryotes (micro-eukaryotes and meiofauna), is central to delivering ecosystem services in these environments. We developed a benthic bioindicator tool based on eDNA considering the representativeness and variety of littoral and retro-littoral wetlands along the Mediterranean and Atlantic coasts of southwest Europe (France, Spain and Portugal). By characterizing benthic communities through 16S and 18S rRNA gene sequencing, physicochemical parameters, and pollutants (metals, pesticides, hydrocarbons), we assessed various environments related to coastal management, rehabilitation, and biodiversity protection. Relationships between benthic communities and environmental parameters were evaluated via different analyses (e.g. LEfSe, TITAN, network). Potential benthic bioindicators were identified for monitoring the ecological quality of wetland ecosystems, addressing environmental threats such as pollution, eutrophication, and salinization. Our focus on organism assemblages offers insights into trophic webs and ecosystem functioning. Co-occurrence networks highlighted connections among organisms across three domains of life, particularly between photosynthetic eukaryotes and prokaryotes. This holistic approach enriches our understanding of microbial interactions and ecosystem dynamics. Furthermore, benthic eDNA bioindicators enable early detection of disturbances and can predict declines in the ecological functions of wetlands affected by global changes.

O059. Microbial biofilms as indicators of environmental change in English rivers.

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For over a decade the Environment Agency has been developing DNA based methods for monitoring the health of freshwater ecosystems. Having developed metabarcoding approaches for the assessment of diatoms in rivers and fish in lakes, focus has now shifted from higher organisms to the recognition of microbes as potential bioindicators of ecosystem health. Microbes play important roles in freshwater ecological processes however,

understanding of the environmental drivers that shape their distribution is poor and have not historically been part of routine biomonitoring. Our aim is to enhance understanding of the distribution of microbes, their functional roles and contribution to ecosystem health and resilience. Over time this work could lead to the development and integration of new microbial bioindicators into monitoring programmes to guide more targeted management interventions. We generated a national-scale, metabarcoding dataset capturing the diversity of bacteria (16S), fungi (ITS), phytobenthic algae (rbcL), and microeukaryotes (18S) in over 1642 freshwater biofilm samples, at 699 sites from rivers across England. Metagenomic sequencing was applied to a subset of 450 biofilms to uncover the functional diversity of the benthic biofilm communities. Landscape characteristics and pressures were identified and mapped using monthly co-located water quality data and spatial datasets of types of landcover. Traditional statistical approaches and random forest regression modelling was applied to the dataset. We will present results of the environmental impacts of pressures on the overall microbial community as well as preliminary results identifying potential microbial bioindicators of specific environmental pressures. We also highlight ongoing areas of data exploration: trends in microbial biogeography, the distribution of pathogens and antimicrobial resistance and linking functional genes and genomes with ecological processes and environmental variables.

O060. Artificial intelligence assisted modelling reveals that species properties rather than species diversity determine community responses to environmental change

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Disturbances are major drivers of microbial community functioning and composition. According to the theory, both the life histories of individual community members (concerning their niche-breadth and ability to recover after disturbances) and species diversity are relevant for the community-level response to disturbances. We aimed to demonstrate that genomic traits indicating life histories of microbial populations, for instance their genome size, in combination with species diversity measures, are meaningful predictors for community dynamics in response to environmental change. We further aimed to examine whether indicated life histories or diversity parameters were more important as explanatory variables to predict community responses to environmental change. We quantified the functional resistance of heterotroph production to salt disturbances, as well as compositional resistance and functional resilience following dilution, from 100 aquatic microbial communities sampled during a year across 13 sites. Diversity measures and community weighted means of 12 genomic traits were determined from amplicon sequences. Artificial intelligence assisted modelling revealed that all three response variables were well predictable from the explanatory variables (correlations between predicted and measured values: r = 0.61-0.82). Genomic traits consistently held greater importance in the resulting models compared to diversity measures. This finding corroborates our observation that eutrophic sites exhibited highest community level resistance and resilience, and were inhabited by species that according to their genomic traits were highly resistant and resilient,

while their communities tended to be less diverse than oligotroph communities. We conclude that the life history of community members holds greater relevance for community-level resistance and resilience than diversity.

O061. Graph theory at the service of assessing the ecological status of lake ecosystems based on phytoplankton communities

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Lake ecosystems play an essential role in climate, through the exchange of heat and water with the atmosphere, and in the global carbon cycle, being both a source and a sink of carbon. Lakes are also essential to human, providing numerous ecosystem services. The functioning of lakes, as well as their stability, resistance and resilience in the face of environmental disturbances, is mainly underpinned by the biodiversity they harbour, notably phytoplankton community. However, human activities are causing an unprecedented loss of biodiversity, particularly in inland water bodies. In this context, assessing the ecological status of lakes is an important task in order to evaluate their state of degradation as well as to determine the origin of degradation. Approaches based on environmental DNA (eDNA) have shown their relevance in addressing such questions. Based on this type of data, statistical modeling approaches can be used to construct ecological networks (co-occurrence), and graph theory to understand their structure (topology). Recent studies have shown that network topology can be influenced by anthropogenic pressures, and can therefore be used as an indicator of these pressures. In this context, we propose to develop an indicator of the ecological state of lake ecosystems based on the measurement of graph topology metrics from eDNA data targeting phytoplankton. More precisely, our work is focused on centrality metrics that characterize the topological role of each node (or ASV) at the scale of networks. This purpose was possible by using a dataset from over 100 lakes across France.

O062. Sediment microbial communities and their association networks differed depending on their disturbance level

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Estuaries are dynamic, complex and productive aquatic ecosystems where marine, freshwater and specific estuarine biomes meet. Millions of people depend on the resources they offer (fisheries, transport, recreational activities), which puts them under high anthropogenic pressure. In order to monitor and regulate such pressures it is essential to better understand the functioning of these ecosystems. We collected eDNA sediment samples time series from six estuaries from the Basque coast (Spain) submitted to different disturbance levels, compared their prokaryotic communities and reconstructed their ecological association networks, that were then used to build consensus networks of

estuaries with the similar disturbance levels. Microbial communities from less disturbed estuaries were more stable across time, while sharp changes were observed in more polluted estuaries. Consensus networks showed common associations occurring in estuaries with similar disturbance levels, and taxa with key roles in the community, i.e. keystones, connectors and drivers. These differed between consensus networks, and included both high and low abundance taxa. Topological properties of consensus networks also differed with the disturbance level. For example, the number of nodes, edges and connectance, were higher in less polluted estuaries, whereas modularity was higher in more polluted estuaries. In the light of these results, we can conclude that time series are a powerful approach to study microbial communities, that can reveal community stability, and together with modeled association networks they can highlight environmental changes in estuarine ecosystems.

O063. Machine learning-driven analysis of metabarcoding data to identify anthropogenic trace substances in aquatic ecosystems

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Aquatic microbial communities play crucial roles in ecosystem functioning, providing a more comprehensive reflection of biological systems than single organisms. This makes them especially promising for evaluating ecosystem health. Although phytoplankton has been used in biomonitoring, bacterial communities offer additional insights. Despite their potential, bacterial communities and other microbial taxa, such as those revealed through 18S rRNA metabarcoding data, have not yet been fully integrated into official biomonitoring programs. In this study, we demonstrated that using 16S and 18S rDNA metabarcoding data combined with machine learning enables the prediction of anthropogenic trace substances. Over one year, eDNA samples for 16S and 18S rRNA gene metabarcoding were collected twice weekly from 14 locations along the Warnow Estuary and Baltic Sea coast, with measurements of over 40 anthropogenic trace substances, such as pharmaceuticals, herbicides, and UV filters. We trained Random Forest models to predict and quantify pollutants from the metabarcoding data, achieving high accuracy for a subgroup of contaminants. Using further regression analyses, we identified taxa that could be used as early warning indicators of substance concentrations exceeding ecotoxicological thresholds. Because the datasets used in this study span fresh water and marine habitats over the entire annual cycle, we expect the results to generalize easily to other locations. Based on our findings, we propose that the integration of eDNA metabarcoding and machine learning will become a vital component of official biomonitoring programs, providing a scalable and efficient method for assessing ecosystem health and impacts of anthropogenic pressures in aquatic environments.

O064. Estuarine microbenthos as ecosystem health indicators, from the Basque estuaries to the whole Bay of Biscay, the world, and beyond

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Estuaries and salt marshes are diverse and dynamic ecosystems, often heavily impacted by stressors such as eutrophication, pollution and hydrological alterations. This has resulted in degradation of many sites worldwide, with reduced biodiversity and water quality, increased harmful algal blooms, collapsed coastal fisheries and reduced protection from floods and storms. While environ-mental legislation and management can improve this situation, it relies on frequent assessments of biodiversity and ecosystem status. As in many types of environments, molecular methods have an important role in scaling up such monitoring regimes, and improving problems such as shortage of taxonomic expertise. However, DNA-based identification of established macrobenthic bioindicators have several shortcomings including considerable spatial patchiness due to the small amount used for DNA extraction, lack of reference sequence data and non-target amplification. Another promising approach is to instead target benthic microorganisms through de novo inference. Here, we present preliminary results from ongoing work to leverage a large collection (>1800 samples) of new and published prokaryotic 16S rRNA metabarcoding data from estuary sediments from around the world, with associated physicochemical and biological data summarised as as a single Pressure Index (PI) indicating total environmental pressure. By using Random Forest machine learning in a cross-validation manner, we demonstrate that we can train a classifier to predict environmental status based on prokaryotic community composition with limited accuracy but highly significant correlation to the ground truth (PI), in spite of the existence of strong gradients with apparent influence far higher than typical anthropogenic pressure (mainly salinity and climate). This even holds true when limiting training data to Australia and setting aside all European data for validation, demonstrating that this approach is robust to imbalanced or heterogeneous training data, given sufficient size. This demonstrates that eDNA metabarcoding of microbenthic communities has a strong potential for biomonitoring and development of novel ecological status prediction tools.

O065. Biodiversity time machine: a holistic approach to monitoring and forecasting freshwater ecosystems

Luisa Orsini (University of Birmingham, UK), Niamh Eastwood (University of Birmingham, UK), Jiarui Zhou (University of Birmingham, UK), Arron Watson (University of Birmingham, UK)

Biodiversity, encompassing taxonomic, functional, and genetic diversity, is essential for maintaining resilient ecosystems and supporting human well-being through services like food, medicine, and clean air. Despite global efforts such as the Kunming-Montreal Framework, biodiversity is declining rapidly, with freshwater ecosystems being the most affected. Lakes and ponds, especially vulnerable to land-use changes and urban runoff, experience biodiversity loss at two to three times the rate of terrestrial and marine systems. These

ecosystems often go unrecognized in conservation efforts, particularly the 'invisible fraction' of biodiversity—small invertebrates, bacteria, and fungi—that play critical roles in ecosystem functioning. Current biodiversity monitoring focuses on species presence but neglects long-term processes and species-species interactions. Moreover, key drivers of biodiversity loss, like pollution, climate change, and invasive species, are often studied in isolation, missing the interconnected dynamics that shape ecosystems. We have developed a holistic framework that links biodiversity dynamics to abiotic changes using sedimentary lake archives as 'time machines' and artificial intelligence. Applied to a lake with over 100 years of human impact, we found that insecticides, fungicides, and climate extremes explained up to 90% of biodiversity changes. Extending this to 52 lakes sampled across the landscape, we confirmed that insecticides, fungicides, and physico-chemical factors are major drivers of biodiversity loss. Looking ahead, we are expanding this framework to multiple lakes, combining long-term sediment records with AI to assess how pollutants, land use, and climate change drive biodiversity shifts. Our goal is to create a predictive biodiversity forecast framework that projects the future impacts of environmental stressors on ecosystems. This model will guide targeted conservation efforts by predicting how climate change, pollutants, and land-use changes will interact to shape biodiversity. Ultimately, our monitoring and forecasting framework offers a new standard for managing freshwater ecosystems, helping to safeguard their biodiversity and the ecosystem services they provide.

O066. Exploring the landscape-level drivers of lake biodiversity using data-driven analysis of environmental DNA

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Major threats to biodiversity, such as chemical pollution, climate change and changing land-use occur and interact across spatial scales. However, the complex interaction of abiotic and biotic factors make the identification of the most impactful environmental stressors difficult, impeding our ability to prioritise environmental intervention efforts. Freshwater lakes, which deliver key ecosystem functions and services, are particularly at risk from these human driven environmental stressors, as lakes act receivers of pollution from the landscape, such as agricultural and urban runoff. In this study, we multi-marker metabarcoding of environmental DNA to measure whole-community biodiversity in 52 lakes across England. We combine this with environmental data (physico-chemical parameters, lake typology, pesticide usage) using explainable multi-view learning, a machine learning approach, to identify landscape-level drivers of community biodiversity in lake ecosystems. We identified plant protection products, such insecticides and fungicides, as the most important factors driving biodiversity dynamics, followed by physico-chemical parameters. Our holistic, data-driven approach provides insights into large-scale biodiversity changes and could inform efforts and interventions to protect biodiversity from harmful pollutants.

O067. From anarchy to clarity, data pre-processing and statistical choices influence quantitative environmental DNA (eDNA) analyses.

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Environmental DNA (eDNA) analyses have shown great potential to increase species detections sensitivity and even estimate their abundances. Given the rapid growth, continuous method development and optimisation a diverse array of methods have emerged to capture and analyse eDNA. While considerable effort has been devoted to refining field and laboratory protocols, understanding the consequences of data pre-processing and statistical choices remains a notable gap, especially when it comes to the analyses of quantitative eDNA data. Yet these insights are essential to develope best-practice guidelines that can harmonize analytical workflows. To fill this gap, an extensive literature search was conducted focussing on species-specific quantitative eDNA studies to assess the diversity of choices made during the data-analysis workflow. Subsequently, common strategies were applied to studies whose raw data were available to formulate general recommendations for improving the reliability and reproducibility of quantitative eDNA analyses. Overall result indicated that within the current literature statistical methods are not always clearly described and raw datasets are rarely made publicly available. Furthermore, the choice of the statistical test used to assess correlations and the pre-processing of the data can substantially influence the ability to detect positive correlations and the estimated effect sizes. Overall, to help advance the eDNA field we recommend: (i) Increased transparency in method descriptions and data availability. (ii) Assessing correlations through the use of mixed effect models that can accurately account for data characteristics. (iii) Avoiding pre-processing of quantitative eDNA data especially in combination with sub-optimal statistical tests. Finally, the proposed guidelines will enhance the reliability of quantitative eDNA analyses and thus their uptake in conservation and policy decisions.

O068. Modeling the interactions between decay and dispersion of eDNA in the Bay of Biscay

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Environmental DNA (eDNA) has emerged as a new tool for fisheries management and biodiversity monitoring, offering novel insights into marine ecosystems. However, linking eDNA concentration to species abundance remains a key challenge. Understanding the biotic and abiotic factors that influence eDNA production, decay, and transport in marine environments is still limited, which hinders its application for estimating marine species abundance. This study aims to enhance our understanding of eDNA decay and transport, focusing on the Bay of Biscay, Northeast Atlantic. Specifically, we (1) explore and compare various temperature-dependent decay rate relationships, and (2) simulate the drift and dispersion of eDNA under different scenarios, including both fixed and mobile source movements with varying decay rates. Using a Lagrangian particle tracking approach implemented in the Connectivity Modeling System (CMS), we model eDNA drift in the Bay of Biscay between April 2023 and March 2024. Our findings reveal a pronounced spatial effect

across three geographically spread study sites, which surpasses temporal (monthly) variations. eDNA from a fixed source (e.g., fish shedding in a localized area) is dispersed and transported further from its release point compared to eDNA from a mobile source. In a fixed source scenario, eDNA dispersal is influenced only by temporal variation, whereas mobile sources experience the combined effects of both spatial and temporal variation as the fish moves. On average, eDNA has a higher probability of detection for up to 7 hours and can travel distances of up to 10 km. We also observe notable differences in dispersion patterns and a strong influence of depth on eDNA transport. We show that the choice of decay rate is crucial, significantly affecting modeled transport outcomes and highlighting the need for precise decay rate estimation at the species level for expected in situ temperatures. Further research into additional eDNA decay processes is essential to refine our understanding of eDNA dynamics in oceanic environments and improve the predictive power of eDNA-based monitoring.

O069. Linking metabarcoding quantitative information to fish environmental DNA concentration in Neotropical rivers

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Environmental DNA is now well established for evaluating the presence/absence of a single species or whole communities, but the relevance of eDNA in providing quantitative measures of biodiversity is still largely debated. Linking fish biomass to eDNA data is particularly uncertain because of two main sources of uncertainties that should be considered independently. The first is the link between species biomass and eDNA concentration, which is influenced by complex interactions between biological (physiological, behavioural) and environmental (temperature, and physicochemical parameters) aspects. The second is the link between eDNA concentration in the water and read numbers, which can be affected by eDNA sampling, extraction, amplification and sequencing procedures. Here we focussed on this second source of uncertainty by investigating the link between the quantity of DNA detected by metabarcoding and the concentration of eDNA in the natural environment measured using digital PCR (dPCR). Indeed, even if the number of reads has been used as the most common proxy, many biases are known to distort this number. We sampled eDNA in 34 sites belonging to two distinct river systems (Oyapock and Sinnamary Rivers, French Guiana) and investigated the relation between the number of metabarcoding reads, the number of positive metabarcoding PCR and the DNA concentration in the water measured using dPCR. Results showed that the relationship between the number of reads and eDNA concentration was significant for only one of the two considered species. In contrast, the number of positive PCR significantly increased with an increasing number of copies/µL for both species in the two independent river systems. Our study demonstrates that the number of positive PCR in a metabarcoding analysis can be a relevant proxy of the eDNA concentration of a targeted species in the natural environment.

O070. Three-year monitoring of lake sturgeon (*Acipenser fulvescens*) occurrence in a spawning ground using environmental DNA

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In Canada, apart from the Lower St. Lawrence River population, most lake sturgeon (*Acipenser fulvescens*) populations are declining, mainly because of over-harvesting, pollution, and alterations to hydrological regimes. Spawning grounds are particularly affected by anthropogenic disturbances and close monitoring of lake sturgeon presence and abundance in these habitats is crucial for the protection of this at-risk species. However, conventional surveying methods such as adult capture are labour-intensive and invasive. Consequently, assessments of lake sturgeon occurrence in spawning grounds are infrequent and conservation efforts are hampered by a lack of information. A potential alternative monitoring method is the analysis of genetic material shed by lake sturgeons in spawning ground water, i.e., environmental DNA (eDNA). Population monitoring methods based on eDNA are often cheaper and faster, which could allow for more frequent and extensive data collection. The objective of this study was therefore to evaluate eDNA as a tool to monitor lake sturgeon occurrence in spawning grounds. For three years (2022-2024) throughout the spawning period (May-June), we sampled water from the Chaudière River's spawning ground (Quebec, Canada) which receives one of the largest spawning runs of lake sturgeons in Quebec, and we quantified lake sturgeon eDNA using quantitative PCR. In 2024, we also counted lake sturgeons in a section of the spawning ground using a drone. We found that eDNA concentrations peak when the water reaches the spawning onset temperature (12°C), indicating that eDNA is correlated with sturgeon spawning activity, a result consistent with the count carried out using the drone in 2024. Our study indicates that eDNA is useful for assessing lake sturgeon occurrence in a spawning ground, but our results should be replicated in spawning grounds that receive smaller spawning runs before eDNA can be adopted as a monitoring tool for this species.

O071. Reproductive and migratory patterns in fish revealed by MBC analyses of monthly samples.

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Water samples collected on monthly basis over a year in four aquatic habitats (river, pond, lake and a marine site) were analysed by metabarcoding to reveal spawning and migration patterns in fish populations. The 200+ collected samples showed spawning patterns in more than 40 fish species. We recalculated fluctuations in ratios of reads for each species separately

to also include species with lower “relative biomass”. The spawning patterns from the eDNA results, matched with background data regarding temperature dependent onset of reproduction and existing knowledge of reproduction in some of the species. The results showed that MBC analysis of monthly sampling combined with sound ecological knowledge provides large amount of data and ecological information which will be described in detail.

O072. Unlocking demography: developing an eDNA-based toolkit to measure sex ratios.

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Given the rapid rate of human impacts globally, monitoring population sustainability is of vital importance. Population demographic information, particularly in relation to the ability to reproduce, like birth/death rates, matting patterns, and sex ratios, are important parameters to understand population viability as a whole. Sex ratios in particular can influence the reproductive success of individuals, and when biased, can impact the growth and stability of a population. For instance, anthropogenic stressors such as global warming and habitat disturbances can distort population sex ratios through sex-biased heat tolerance or even by influencing sex allocation. But despite being an important factor for accurately estimating population viability, sex ratios are often overlooked possibly because traditional monitoring approaches are laborious, time consuming, likely biased and often invasive or destructive to study organisms. Environmental DNA (eDNA) is a novel non-invasive method of monitoring species or communities by analyzing the genetic material present in an environmental sample such as water, soil, or air and has potential to overcome these hurdles. However, its applications typically focus on taxonomic identification or presence/absence and are mainly based on mitochondrial DNA (mtDNA). Our research group is developing an eDNA-based toolkit to measure sex ratios of populations through the detection of nuclear DNA (nDNA) in environmental samples. To develop our toolkilt from scratch, we use the Balkan crested newt (*Trituturs ivanbureschi*) as our sudy organism. We first identify male specific genetic markers by analysing genomic sequencing data of *T. ivanbureschi*, we further validate these markers and lastly test them on mock population samples, as well as mesocosm eDNA samples. Our method could offer a way to measure sex ratios and facilitate conservation efforts with minimal disturbance to the organisms and environments under study.

O073. Species-level detection and population genetic inference of small cetaceans from environmental samples, using mitochondrial and RADseq-derived markers

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Oceanic dolphins (Delphinidae) and other small cetaceans are notoriously difficult to differentiate using mitochondrial DNA markers typically employed in eDNA studies, such as the COI and D-loop regions. Collecting tissue samples from these species poses significant challenges due to the high cost of oceanic expeditions, the unpredictability of locating individuals, and the invasive nature of skin biopsies. Therefore, developing species-specific genetic markers that can serve as reliable barcodes, with the potential for use in population genetic studies, would be highly beneficial. In our study, we assess the utility of the mitochondrial D-loop for the accurate detection and characterization of population-level genetic diversity in the common dolphin (*Delphinus delphis*). By comparing eDNA samples, with haplotypes obtained from skin biopsy samples collected simultaneously at 14 sites in the Bay of Biscay in 2022 and 2023, we aim to evaluate the method's reliability. Additionally, we are using loci identified through ddRAD sequencing to develop markers for species-specific detection across eight delphinid species, as well as testing the efficacy of these markers in population genetic inference in the common and striped (*Stenella coeruleoalba*) dolphins.

O074. Population genetic analyses of a key invertebrate species using mitochondrial and nuclear DNA markers

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Aquatic ecosystems face drastic biodiversity declines. However, most DNA-based bioassessments focus on species detection, ignoring the intraspecific genetic diversity, which can inform about species' evolutionary history and population structure. To move beyond species detection and towards eco-evolutionary insights, we investigated the population genetic structure of the freshwater amphipod *Gammarus fossarum*, presenting a hyper-diverse species complex. Here, we studied the population genetic structure of *G. fossarum* clade 11 in the Kinzig catchment (Rhine-Main-Observatory, Long-term Ecological Research site) in Hesse, Germany, using two different molecular methods: sequencing of the mitochondrial cytochrome c oxidase subunit I (COI) gene and generating genome-wide single nucleotide polymorphism (SNP) data using double digest restriction-site associated DNA sequencing (ddRADseq). With COI data, we found a high genetic diversity of 12 haplotypes within 364 specimens from 15 sites and a prominent small-scale isolation pattern with endemic haplotypes in the headwater regions. While this could have been only a historical pattern, high-resolution nuclear SNP data from 300 of the specimens confirmed the strong isolation of the populations, revealing 13 isolated genomic clusters along the 15 sampling sites. These results show that headwater populations of *G. fossarum* can be strongly isolated despite their generally high abundance and observed mobility in many streams.

Understanding the drivers of this fragmentation and assessing the eco-evolutionary as well as the conservation genetic consequences requires population genetic diversity data. Thus, our study highlights the importance of omics-based data for monitoring freshwater communities and identifying conservation priorities.

O075. Hybrid horizons: detecting hybridization in natural populations using a novel eDNA toolkit

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Hybridization plays a key role in the evolution of biodiversity by generating new species, altering genetic diversity, and, in some cases, threatening species integrity. Effective monitoring of hybridization is essential for understanding evolutionary dynamics and mitigating species loss due to human-mediated hybridization. Traditional methods rely on sampling numerous individuals, which is costly, time-consuming, and challenging for elusive or rare species. Environmental DNA (eDNA) offers a promising alternative for studying hybridization but requires the detection of nuclear eDNA, which is scarcer and more prone to degradation than the widely used mitochondrial eDNA. While a few recent studies have successfully recovered sufficient nuclear eDNA to assess intraspecific variation, the use of eDNA for hybridization monitoring remains unexplored. Our research group is developing an eDNA-based toolkit to detect hybridization, overcoming the limitations of conventional methods. We tested our toolkit in mesocosm experiments using pure individuals of *Triturus ivanbureschi* and *T. macedonicus*, along with F1 hybrids produced through captive breeding. Additionally, we conducted a field trial by sampling five natural populations within and outside the hybrid zone between these species in Serbia. Toolkit performance was validated by comparing genotypes obtained from eDNA samples with those from direct individual genotyping. In mesocosm samples, alleles inferred from eDNA were highly correlated with those from genotyped individuals. However, in field samples, only *T. ivanbureschi* alleles were detected, while *T. macedonicus* alleles were absent. Our method has the potential to provide an efficient, non-invasive approach for monitoring hybridization events on biodiversity.

O076. Repeated river–lake introgression in the adaptive radiation of Sailfin silverside fishes in Lake Matano, Sulawesi

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Adaptive radiations significantly contribute to the world's biodiversity, yet much is still unknown about the genetic and ecological factors underlying these rapid successions of speciation. It has been suggested that hybridisation can facilitate the rapid speciation process by producing genetic diversity on which diversifying selection can act. Hybridisation has been shown at the root of adaptive radiations, for example in the Malawi cichlid radiation. Yet comprehensive systems, in which the effects of genetic exchange can be studied in all members of the radiation, are still rare. In lake Matano, Sulawesi (Indonesia), one of the oldest and deepest lakes in the world, Sailfin silverside fishes (Telmatherina) form an adaptive radiation of ~10 species, each with distinct behavior, habitat, and diet. We used whole genome sequences to reconstruct the phylogenomic relationships and infer past and ongoing introgression within this adaptive radiation. While genome wide tests confirmed the two known monophyletic clades, sharpfins and roundfins, we found mismatches between morphology-based taxonomic assignments and genome-wide genetic relationships. In addition, we found signs of both old and ongoing introgression between river-dwelling *Telmatherina bonti* and the lacustrine sharpfin group, as shown in elevated D-statistic, f4-ratio and f-branch statistics. Levels of excess allele sharing declined with increasing distance from the river inlet, indicating ongoing introgression at the lake-river interface. This ongoing introgression, together with admixture between members of the lacustrine groups forms a solid basis for further studies on the effects of genetic exchange on speciation in an adaptive radiation.

O077. Technology Readiness Level of biodiversity monitoring with molecular methods – where are we on the road to routine implementation?

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Human activities are causing rapid biodiversity loss across ecosystems, affecting human wellbeing and crucial ecosystem services. Traditional biodiversity monitoring tools cannot keep up with the increasing demands of monitoring due to their limited spatial or temporal coverage, high costs, and lack of taxonomic expertise. Thus, implementation of novel molecular monitoring methods such as environmental DNA (eDNA) and DNA metabarcoding, are necessary. We used the Technology Readiness Level (TRL) framework to evaluate the maturity of molecular monitoring methods, providing a structured assessment of their

readiness for routine use. In a systematic literature review 420 articles fulfilling the study criteria were assessed and both individual studies and method categories ranked according to the TRL scale. The findings revealed a growing number of studies, particularly in aquatic environments, with most studies validating molecular technologies on a small scale but lacking large-scale system demonstrations. Aquatic eDNA-based methods targeting fish showed overall higher technology readiness compared to other sample types and taxa, and applications of molecular monitoring methods ranked into the highest TRL were predominantly freshwater studies. Key barriers to the broader implementation of molecular methods to monitoring include the need for international standards, better quantitative estimates, and comprehensive reference libraries. National and international cooperation is crucial for establishing common standards, ensuring reliable and comparable results, and expediting the routine use of molecular methods in biodiversity monitoring. Recent efforts towards international standardization are encouraging, but further coordinated actions are necessary for the global implementation and acceptance of these methods.

O078. Preliminary results from the European Marine Omics Biodiversity Observation Network (EMO BON): long-term genomic monitoring and FAIR stakeholder reporting

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There are many individual biological observation stations in Europe, however there are few and inconsistent links between them. The European Marine Omics Biodiversity Observation Network (EMO BON) is an ESFRI (European Strategy Forum on Research Infrastructures) initiative, coordinated by the European Marine Biological Resource Centre-European Research Infrastructure Consortium (EMBRC-ERIC) to unite marine stations under one centrally organised observation network that uses shared protocols, international standards and agreed policies. EMO BON is employing omics methodologies for accurate biodiversity monitoring and reporting; it aims to establish a coordinated, long-term, marine biodiversity observation network. It was launched in 2021, and it currently includes 17 marine stations, in 9 countries, ranging from the Arctic to the Red Sea, which regularly collect samples from three different habitats (water column, soft substrates, and hard substrates) and three different communities (microbes, meiofauna and macrofauna). EMO BON generates high-quality FAIR genomic biodiversity data that are being made periodically available to all interested parties (e.g., researchers, managers, policy makers, industries etc.) and thereby support constructive dialogue towards a holistic understanding of our ocean. EMO BON is an OBON (Ocean Biomolecular Observing Network) endorsed project and thus is it one of the UN Ocean Decade Actions. EMO BON has become the European contribution to the global marine biodiversity observation efforts and plans to collaborate and integrate further with other global entities. Preliminary results, based on 4 TB of data from 700 samples, will be presented as example case studies of the added value of including genomic data into conventional monitoring schemes.

O079. Taxonomy-free approach to diatom indicators based on three eukaryotic markers

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Diatoms are among the biological indicators used for quality and health assessment of water ecosystems. Unfortunately, traditional microscopic analysis poses challenges due to misidentifications, incurring in economic losses. DNA metabarcoding offers a promising alternative, enabling efficient, rapid, and objective characterisation of numerous samples to assess the water quality of rivers and lakes. However, the potential of metabarcoding for biomonitoring remains unfulfilled because a significant part of the sampled DNA cannot be assigned to species due to incomplete reference databases. We propose a taxonomy-free diatom-based approach to assess ecological status as an efficient strategy to retrieve biological and ecological information carried by the metabarcoding-detected sequences, bypassing the gaps in reference databases. Diatom metabarcoding employs various markers,

including 18S, rbcL, ITS, and COI. Among these, rbcL is commonly used in freshwater diatoms, while 18S is typically used in marine samples. COI is a promising marker for diatom identification, offering higher resolution than rbcL, having the ability to detect cryptic species and intra-species diversity. Our approach uses a taxonomy-free, Machine Learning workflow, based on Random Forest classifiers, to identify which sequences from multiple metabarcoding markers (COI, 18S, and rbcL) are ecologically informative. This workflow facilitates the characterisation of indicative values not only for diatom operational taxonomic units but also for other ecologically informative microeukaryotes. It enables the reconstruction of their co-occurrence networks and expands the utility of diatom indicators across diverse ecosystems, from peat bogs and littoral seawater to marine sediments.

O080. A novel framework for phytoplankton biomonitoring: Trait assignment of 23S rRNA sequences

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Phytoplankton is a key biological group used to assess the ecological status of lakes in several legislative water management plans. Two cutting-edge approaches for community characterization are DNA metabarcoding and trait-based analyses. While the former provides a fast, cost-effective and high-throughput methodology for identifying communities, the latter reveals the structure of communities through bio-ecological traits. The main aim of this study was to combine these approaches to directly assign traits to amplicon sequence variants. To achieve this, we used the newly developed Phytool v3 reference database. Using a in silico tests, we assessed the efficiency and reliability of our approach. We found that (i) a greater number of sequences with better reliability can be assigned to traits than to genus or species level and that (ii) traits are conserved in the phylogeny with varying extent. Then, we tested the usefulness of direct trait assignment on environmental samples from lakes. The test showed a greater number of successfully assigned sequences and a good ecological interpretation of community structures in the different environments. Furthermore, we identified three factors (completeness of the reference library, sequence similarity and the number of neighbours in the reference database) which, depending on the trait under consideration, interfere with the assignment success of our approach. While DNA metabarcoding data can be exploited in many ways depending on the objectives, our study showed that an innovative framework based on direct trait assignment of sequences could overcome gaps in reference databases and further improve our knowledge of phytoplankton community structure.

O081. Environmental DNA approach to assess phytoplankton communities in lake environments

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Aquatic ecosystems play an important role in the well-being of human populations through ecosystem services. However, the development of human activities increases the

eutrophication of lakes and rivers having a negative impact on human health. Thus, maintaining clean ecosystems without altering their biodiversity is one of the main issues nowadays. The Water Framework Directive (WFD) require the monitoring of ecological quality in order to assess the level of pollution in lakes following anthropogenic pressures. The assessment of ecological status is based on the use of biological groups playing key roles in the functioning of aquatic ecosystems, and phytoplankton is one of them. Classically, the bioindicators built with phytoplankton are based on microscopic count data. However, cells with small size (e.g. picophytoplankton), cryptic diversity and rare taxa are underestimated with this approach. On the other hand, eDNA approach can overcome these limitations, and for example, an indicator based on the estimation of ecological profiles of diatoms identified using this approach, has already been successfully used to assess the ecological status of rivers. So, in this study, we aim to identify phytoplankton in order to develop a method and tools to analyse ecological status of lakes by testing the same type of biological indicator on lake phytoplankton communities. This was made possible by the use of a dataset from around 100 waterbodies across France. Phytoplankton communities were characterized using an eDNA approach (metabarcoding based on 23S rRNA). Analyses were carried out without taxonomic assignment (i.e. taxonomy-free approach). The using of a taxonomy-free approach was explained by the lack of data in reference libraries reducing the percentage of assignment to precise taxonomic levels. The indicator obtained by taxonomy-free approach enables to significantly predict the environmental gradient (based on physico-chemical variables), and could be an alternative to traditional microscopic methods.

O082. Exploring the Potential of eDNA-Metabarcoding as an Alternative to Conventional Fish Monitoring under the Water Framework Directive

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Fish are a key biological quality element for assessing ecological health of waterbodies under the Water Framework Directive (WFD). Conventional methods like electrofishing and seine fishing are used to calculate Ecological Quality Ratios (EQRs) based on standardized protocols. eDNA-metabarcoding offers a promising alternative with higher sensitivity, lower costs, and less invasive sampling. A comprehensive project in the Netherlands, in collaboration with STOWA and waterboards, evaluated eDNA-metabarcoding as a substitute for traditional WFD fish monitoring. From 2016 to 2020, 81 waterbodies—including streams, rivers, lakes, canals, and brackish waters—were sampled using both methods. Sampling strategies were developed. Mixed water samples from three transects provided accurate results at reduced costs. Additionally, eDNA data were also incorporated into WFD indices, producing comparable EQRs for streams and linear waters, though weaker correlations were found in lakes. A large follow-up project, funded by the Dutch Ministry of Infrastructure and Water Management (I&W) is now underway and aims to expand the eDNA dataset by sampling additional waterbodies in the Netherlands and reference sites across Europe, generating a robust dataset from which eDNA-based indices can be developed. The project will compare methodologies across labs to establish a standardized protocol, ensuring reproducibility and building lab capacity for widespread implementation. This presentation will discuss the advantages and limitations of eDNA, and outline steps for integrating eDNA-metabarcoding into standardized WFD monitoring.

O083. The Fellowship of the Ring Test confronting a method to rule them all: testing the transferability and comparability of diatom DNA metabarcoding protocols for biomonitoring

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DNA metabarcoding of benthic diatoms has become an effective tool for large-scale biomonitoring within aquatic monitoring networks across multiple countries, reaching a level of technical readiness for routine use. However, differences in protocols across laboratories, both within and between countries, limit the comparability and operational transferability of results. To address these challenges, a collaborative study was conducted, focusing on DNA extraction and PCR amplification steps to evaluate the transferability and reproducibility of a standardized protocol across laboratories and the variability introduced by differing protocols in the scientific community. Guided by a reference laboratory (CARRTEL, INRAe), 17 participants from 14 countries performed DNA extraction and PCR amplification in parallel, using both a fixed protocol and their own lab-specific protocols. The experiments were carried out on two biofilm samples (river, and lake), and a mock community, to specifically assess the impacts of variability in these steps. Results revealed a high level of consistency in ecological assessment, demonstrating that a reference protocol could be reliably transferred across

laboratories. Furthermore, despite variations in the lab-specific protocols, identical ecological assessments were achieved across participants, supporting an "all for one, but prove them all" strategy. This approach suggests that while distinct protocols may be operationally feasible, maintaining minimum standard requirements controlled through accreditations is an essential way to ensure consistency and reliability in diatom DNA metabarcoding for routine biomonitoring. This approach provides an alternative to the “one protocol to rule them all” strategy, preventing the danger of technological “lock-in” where a dependence regarding specific equipment, consumables, and reagents can appear and settle down over time.

O084. Optimizing MSFD pelagic habitat indicator workflows to accommodate genetic monitoring data

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The Marine Strategic Framework Directive was implemented in 2008 by the European Union to achieve good environmental status (GES) for EU marine ecosystems. The directive defined 11 qualitative descriptors to address the concept of GES and thereby protect marine ecosystem biodiversity and health as well as the ecosystem services and the socio-economic activities they provide. Under the MSFD – Descriptor 1 – Criteria 6, pelagic habitat biodiversity indicators were developed by OSPAR. Since their initial use in the Intermediate Assessment 2017 they have been further developed and clearly defined as three indicators; PH1 – plankton lifeform index ratios, PH2 – plankton biomass and/or abundance, PH3 – plankton diversity indices. Currently the pelagic biodiversity indicator calculations are primarily based on conventional plankton monitoring datasets (CPR, satellite, and image data). Within the Marco-Bolo project (funded under the Horizon Europe Programme), we aim to optimize the indicator workflows to also account for genetic monitoring data. Different long-term genetic datasets will be tested and compared to either traditional observations or AI processed imaging data. Additionally, a Data Analysis Challenge was held to assess how varying bioinformatic pipelines might impact the outputs of the pelagic habitat biodiversity indicators.

O085. The carbon footprint of diatom molecular research

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Carbon footprints (CF) are usually drawn up on the scale of states, sectoral activities (e.g. agriculture) or institutions. However, in order to reduce greenhouse gas (GHG) emissions, it is interesting to investigate finer scales. And to fully understand all the issues related to the adoption of new technologies, their environmental impact is an element that needs to be considered. In this exploratory study, we are looking at the CF of diatom metabarcoding, on the one hand at the scale of research projects that have used this technology, and on the other hand at the scale of the data acquisition process leading to taxonomic inventories. However, there are no freely available tools for carrying out CF at these scales. We therefore adapted the tools provided by ADEME (The French Agency for Ecological Transition) and the French research initiative Labo1point5. On the one hand, the CFs of a panel of 29 research projects focusing mainly on tropical aquatic ecosystems, some of which using molecular methods, were produced. These CFs were used to establish a typology of projects, based on the contribution of different GHG emission items. In addition, the CFs associated to the diatom metabarcoding process were produced for 3 structures. They were compared to the CFs from the conventional method of acquiring diatom taxonomic data, using microscopy. Compared to microscopy, metabarcoding involves a large number of stages and a wide variety of operating procedures. Possible solutions to limit GHG emissions are suggested, and the main aim of this presentation is to open up a discussion on the subject, with a view to developing practices that are as carbon-free as possible, in anticipation to the routine and large-scale deployment of molecular methods. The presentation is accompanied by a poster giving the tools needed for each participant in the AquaEcOmics meeting to carry out the CF of their own research projects and metabarcoding processes.

O086. Local accumulation of organic matter in marine sand extraction areas drives changes in sediment prokaryotic communities with potential consequences for nitrogen cycling

Martijn Callens (ILVO, Belgium), Nanou Goedefroo (ILVO, Belgium), Annelies De Backer (ILVO, Belgium), Sofie Derycke (ILVO, Belgium)

Sand is the second most exploited resource in the world and is extracted in high volumes from coastal environments for construction, land reclamation and flood protection. Although the impact of this activity on larger benthic animals has been well-studied, its impact on sediment prokaryote communities – essential for many ecosystem services – is unknown. We assessed the impact of sand extraction on the abundance, taxonomic-, and functional composition of sediment prokaryotic communities in the North Sea. Using a combination of shotgun metagenomic sequencing and digital PCR, we analyzed one-centimeter depth slices from the top six centimeters of the sediment in a sand extraction and non-extracted reference zone. By using quantitative microbiome profiling through anchoring relative abundances to digital PCR measurements, we were able to assess compositional effects and gained better insight

in ecological responses of the prokaryote community to sand extraction. Sand extraction significantly affected the taxonomic composition and relative abundance of functional genes in sediment prokaryote communities. In-depth analysis of genes involved in nitrogen metabolism pathways indicated that fixation, recycling, and retention of nitrogen in the sediment might be elevated in the sand extraction zone. Prokaryote community features showed a higher variability between stations located in the sand extraction zone compared to those located in the reference zone. A large fraction of this variability was explained by the accumulation of fresh organic matter, which was the main driver of the observed effects. The significant effect of sand extraction on sediment prokaryote communities, and the implications for ecosystem functioning, show that the inclusion of prokaryote communities in environmental monitoring allows for a more complete picture on how human activities are affecting marine ecosystems.

O087. Impact of 10 years of deforestation and population growth on biodiversity seen through the lens of metabarcoding

Anthony Foucher (LSCE-IPSL Univ. Paris-Saclay, France), Olivier Evrard (LSCE-IPSL Univ. Paris-Saclay, France), Jonathan Marescaux (E-BIOM, Belgique), **Valentin Ambroise** (E-BIOM, Belgique)

Mayotte is a small tropical island situated between Madagascar and Mozambique. Being an overseas department of France, it benefits from a high GDP per capita for the region, making it a major destination for immigration. As such, in only 10 years, its population has soared (+ 80% population between 2002 and 2021), leading to a staggering increase in deforestation rate and an intensification of agricultural practices. These changes of land use significantly impacted biodiversity, increasing connectivity and driving changes in ecological communities. The Dzoumogné reservoir, one of the two reservoirs supplying drinking water to the island has been a witness of this increased pressure on biodiversity. Indeed, up to 2012, its catchment was largely covered by natural vegetation and managed with traditional extensive agriculture. However, by 2022, the forest cover as well as the traditional agriculture had significantly decreased at the expense of monoculture. This study used sedimentary DNA (sedDNA) as an innovative approach to monitor the ecological changes in this region during the last 10 years. A sediment core was taken from the Dzoumogné reservoir and dated using X-ray fluorescence analysis. 12 subsamples dating between 2012 and 2021 were chosen for sedDNA analysis. sedDNA showed an increase in some species directly related to intensive farming and agriculture, such as cattle and banana, while species associated with traditional agriculture decreased. sedDNA also revealed the appearance of some animal and plant species in the sedimentary records (such as Lemurs) resulting from an increased fragmentation of the bordering forest. In addition, some recent water crisis event also left some visible traces on the algal community structure. In conclusion, this study highlights the usefulness of sedDNA to monitor changes in biodiversity and landscape connectivity on a recent scale.

O088. The power, challenges and integration of Omics-based ecological insights as a cornerstone for invasive species management in aquatic environments

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The integration of omics methodologies into invasive species surveillance programs offers significant potential for enhancing the management of aquatic ecosystems. Omics techniques deliver crucial insights into the presence, abundance, and ecological impact of invasive species, yet their adoption by decision-makers and ecosystem managers remains limited. This presentation seeks to bridge this gap by addressing key topics relevant to both invasive species managers and omics practitioners. We will demonstrate how omics methodologies can be integrated to improve the detection, quantification, and management of invasive species, thereby supporting conservation and eradication efforts. Key points include: i) strategies to enhance the spatial and temporal resolution, as well as the robustness, of eDNA detection, ii) methods to refine abundance and population size estimates using eDNA data, and iii) insights on using eDNA data to assess the impact of invasive species on native communities, integrating these with remote sensing and environmental data to improve sensitivity and suitability modelling. These concepts will be illustrated with numerous innovating examples from our eDNA research on invasive species in freshwater ecosystems. Our ultimate goal is to empower invasive species managers with the information needed to make informed decisions on whether, when, and how to implement omics-based methods in their surveillance and eradication initiatives. By understanding the strengths, challenges, and limitations of these methods, managers can more effectively leverage them to combat invasive species and safeguard native ecosystems.

O089. Omics in the service of characterising the eukaryotic plankton community in the Adriatic Sea

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As the northernmost part of the Mediterranean Sea and an extremely dynamic marine ecosystem with contrasting environmental characteristics from north to south and striking coastal influences on the east and west coasts, the Adriatic Sea represents a challenge and a pleasure for the study of plankton diversity and ecology. Throughout the long history of plankton research and monitoring in the Adriatic, data collection and analysis have been based primarily on traditional methods of taxa identification such as light microscopy. Omics methods are still rarely used in plankton research in this area and are not yet standardised for routine monitoring of the plankton community. Here we present an integrative approach to plankton community research based on metabarcoding and metatranscriptomics in the

Adriatic Sea, which has been conducted since 2020. The high frequency of sampling (annual monthly sampling) and the diversity of sampling stations covered (coastal and open sea) allowed a detailed and reliable characterisation of the Adriatic plankton. The broad taxonomic spectrum from protists to metazoans and the high diversity and richness in each of the recorded groups allowed an extremely valuable characterisation of the overall Adriatic eukaryotic plankton community. At the same time, metatranscriptomics provided information on physiological activity, highlighting the ecological behaviour of the community in a dynamic marine ecosystem such as the Adriatic Sea. The results obtained serve as a valuable dataset for the multi-layered characterisation of complex plankton community interactions and ecological networking as well as a basis for the standardisation of omics methodology in the Adriatic Sea.

O090. Under-ice methanotrophy may offset Baltic sea ice methane fluxes

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Sea ice is an active component of Earth's climate and oceanic systems, e.g. altering the energy flow between the atmosphere and sea as well as limiting gas exchange. Recent evidence suggest that Baltic Sea ice could serve as a temporary storage for greenhouse gases (GHGs) such as methane and N_2O. Sea-ice brine is a habitat for diverse microscopic organisms including archaea, viruses, protists and bacteria. These bacteria decompose particulate organic matter, remineralize nutrients and convey carbon to the upper trophic levels through microbial loop. However, their specific metabolic functions still are still largely unknown. The aim of this study was to investigate the microbial community composition and activity using metagenomics and metatranscriptomics with special emphasis on detecting potential methanotrophic bacteria from sea ice and under-ice water. The results show that under-ice water had pmoA transcripts pointing to active methanotrophy in the under-ice water. Our results suggest that sea ice may hinder the methane flux from seawater to atmosphere if the methane trapped under semi-permeable ice is consumed by methanotrophic bacteria.

O091. Understanding emerging ecosystems: tracing biodiversity and ecological change in a periglacial Arctic lagoon using eDNA

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The Arctic, known as a sentinel for global environmental changes, is undergoing rapid transformation due to climate change, with glaciers retreating and unveiling new ecosystems. These emergent landscapes present a unique opportunity to study ecosystem formation and dynamics in a region highly sensitive to environmental shifts. This study focuses on a periglacial Arctic lagoon in Eidembukta Bay, Svalbard, which has transformed from a glacier-dominated coastline to a distinct lagoon habitat over the past eight decades. As the glacier receded, this previously ice-covered region became a biologically diverse system, featuring charismatic apex species like polar bears and harbor seals. However, much remains unknown about the biodiversity patterns at lower trophic levels and the ecological interactions shaped by the lagoon's complex hydrological gradients. To address these gaps, we employed environmental DNA (eDNA) metabarcoding—a cutting-edge technique that enables comprehensive biodiversity assessment from environmental samples such as water and sediment. This non-invasive method allows us to examine both prokaryotic and eukaryotic diversity and analyze ecosystem structures across different trophic levels. We aimed to elucidate the lagoon's ecological zonation and functional dynamics, testing key hypotheses regarding species accumulation, biodiversity gradients, and functional structures across varying hydrological and sedimentary conditions. Our findings provide critical insights into how emerging Arctic ecosystems develop and function. By leveraging eDNA metabarcoding, this study advances our ability to monitor remote and rapidly changing environments, offering new perspectives on the biodiversity and ecological dynamics of post-glacial habitats.

O092. Using multi-omics to illuminate responses of sediment microbial communities to hydrological changes in lotic systems and their consequences on carbon cycling

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Sediment microbiome play a major role in regulating carbon fluxes in aquatic ecosystems. Therefore, understanding how climate change affects the structure and functioning of microbial communities is central to anticipate the functional trajectory of hydrosystems, and their capacity to act as carbon source or sink. In large rivers, such as the Rhône, climate projections predict both an increase of water temperature and a reduction of the water flow, especially in the river side arms, more or less connected to the main channel. The present work aims at investigating responses of sediment microbial community to hydrological changes and their consequences on carbon cycling. For this, we first used a metabarcoding approach to characterize the temporal variation in prokaryote communities (Bacteria and Archaea) in 13 side-arms of the Rhône River characterized by a gradient of hydrological connectivity. Results showed that GHG emissions were more important in the less connected habitats. In the same vein, microbial communities exhibited contrasted taxonomic structure contrasted among hydrological regimes, and were related with environmental parameters shifts and greenhouse gas (GHG) fluxes measured in situ. In addition, preliminary metagenomics and metatranscriptomics analyses were conducted on a small subset of samples to explore how microbial pathways were affected by changes in hydrological connectivity. Metagenomics data showed that the pool of genes present in sediment were

poorly influenced by the hydrology, with no major difference observed for genes involved in carbon and methane cycling. Contrarily, metatranscriptomics data showed different active microorganisms and contrasted expression patterns for carbon and methane pathways among hydrological regimes. Archaea were more active and methanogenesis pathway was more expressed in the most disconnected sites, but we also observed differences in the expression of carbon-related microbial pathways among fully connected side-arms with low GHG emissions. Overall, this work showed the potential of multi-omics approaches to decipher the taxonomic and functional responses of sediment microbial communities to climate change, and highlight the mechanistic basis underlying the alteration of biogeochemical processes they support, but also the challenges for their wider applications.

O093. Uncovering Effects of Environmental Change through eDNA Metabarcoding: A Long-Term Perspective

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Biodiversity monitoring has become crucial for understanding and predicting how ecological systems are affected by ongoing environmental change. Freshwater ecosystems are particularly well-suited for environmental DNA (eDNA) monitoring, as many of the organisms are small, making traditional species detection methods time-consuming and labor-intensive. Despite their size, these organisms play a vital role in maintaining ecosystem health. Phytoplankton are important primary producers and essential for assessing freshwater ecosystem dynamics. We present insights from a long-term monitoring dataset collected from Lake Müggelsee in Berlin, Germany. Since 1979, this lake has been extensively monitored for both abiotic and biotic parameters. For the last decade, traditional species identification has been complemented with eDNA monitoring, allowing for a comprehensive view of ecosystem changes. This combined dataset provides a unique opportunity to examine the suitability of eDNA to study the effects of environmental change over an extended period. Our findings revealed a rise in average water temperature over the past decade, consistent with broader climate trends. To date, the analysis of phytoplankton species richness through eDNA has revealed 334 species and a decline in richness over time, while traditional morphological identification indicated an increase in species richness. We hypothesize that eDNA detects more rare species that traditional methods may overlook. These results highlight the value of integrating multiple methods, such as eDNA and traditional morphological approaches, to capture complementary insights into species composition and ecological trends.

O094. Predicting cyanotoxins concentrations in lakes and reservoirs using microbial community information

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Climate change and human activities are increasing the pressure on our water resources, challenging the preservation of water quality. Cyanobacteria are naturally present at low levels in water bodies. But due to increased eutrophication (nutrient input from agricultural or municipal releases) and climate change, some cyanobacteria bloom in aquatic ecosystems. These blooms are a considerable threat as cyanobacteria can potentially develop and release toxins which are harmful to human and animal health. Unfortunately, the intensity and frequency of these blooms are increasing likely due to human activities and are already generating economic and human health impacts with high societal costs. Monitoring, predicting and preventing blooms is therefore a priority, but also presents a significant challenge. Blooms are composed of a high diversity of cyanobacteria with different niches preferences and dynamics. Here, we hypothesized that blooms could be viewed as a biological disturbance, measurable by their impact on the surrounding microbial community. This disturbance could take different form – from taxa composition to genes frequency - and therefore could be used as toxic bloom biomarkers. To identify these early warning signals of a toxic bloom, we used data generated from the Genome Canada-funded ATRAPP project, focusing on eight lakes, for a total of 834 metagenomes. Our first set of analyses, Joint Species Distribution Modeling, aimed at improving our understanding of how toxins respond to environmental variables. We found that seasonality was a key variable in explaining the presence-absence and continued rise in cyanotoxins, followed by ammonia and total phosphorus concentrations. To further examine toxins seasonal succession and evaluate toxins co-occurrence, we ran a Latent Dirichlet Allocation. We found that certain toxins appeared earlier in the season in many lakes (microcystin-LR and –LY), and were replaced by a different community of toxins by summer (microcystin-LA, anabaenopeptins). To finally predict total microcystin concentrations and the time until toxicity reached the WHO guideline of >1 ug/L, we used of Bayesian Additive Regression Trees with microbial data as predictive variables. All observed species of cyanobacteria (Spearman correlation of observed vs. predicted values = 0.84) or the top 500 non-cyanobacterial species (correlation = 0.83) showed similar results. Predictions of the time until the WHO guideline was reached were more uncertain (correlation = 0.7 using cyanobacterial taxa or 0.67 using non-cyanobacteria), but still significant.

O095. Multiomics approach to characterize the link between the structural biodiversity and the microbial activity along the natural dynamic of freshwater periphytic biofilms and its response to the chemical stress : Main Outcomes from the MICROBIOMIQ projectt

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Facing global changes, a major challenge is to understand the response of environmental microbiomes to multiple stressors in order to preserve ecosystem functions and services. In particular, as regard chemical contamination of aquatic systems, one critical need is to characterize how the fluctuation of microbial community functioning and structure due to environmental changes might modulate their sensitivity to micropollutants. To address these challenges, the combination of metabolomics and metagenomics is particularly relevant as it allows for the assessment of microbial activity, and taxonomic and genic diversity, respectively. In this context, MICROBIOMIQ aimed (i) to characterize the synchronicity between the natural dynamic of microbial activity (meta-metabolome) and structural biodiversity (species and genes) over a course of a year; (ii) to highlight the impact of such changes on the sensitivity of the meta-metabolome and the photosynthesis to a model herbicide. To do so, biofilms were colonized monthly on glass slides in a pilot pond. After quenching and freeze-drying, samples were split for both omics. In parallel, at each month, a part of the collected biofilm was exposed during 4h to serial dilution of terbuthylazine (TBA) to assess the community sensitivity to this herbicide. Our results revealed asynchronistic and uncorrelated shift of meta-metabolome and microbial composition for both richness and evenness suggesting different assembling mechanism. Then, ordination and PERMANOVA showed significant phylogenetic and meta-metabolome discrepancies between the months, further highlighting strong influence of the temperature in microbial composition. DIABLO analysis confirmed such influence whereas the meta-metabolome assemblage was driven by other factors (e.g. NO_3). These fluctuations likely influenced the change of sensitivity to TBA mainly noted on the metametabolome. Further investigations are ongoing to elucidate the contribution of internal (metabolism) vs external factors (water properties) in this shift likely related to the co-occurrence of microbes and metabolites with peculiar climatic conditions. Altogether, our results will support better understanding of potential functional impairment of microbiomes by the chemical stress in the global changes context.

O096. Occurrence of antibiotic-resistant bacteria in household plumbing system

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The presence of antibiotics (Ab) and antibiotic-resistant bacteria in household water is a growing public health concern with significant implications for a wide range of stakeholders. Ampicillin is a widely used antibiotic in healthcare, pharmaceutical, and agricultural sectors. These industries, as key stakeholders in antibiotic management, play a critical role in the proper disposal of antibiotic residues. However, inadequate disposal practices lead to the release of ampicillin residues into the environment, which contributes to the development and spread of ampicillin-resistant bacteria. This environmental contamination promotes antibiotic resistance, posing a significant threat to public health and ecosystems, as resistant bacteria can thrive and spread more easily. This study investigated the occurrence of various opportunistic pathogens (OPs) resistant to ampicillin in the drinking water systems from

household pipelines. We isolated 42 ampicillin-resistant bacteria from water samples (collected from kitchen taps, bathroom taps and showerheads) from 30 houses, Birmingham, UK. Isolated bacteria were characterized by acid-fast and Gram staining and identified by 16S rRNA gene sequencing to quantify the microbial load in water samples from three sites at 30 houses. All 42 bacteria were negative in acid-fast staining, and all were Gram-negative, with one exception. Blast search of 16S rRNA sequences revealed that ten isolates belong to *Cupriavidus*, six to *Delftia*, five to *Pseudomonas*, four to *Bosea*, two each to *Xenophilus*, *Microbacterium*, *Sphingomonas*, *Methylorubrum*, *Methylobacterium* and *Microbacterium*, and one each to *Burkholderiaceae*, *Acidovorax*, *Paracoccus*, *Caulobacter* and *Arvibacter*. *Cupriavidus*, *Delftia* and *Pseudomonas* were found in all household samples as most common genera. The microbial load was high in showerhead compared to other two sites. Based on our knowledge, this is the first report introducing *Arvibacter aurantiibacter* and *Methylorubrum extorquens* as ampicillin resistant bacteria from household water systems. Overall, our results demonstrate that the drinking water from households in Birmingham contains various bacteria resistant to ampicillin and provides valuable information for a better understanding of microbial load and antimicrobial resistance profiles in household plumbing systems. These findings can provide insights into assessing different factors like improving wastewater treatment and considering the pipeline materials in household plumbing systems. Additionally, public awareness and various stakeholders' collaboration can be aligned with policies by governments to reduce antibiotic use to address public health issues more effectively.

O097. Metagenomic insights into the functional microbial diversity of the lower stretch of the River Ganga: mapping antibiotic and metal resistance genes

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The banks of the lower stretch of River Ganga are home to several towns and megapolis representing high density of human population. A stretch of 50 km represented by prefixed stations of the lower part of Ganga (Ganga Environmental Time Series- GETS) was monitored spatiotemporally using eDNA metagenome-based Nanopore sequencing to elucidate structure of microbial communities along with mapping of antibiotic-resistant genes (ARGs), metal resistance genes (MRGs) and mobile genetic elements (MGEs). Besides, in situ environmental parameters, concentration of dissolved nutrients, metals and metalloids were measured. The concentration of dissolved oxygen ranged from 3.4–6.2 mg l^{-1}, indicating deteriorating water quality corresponding to high population density. Dissolved nitrate concentrations were higher in some stations reflecting direct release of untreated municipal sewage into the river. In particular, concentration of metals such as Cd (2.34–38.52 ppb) and metalloids such as As (0–218.7 ppb) were found to be alarmingly high in surface water. *Gammaproteobacteria* was encountered ubiquitously while rare bacterioplankton represented by eleven classes showed site specificity. Several genes belonging to ARGs were identified and multidrug resistance genes (MDR) were found in all the studied stations exhibiting high abundance. The 'hotspots' of ARGs were widespread, possibly owing to rampant usage of personal health care products that may have contributed to observed ARG abundances. High abundance of MRGs comprising arsenic (∼12%) and copper (∼12%) were

also identified showing strong correlations with the abundance of ARGs. Correlation and network analysis revealed the potential role of MGEs in the dissemination of ARGs. ANOSIM and cluster analyses supported patchy distribution patterns of microbial communities. The RDA plot showed possible influences of environmental parameters in shaping the microbial community structure and towards dissemination of functional genes such as ARGs and MRGs in the lower stretch of the River Ganga.

O098. Multi-compartment impact of micropollutants and particularly antibiotics on bacterial communities using environmental DNA at river basin-level

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Bacterial communities respond to environmental conditions with diverse structural and functional changes depending on their compartment (water, biofilm or sediment), type of environmental stress, and type of pollution to which they are exposed. In this study, we combined amplicon sequencing of bacterial 16S rRNA genes from water, biofilm, and sediment samples collected in the anthropogenically impacted River Aconcagua basin (Central Chile, South America), in order to evaluate whether micropollutants alter bacterial community structure and functioning based on the type and degree of chemical pollution. Furthermore, we evaluated the potential of bacterial communities from differently polluted sites to degrade contaminants. Our results show a lower diversity at sites impacted by agriculture and urban areas, featuring high loads of micropollution with pesticides, pharmaceuticals and personal care products as well as industrial chemicals. Nutrients, antibiotic stress, and micropollutant loads explain most of the variability in the sediment and biofilm bacterial community, showing site-specific increase of bacterial groups known for their capabilities to degrade various organic pollutants and also selecting for taxa known for antibiotic resistance. Moreover, potential ecological functions linked to the biodegradation of toxic chemicals at the basin level revealed significant reductions in ecosystem-related services in sites affected by agriculture and wastewater treatment plant (WWTP) discharges across all investigated environmental compartments. Finally, we suggest transitioning from simple concentration-based assessments of environmental pollution to more meaningful toxic pressure values in order to comprehensively evaluate the role of micropollutants at the ecological (biodiversity) level.

O099. A comprehensive pan-European exploration of fungal plastisphere dynamics along river-to-sea continuums

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Microplastics (MP) provide a persistent and buoyant substrate that can act as a raft for microorganisms, including prokaryotes and microeukaryotes. A significant concern regarding

the plastisphere is the potential dispersal of freshwater microorganisms, including invasive species and pathogens, into the sea. Here, we investigated the fungal plastisphere communities across river-to-sea continuums in nine major European rivers. These rivers were sampled over a seven-month period during the Tara Microplastics mission. Our research focused on examining the fungal communities associated with floating plastic debris, collected with manta nets, as they transitioned along steep salinity gradients from areas downstream and upstream of the first densely populated city to the sea. Additionally, we immersed pristine plastics (PriP) at each site for a month, in order to compare the mature biofilms on PriP with the plastisphere on floating MP along with the fungi living in the surrounding water at the same sites. Distinct niche partitioning was evident, with plastic-associated fungi clearly differing from those in surrounding waters. Additionally, significant variation in community composition across rivers suggests that each river harbors a unique microbial signature. Salinity also emerged as a key structuring factor, delineating a clear distinction between samples collected at low salinity and those from estuarine or marine environments. Finally, multidimensional analyses revealed that water samples form a distinct group, in sharp contrast to floating MP samples, while PriP immersed for one month are highly dispersed, overlapping with both floating MP and filtered water samples. This pattern suggests that a one-month colonization period allows fungal communities to establish themselves in line with the surrounding communities. The unknown history of floating MP, with likely varying water residence times, results in a patchy distribution, distinguishing them from seawater and PriP communities. This indicates the dynamic nature of fungal communities associated with floating MP and a potential lack of resilience. This is further confirmed by the significant differences between freshwater and marine communities, indicating drastic evolution along the land-sea continuum.

O100. Genome-wide responses to chronic Bti and copper exposure in a laboratory culture of Chironomus riparius

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Aquatic environments often experience persistent contamination from human activities, introducing pesticides, heavy metals, and organic pollutants that exert adaptive pressure on benthic organisms. Predicting species' responses to environmental challenges requires a detailed understanding of their adaptive capacities under chronic stress. Evolve and Resequencing (E&R) studies integrate experimental evolution with high-throughput omics approaches, advancing knowledge of the genetic mechanisms underlying adaptive processes. Here, we present findings from an E&R study on the model organism *Chironomus riparius*, exposed to two common stressors: the biocide *Bacillus thuringiensis israelensis* (Bti), widely applied to natural water bodies for mosquito control, and copper, a frequent agricultural contaminant introduced to aquatic systems due to fertilizer and pesticide runoff. Using whole genome sequencing of pre-exposed and naïve populations, we assessed whether *C. riparius* exhibited measurable genetic adaptation to chronic Bti and copper exposure after about eight generations. In addition, common garden experiments were conducted to determine fitness differences between pre-exposed and naïve populations under varied exposure scenarios. Results from common garden experiments indicated that responses of both pre-exposed and

naïve populations to the varied exposure scenarios were largely similar - a finding supported by population genomic analyses, which suggest limited adaptive potential in our *C. riparius* culture. These findings highlight the potential constraints on rapid adaptation under persistent chemical stress and underscore the need for understanding mechanistic limitations in adaptive responses. We discuss both the advantages and limitations of using E&R approaches to study adaptive responses to environmental stressors, with a particular focus on applications in ecotoxicology, emphasizing their relevance in ecological risk assessments and biodiversity conservation.

O101. Making the invisible visible: molecular methods to trace the success of the restoration of river Emscher

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The river Emscher, a tributary of the Rhine, was used as a channelized, open sewage for industrial wastewater for over a century. Even until recently, untreated wastewater from households was still discharged directly into the river. A generation program to restore the ecological state of the river was launched in the 1990's leading to substantial improvements to the Emscher. A key step in the restoration efforts was January 2022, when untreated sewage discharges were stopped and subsequently, in November 2022, a 5-m barrier blocking the mouth of the Emscher, was removed, promoting migration of species between both rivers. Conventional methods assessing restoration effects are often focused on a specific species or taxonomic group. However, changes in biodiversity due to stream restoration are diverse and can affect the entire tree of life and novel broad genetic methods might be better suited to investigate these changes. Environmental DNA (eDNA) analysis, which utilizes DNA from organisms, has proven a powerful tool for biodiversity analysis in freshwaters. We sampled the river Emscher monthly for two years starting from February 2022, directly after the sewage discharge in the Emscher stopped. Sampling was conducted at 18 sites in the Emscher catchment. At each location 1L of water was collected and filtered for eDNA. Diversity was assessed using two genetic markers, one designed to identify the fish community (12S), the other for the freshwater invertebrate community (COI). In this talk, we will present the first results obtained from this longitudinal time-series monitoring program using eDNA. In this analysis, we particularly focus on the time species (native and non-native) are reported for the first time in the Emscher, as well as total richness change, nestedness and turn-over. We will show the benefits of using eDNA metabarcoding as an additional tool to study biodiversity change in stream ecosytems and provide recommendations on the interpretation of the data in anthropogenically highly-impacted systems.

O102. From ice to heat: variation in phenotypic and transcriptomic response of Arctic Charr populations from contrasted environments

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Climate change poses significant challenges to biodiversity, particularly for cold-adapted species like Arctic charr (*Salvelinus alpinus*). This study explores the phenotypic plasticity and adaptive divergence of four Arctic charr populations from different lakes, analyzing their responses to rising temperatures through morphological traits and transcriptomic analysis. Building on previous research by Mari et al. (2019), the study involved fertilizing eggs from charr populations of Lake Geneva, Lake Constance, and Lake Pavin, then subjecting them to two temperature conditions (5°C and 8.5°C) in a common garden experiment. Results revealed population-specific responses, with Lakes Constance and Sainte-Croix exhibiting higher genetic diversity and broader adaptive capacities compared to Lake Geneva's reduced diversity. Transcriptomic analyses highlighted significant differences in gene expression, particularly in stress responses (e.g., HSPs, SGK-1), ionic regulation (NaKATPaseα1a), and oxygen transport (HBB4). QST-FST comparisons suggest strong selection pressures driving local adaptations, especially in Lakes Pavin and Sainte-Croix. These findings underscore the importance of genetic diversity and local adaptation in mitigating climate-induced stress. They also provide valuable insights into the evolutionary trajectories of Arctic charr populations, emphasizing the need for conservation strategies tailored to each population's unique genetic and ecological context.

O103. Environmental DNA-based profiling of benthic microbial communities along a crude oil spill gradient in a coral reef in the Persian Gulf

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Coral reef ecosystems in the Persian Gulf are frequently exposed to crude oil spills. We investigated benthic bacterial and eukaryote community structures at such coral reef sites subjected to different degrees of polycyclic aromatic hydrocarbon (PAH) pollution using eDNA metabarcoding. Both bacterial and eukaryote communities responded with pronounced shifts to crude oil pollution and distinguished control sites, moderately and heavily impacted sites with significant confidentiality. The observed community patterns were predominantly driven by Alphaproteobacteria and metazoans. Among these, we identified individual genera that were previously linked to oil spill stress, but also taxa, for which a link to hydrocarbon still remains to be established. Considering the lack of an early-warning system for the environmental status of coral reef ecosystems exposed to frequent crude-oil spills, our results encourage further research towards the development of an eDNA-based biomonitoring tool that exploits benthic bacterial and eukaryote communities as bioindicators.

O104. Aquatic biodiversity on Reunion Island: Responses of biological communities to environmental and anthropogenic pressures using environmental DNA

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Island ecosystems, characterized by isolation and vulnerability, are subject to natural and human-induced pressures. Rapid and effective biodiversity monitoring is crucial for tracking these impacts and adapting conservation efforts. This study focuses on Reunion Island (South-West Indian Ocean), where aquatic biodiversity is threatened by habitat loss, invasive species, and climate change. Stressors, both environmental and human-caused, can affect aquatic community structures. To test this hypothesis, a comprehensive dataset was compiled from

various aquatic habitats, including rivers, ponds, reefs, and coastal waters. Biodiversity data for bacteria, diatoms, invertebrates, and fish were collected using eDNA metabarcoding, while environmental and anthropogenic parameters were recorded through field measurements and local databases. Redundancy analysis was used to identify the spatial distribution patterns of aquatic communities and their variations in response to these parameters. Results showed a significant distinction between freshwater and marine communities, with rivers and ponds hosting fewer taxa than marine environments, reflecting unique ecological patterns. In freshwater systems, fish and invertebrate communities are significantly driven by conductivity, temperature, and metals such as arsenic and barium, while diatoms and bacteria are primarily influenced by oxygen levels, atrazine, and perfluorooctanesulfonate. In marine environments, community composition is primarily affected by turbidity and conductivity. This study demonstrated that eDNA methods are effective for routine monitoring of large taxonomic groups, enabling the detection of biodiversity changes related to water chemistry in watersheds. These approaches, commonly used on continents, are also effective in monitoring biodiversity on tropical islands threatened by human activities

O105. Navigating the seven challenges of taxonomic reference databases

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DNA metabarcoding has revolutionized biodiversity assessment by enabling the identification of multiple species from environmental samples. However, a critical bottleneck for accurate taxonomic identification in aquatic environments is the quality and completeness of reference databases. Taxonomic identification of organisms from their genetic material relies strongly on reference databases that link genetic sequences to taxonomic names. These databases vary in completeness and availability, depending on the taxonomic group studied and the genetic region targeted. The incompleteness of reference databases is an argument that is often cited by researchers and practitioners to explain the nondetection by metabarcoding of species supposedly present in the environment. However, there exist further and generally overlooked issues with reference databases that can lead to false or inaccurate inferences of taxonomic assignment. In this presentation, we will review and discuss the seven key challenges of using reference databases for taxonomic classification in metabarcoding projects, including mislabelling, sequencing errors, sequence conflict, taxonomic conflict, low taxonomic resolution, missing taxa and missing intraspecific variants. For each of these challenges, we will discuss the possible unwanted consequences and the solutions that can be implemented to limit them. Recognizing these challenges and implementing existing solutions should allow us to continue improving genetic reference databases and the results of taxonomic classification of DNA sequences obtained through metabarcoding.

O106. dbDNA - A phylogeny- and expert identifier-driven grading system for reliable taxonomic annotation of (meta)barcoding data

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Identifying specimens to species or higher taxonomic level is a key component of biological monitoring. In recent times, species identification has been facilitated through high-throughput genetic methods, in particular DNA metabarcoding. One key aspect, that limits direct comparability of data and in particular hampers the defensibility of the results as part of regulatory monitoring tasks are uncertain taxonomic assignments via reference sequence databases. While these should ideally be complete, open, well-maintained, curated and updated continuously, they often lack quality assurance of taxonomic annotations. Existing solutions to this problem are researchers, industry and agencies creating their own, often closed reference databases. This is highly problematic as in closed databases the direct comparability of data sets is limited, and by this the key advantage of (meta)barcoding as a simple comparable, open tool gets lost. In view of the problematic situation and the need to also formally implement metabarcoding into regulatory monitoring programs, authoritative reference databases that are compiled according to community standards, have a version number, quality control and are maintained over time are needed. The ultimate goal of the dbDNA project was developing a system that aids in achieving robust taxonomic annotations when using DNA (meta)barcoding. The strategy reaching that goal is twofold, creating a pipeline that allows for grading individual sequences deposited in reference libraries as well as using graded references sequences to generate curated lists of reference sequences. The backbone of the developed pipeline are criteria for robust taxonomic annotations worked out by experts in two workshops. We will introduce the developed pipeline and criteria in detail and demonstrate the effectiveness of the approach on an existing taxa list for monitoring freshwater invertebrates in Germany.

O107. Challenges in the investigation of the diatom community of saline habitats: A case study of Plava Banja (Serbia)

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Plava Banja is an artificial saline lake in Vojvodina province (Serbia). It was formed during clay excavation by a local company, later abandoned and covered with gravel. In 2023, 12 diatom samples were collected during three seasons of sampling (spring, summer, and autumn) from reeds and artificial bricks. Two approaches were used to analyze the samples: traditional microscopic analysis and high-throughput sequencing (HTS) of the rbcL gene. This study aimed to see a difference in diatom diversity between these two approaches. In parallel with the analysis of the diatom community, physicochemical analyses of the water were also carried out. Plava Banja is characterized by elevated concentrations of sulfates, chlorides, alkaline pH, and high conductivity values. Around 55 taxa are recorded by microscope, and 82 taxa with molecular analysis. According to both analyses, *Nitzschia* was one of the most represented genera across the samples, but with mostly unassigned sequences. Traditional

microscopic analysis showed higher diversity within the genus *Craticula* than molecular analysis. Out of 7 *Craticula* taxa identified by a microscope, two were recorded as dominant through seasons: *Craticula aff. simplex* (relative abundance 2.66 – 90.68%) and *Craticula aff. halophila* (relative abundance 0.24 – 36.65%). According to molecular data, only one Craticula ASV is noticed. The second significant discrepancy in diversity was observed among the genus *Navicymbula*. Three *Navicymbula* taxa (*N. pusilla*, *N pusilla* var. *lata*, and *Navicymbula sp*.) were recorded in Plava Banja by microscope. However, none of the obtained sequences were assigned to the genus *Navicymbula* because this genus is not included in any rbcL reference database. All three *Navicymbula* identified under the microscope were noticed in the same samples with different relative abundance (*N. pusilla* 1.24–24.34%, *N pusilla* var. *lata* 0.49–10.87% and *Navicymbula sp.* 0.25–14.80%).

O108. eDNA-based assessment of phytoplankton community structure and dynamics in a saline lake in Serbia: comparison with microscopy-based method

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Continental saline lakes are among the most endangered aquatic ecosystems, as a result of intensifying human pressures. However, these ecosystems are home to remarkable biodiversity that performs important ecological functions. Our understanding of the structure and development of their biological communities, such as phytoplankton, remains limited, hindering efforts to preserve the biodiversity of these fragile habitats. A significant challenge in studying these communities is the dominance of small-cell size plankton (e.g. picoplankton) that is underrepresented when assessment relies solely on microscopy. However, coupling a high-throughput sequencing method with a DNA metabarcoding approach provides access to this hidden phytoplankton diversity. The aim of our study was to use DNA metabarcoding (targeting 23S rRNA gene) and microscopy methods to study phytoplankton in a saline lake in Serbia. The samples were collected from four different sites within lake Pečena Slatina during spring, summer, and autumn 2023 to study the spatiotemporal dynamics of the community. A large difference between the two inventory lists (microscopy and metabarcoding) was

observed. The metabarcoding approach yielded 344 ASVs, of which 156 were assigned to the genus level, and overall, 70 taxa were detected in eight phyla. On the other hand, 26 taxa belonging to four phyla were detected by microscopy. Both methods captured spatial and temporal variation in the phytoplankton community, but a better resolution was obtained by metabarcoding. During summer, coccal small-sized cyanobacteria dominated, while in autumn samples, filamentous Cyanobacteria such as Arthrospira and Anabaenopsis prevailed, both in terms of biomass and the number of reads. Spring samples showed a large discrepancy between the two methods: diatoms were the most frequently counted organisms, while the metabarcoding showed the codominance of euglenoids and cyanobacteria, but with a great percentage of unassigned reads in this season. The findings suggest that while metabarcoding can assess biodiversity in greater detail than microscopy, its effectiveness is currently constrained by gaps in reference barcoding library. Despite these limitations, it still provides a more comprehensive method for evaluating diversity compared to microscopy alone.

O109. MSFD and impact monitoring with eDNA: Insights from case studies in the Belgian Part of the North Sea and Avlékété Beach, Benin

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With increasing anthropogenic impacts and changing climate conditions, monitoring biodiversity in marine ecosystems has become critically important. eDNA is a cost-effective and non-invasive monitoring method, but a standardized framework for reporting and storing eDNA results is currently lacking and the resulting species list highly depends on the available reference database. We demonstrate this with eDNA data from two use cases. The Belgian part of the North Sea (BPNS) is a highly exploited area, and monitoring for the Marine Strategy Framework Directive (MSFD) currently involves morphological datasets. Fish species in the area are well studied and an extensive barcode reference database exists. We reconstructed the spatial patterns of fish communities with eDNA, demonstrating a strong alignment with the long-term trawl monitoring data. This opens up the debate on how to use eDNA for MSFD reporting. The second case study – in collaboration with the dredging company Jan De Nul- uses eDNA to study the impact of a submerged breakwater parallel to the shore - constructed to prevent beach erosion and to create a safe swimming area - on fish communities near Avlékété, Benin. Fish diversity data were collected through examining fishing nets of local fishermen. We developed a custom 12S reference database using local fish fin clips and available reference sequences from NCBI. Among the 50 species detected morphologically, 28 were not detected with eDNA. Of these, only nine had available 12S reference sequences. Taxonomic identification revealed many Pacific species assignments, likely indicating that species in the area have highly similar sequences with their non-native relatives. Despite a reasonably good fish inventory in the Gulf of Guinea, the barcode database remains insufficient for fully representing marine fish diversity of the area with eDNA. These comparative studies underscore the importance of consistent barcoding efforts for developing cost-effective monitoring programs.

O110. Laying the foundations of a national genetic baseline for species identification in support of biodiversity research and public policies in France.

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Biological diversity is crucial for maintaining ecosystem health and resilience, yet it is increasingly threatened by the rapid environmental changes of the Anthropocene. The potential of molecular methods, with their diversity of approaches (DNA barcoding, metabarcoding, eDNA) and applications, makes the development of DNA-based identification capacities a priority for biodiversity-related public policies in many countries. However, the effectiveness of these approaches relies heavily on the completeness and reliability of DNA reference libraries. To address this challenge, France, through its National Strategy for Biodiversity, is leading in the conception and coordination of a comprehensive National Genetic Baseline (NGB). The primary goal of this initiative is to improve the accuracy and reliability of taxonomic identifications using genetic and genomic data from organisms collected across France and its overseas territories. This presentation has two main objectives: (1) to outline the general design and scope of the NGB, emphasizing its role in refining taxonomic identifications based on genetic and genomic data, highlighting both marine and continental aquatic ecosystems, (2) to present a preliminary proof-of-concept that leverages existing DNA sequence databases (e.g., ENA, BOLD, GenBank, UNITE), and connecting these resources with TAXREF, the national nomenclatural and taxonomic repository, to enhance completeness and reliability of DNA reference libraries. Beyond its primary goal, the NGB is expected to support existing national programs that generate genetic or genomic data, gather taxonomic expertise and manage collections of voucher specimens in the context of DNA-based species identification.

O111. A molecular reference database of mitochondrial genomes for freshwater fish in France.

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In the last decade, non-invasive approaches to studying biodiversity, such as eDNA or metabarcoding are being employed extensively and with efficacy in aquatic environments for investigative purposes. Nevertheless, the effectiveness of these approaches hinges upon the availability of a reliable molecular reference database. Voucher specimens stored in museum collections are the cornerstone of establishing a reliable reference dataset. However, the majority of sequences currently accessible in public databases are insufficiently reliable. This is due to several factors, including the absence of vouchers specimens, the lack of available sequences for some taxa, and identification inaccuracies or errors. Despite significant advances in the taxonomic understanding of the fish fauna of France over the past two decades, there remains a dearth of comprehensive molecular data. These precludes the full identification and interpretation of sequence data obtained by eDNA and metabarcoding. The decision was therefore taken to established a molecular reference database, in close collaboration with taxonomic experts, comprising the complete mitochondrial genomes of all freshwater fish species occurring in France. This database will be accessible and searchable by all relevant parties (researches, institutions, NGOs...), with the aim of strengthening molecular expertise and use. Specimens from recent surveys will be combined with those from museum collections in order to ensure the reliability of the sequences. The aim is also to enhance the value of museum collections by including sequences of type specimens within a comprehensive dataset. Finally, the database of complete mitochondrial genomes enables interoperability with the markers commonly used in various ichthyological studies (12S, 16S, COI or Cytb), ranging from taxonomic reviews and eDNA studies to phylogenetics analyses and population genetics studies.

O112. Metabarcoding “by-catch” is precious

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Methodologically, two main types of metabarcoding study can be distinguished: those employing ‘universal’ primers to gain broad insights into community composition, and those using selective primers to target particular groups. The separation is not complete. Universal primers are probably never truly universal. For example, general V4 18S surveys of eukaryotes can be biased against ‘excavates’ because of primer mismatches and introns. Conversely, selective primers are often ‘leaky’, amplifying non-target groups even if binding is suboptimal. During the last 10 years, several sets of selective primers have been developed for Illumina-based HTS of diatoms. All are leaky, amplifying some members of related ochrophyte groups and even green-chloroplast lineages (*Viridiplantae*, *Euglenophyta*). This 'by-catch' can be regarded as undesirable and is often discarded. We argue that this is a missed opportunity and wasteful, because sampling is expensive and, in many respects, unrepeatable. We illustrate this by reference to brown algae (*Phaeophyceae*) recorded ‘accidentally’ in river biomonitoring datasets targeting diatoms. The brown algae are predominantly marine; freshwater browns, though discovered 170 years ago, are inconspicuous and poorly understood. The main genera, *Heribaudiella*, *Pleurocladia* and *Porterinema*, are all detectable with rbcL-based metabarcoding, as is *Bodanella*, hitherto known only from deep subalpine lakes [from the type locality, Lake Constance, where it is now apparently extinct, and three lakes in Austria]. Metabarcoding now reveals its presence in UK and French rivers. Besides the

conservation implications in this case, the potential value of 'by-catch' suggests a need to improve how metabarcoding data are made available.

O113. Overcoming Limitations in eDNA Metabarcoding with Nanopore Sequencing of Whole Mitogenomes

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In the past decade, environmental DNA (eDNA) metabarcoding has become an essential tool for biodiversity assessments. However, it faces several key limitations: (1) amplicon-based metabarcoding is impacted by primer biases; (2) commonly used markers are not always suitable or informative for all relevant taxa; (3) abundance estimations lack reliability; (4) taxonomic assignments depend on the variable quality of reference databases; and (5) limited read lengths can restrict the resolution of taxonomic insights. Recent advancements in nanopore sequencing offer promising alternatives that mitigate some of these challenges. By sequencing eDNA whole metagenomes, nanopore technology circumvents primer biases, while producing longer reads than amplicon-based methods, thereby potentially capturing more informative genetic data. This approach can be enhanced by focusing on mitochondrial sequences, as many commonly used genetic markers for eukaryotes (e.g., COI, 12S, 16S) are located on the mitochondrial genome. Mitochondrial genomes are present at hundreds to thousands of copies per cell, compared to the nuclear genome, with approximately 1% of whole genome sequencing reads originating from mitochondrial DNA. This abundance makes it feasible to assemble whole mitogenomes from only a few thousand nanopore-derived long reads, either from single-taxon DNA or, in the case of high-abundance taxa, directly from eDNA. Using whole mitogenomes for taxonomic assignments in metagenomic sequencing of eDNA allows the utilization of existing databases, the integration of various markers and databases, and even allowing for custom databases. Moreover, this approach also enhances abundance estimations and, through haplotype detection, opens avenues for population genomics studies. We present a method for generating high-quality mitogenomes within a single day using low-effort techniques and demonstrate how these mitogenomes improve taxonomic assignments of eDNA sequences.

O114. FIDO: A new type of autonomous aquatic sampling instrument for 'omics studies

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The study of environmental 'omics can allow insights into biological processes that result in better understanding of aquatic ecosystems. Despite the myriad of 'omic processing available, all studies begin with the mundane: acquiring a sample. Such a seemingly simple activity can severely limit interpretations when samples need to be collected repeatedly or over extended periods of time. For example, weather, access, funding, and personnel shortages can make repeated, extended sampling difficult. Over 18 years ago we developed the Environmental Sample Processor (ESP), a robotic system capable of automatically sampling and processing in situ. While the ESP has repeatedly demonstrated the advantages

of autonomous sampling, its size, complexity, and cost have limited its use at scale. To address this problem, in collaboration with the USGS, we have designed a new type of robotic sampler, initially developed for environmental DNA studies where preservation of samples is the primary goal. This device, called FIDO (Filtering Instrument for DNA Observations), was designed from the start with cost and ease-of-use in mind. FIDO is capable of acquiring 140 samples, and operates from a simple web-interface via cell or satellite linkage. In this talk we will explain the drivers that led to the development of FIDO, as well as it's efficacy at eDNA collection compared to traditional methods, based on results from a recent validation study at the Monterey Bay Aquarium.

O115. Leveraging hybridisation capture for detecting rare events and as a PCR-free metabarcoding approach for vegetation surveys

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Environmental DNA (eDNA) metabarcoding is transforming biodiversity monitoring, particularly in aquatic environments. The current most common approach uses PCR to specifically amplify the DNA of target taxa. As with any approach, PCR-based metabarcoding has inherent biases that significantly influence the set of species that can be detected. Hybridization capture (HC), or target enrichment, is a promising alternative to PCR-based eDNA metabarcoding as it overcomes some of the biases associated with PCR. We evaluated the effectiveness of HC metabarcoding in detecting plant species from water samples across diverse river types, from oligotrophic headwaters to large floodplain rivers. Our study is the first comprehensive evaluation of HC eDNA metabarcoding using four common plant markers (trnL, rbcL, ITS2, ITS1), directly comparing it to PCR-based metabarcoding and field surveys for aquatic vegetation diversity monitoring. The same river water samples, bioinformatic pipeline and reference database were used for the two eDNA approaches, such that differences between the methods arise from their intrinsic properties. Our findings show that HC recovers comparable species richness to PCR-based metabarcoding. However, we show that HC excels at detecting taxa across the four main plant phyla, enabling the most comprehensive vegetation survey to date compared to both PCR-based and observational methods. For instance, HC recovered a significant number of mosses, which are often difficult to identify and frequently overlooked in vegetation surveys but serve as important bioindicators. The three methods recover rare taxa but HC finds additional rare species, also missed in the field surveys. While both eDNA methods offer advantages over traditional field surveys, they also retrieved unique sets of species, highlighting intrinsic methodological biases. The use of one or the other will depend on the objectives of the study. By addressing key technological gaps in PCR-based methods, such as the detection of cryptic diversity and the limitations of primer universality, our study opens up new possibilities for eDNA-based biodiversity assessments. The presentation will discuss the advantages and limitations of hybridization capture, its implications for biodiversity monitoring, and propose strategies for integrating this technique into standard eDNA practices to overcome current challenges.

O116. Enhancing eDNA Metabarcoding Accuracy: Mitigating PCR Bias with Droplet PCR

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Environmental DNA (eDNA) analysis has emerged as a crucial tool for biodiversity monitoring. The data obtained are not only used to estimate community composition but also to infer organism abundance. However, correlations between biomass and eDNA concentrations are influenced by multiple variables, including temperature and the time elapsed since DNA shedding. Moreover, difficulties are also in the quantification of eDNA itself, particularly in eDNA metabarcoding, where accurate quantification is especially difficult. PCR bias, which occurs during the amplification of DNA templates using metabarcoding primers, significantly affects analysis outcomes. Despite this, many analyses proceed without adequately addressing this bias. In this study, we found that droplet PCR (dPCR) substantially reduces the variability in PCR amplification efficiency caused by base mismatches between barcoding primers and DNA templates, thereby improving detection sensitivity and quantification accuracy. Validation using the MiFish primers revealed that, in conventional PCR, the number of sequencing reads sharply declined when the mismatch exceeded two bases, and species with a six-base mismatch were no longer detectable. In contrast, dPCR results more closely matched the proportional input values, allowing the detection of species with up to a six-base mismatch. Even while universal primers are designed to closely match the DNA sequences of organisms in the target taxon, achieving a perfect design is highly challenging. In studies using eDNA analysis, where there is no a priori information on the species or their DNA sequences present in the sample, the dPCR-based sequencing library construction method proposed in this study can help produce more unbiased results by ameliorating the effects of base mismatches. This strategy will lead to analytical results that are more trustworthy and accurate.

O117. Unrestricted metabarcoding with Nanopore long-read sequencing

Patrick Lypaczewski (Department of Microbiology & Immunology, McGill University. Canada)

Current metabarcoding approaches suffer from many limitations either due to (i) technological limitations or (ii) reagent limitations. While Illumina based metabarcoding approaches offer high accuracy and depth, they are limited to at most markers of interest at most ~500 bases in length using 2x300bp MiSeq sequencing or shorter on newer platforms. Oxford Nanopore Technologies has developed an easy-to-use sequencing kit aimed at the full ~1500bp 16S locus but is limited to 24 concurrent multiplexed samples and has been shown to create a slight bias in amplicons due to rigid oligo constructs. Thanks to improvement in technology, Nanopore accuracy now regularly exceeds 99.5% which allows us to introduce short barcodes in primer design. As Nanopore sequencing is not length limited, this is done without sacrificing the length of the marker that can be amplified. These custom barcodes allow for up to 1000 concurrent 16S samples to be sequenced at the standard 100,000 reads per sample while providing species level accuracy with a sequencing cost of 1000-2000 €. Next, we expand our design with a universal primer system allowing for the sequencing of

any amplicon, of any length allowing for metabarcoding of the entire 16S-5S-28S locus or any gene of interest in any species.

O118. Aquatic diversity of microbial eukaryotes assess by Oxford Nanopore Technology and Illumina sequencing

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Biodiversity assessment of aquatic microbial eukaryotes (i.e. protists) using 18S rDNA genes has proved extremely valuable for both ecological studies and environmental monitoring. Over the last two decades, such metabarcoding surveys have been greatly influenced by the evolution of methodological and bioinformatic approaches. Currently, the most commonly used sequencing technology is the Illumina targeting 18S rDNA gene V4 or V9 regions and generating millions of short fragments for each samples considered. More recently, alternative technologies emerged, generating longer fragments, encompassing the entire ribosomal operon, thus providing more accurate taxonomic and evolutionary information. Among the “long read” sequencing options available, the Oxford Nanopore Technology (ONT) is one of the least expensive, produces a large quantity of reads per sample, with an ever increasing read quality. Here we developed a protocol for sample preparation and ONT sequencing of the entire 18S rRNA gene. We applied this protocol to generate data from a mock community, along with fresh water and marine environmental samples. Finally, using a newly developed bioinformatic pipeline, we calculated the relative abundances of the Operational Taxonomic Units generated, which allowed comparing our results with illumina amplicons previously generated following standard procedures. Analyses at both genus and species levels show a good overlap of taxonomic assignations between technologies. Overall, Illumina systematically produce more diversity than ONT, potentially overestimating taxonomic diversity. This is particularly manifest regarding “rare” taxa, yet without impacting main ecological interpretations, a pattern consistent with previous studies. Our study demonstrates the suitability of ONT sequencing for metabarcoding investigations of aquatic protists communities, providing a fast and cost effective approach to generate both ecologically and evolutionary relevant information.

O119. Addressing workflow challenges of ONT sequencing approaches for Riverine Microbial Communities: Long-Read vs Short-Read Sequencing

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Microbial communities are vital components of freshwater ecosystems, recognized as engines of global biogeochemical and nutrient cycles. Advances in DNA sequencing technologies now generate sequence reads of hundreds of kilobases, enhancing taxonomic

characterization at high levels and improving microbiome classification. However, methodological issues influencing the quality of results, accuracy of species detection, and potential biases introduced by short and long-read sequencing remain a concern. Metabarcoding of environmental samples presents numerous challenges and limitations, requiring carefully designed laboratory protocols and analytical workflows to ensure reliable results, especially with Long-Read sequencing. We evaluated the performance of two primers sets targeting the 16S region to characterize microbial diversity in water and biofilm samples collected in four rivers located in Cantabria, Northern Spain. Environmental DNA from the 16S rRNA region was sequenced using Oxford Nanopore Technologies (ONT), comparing full length reads (V1-V9, 1.5 kbp) with short-reads (V3-V4, 464 bp). We identified technical steps as critical sources of bias, distorting the view of actual community composition. These steps include the choice of DNA extraction method, number of PCR amplification cycles, and sequencing depth, particularly when handling long-read data. Bioinformatics decisions, such as quality filtering, trimming primers or adapters, chimeric sequence removal, and determining the appropriate sequence identity and coverage thresholds, play a critical role in shaping the outcomes of microbial community analyses. Long-read sequencing provides the advantage of analysing entire regions, enabling higher specificity in microbial community classification. Additionally, it provides flexibility in sequencing depth, allowing for adjustments in read output to match the complexity of the microbial community and capture both abundant and rare taxa in environmental samples.

O120. Nanopore eDNA Long-Read Metagenomics: An Holistic Window for eDNA Analysis

Olivier Collard (ULB, Belgium) Jean-François Flot (ULB, Belgium) Fabio Stoch (ULB. Belgium)

Since their initial description by Andrew Ogram in 1987, environmental DNA (eDNA) analyses have evolved into a scientific discipline of their own. This technique has shown its capacity for cost-effective monitoring of diverse ecosystems without disturbing existing populations. However, eDNA analysis remains complex and faces several challenges, such as unreliable quantification or the transport of eDNA samples to distant areas, which can lead to false positives. On the other hand, eDNA can be the only sustainable method for regular monitoring in habitats that are difficult to access, such as deep seas or caves. Monte Albo is a Sardinian karst system that is part of a UNESCO Man and the Biosphere reserve. Despite this protected status, the Monte Albo Aquifer lacks an efficient monitoring plan, although such plan is required by the Habitats Directive. To address these challenges, we aimed to achieve two main objectives: to develop an effective eDNA monitoring plan for the karst system of Monte Albo, and to evaluate the use of eDNA metagenomics for a more holistic study of eDNA dynamics. For this purpose, we used hydrophilic polyethersulfone (PES) membranes with a 0.8 µm pore size (Sylphium eDNA Dual Filter Capsule) to filter and preserve eDNA directly in the field. Bulk DNA sequencing was then performed on these samples using Nanopore ligation sequencing, without prior PCR amplification. This method reduces quantification bias and incorporates a temporal and spatial dimension by leveraging fragment size, as DNA degradation can be linked to the time elapsed or distance from the target specimen. For comparison, we also performed COI metabarcoding of each eDNA sample, as well as generated reference sequence libraries from morphologically identified specimens collected from this karst system using a combination of Nanopore COI metabarcoding and genome skimming. Results obtained using Nanopore eDNA long-read metagenomics were consistent

with those obtained using traditional eDNA metabarcoding, while avoiding PCR biases and yielding additional information on the lengths of the eDNA fragments sequenced.

O121. eDNA optimization for regular monitorings of white-clawed crayfish (*Austropotamobius pallipes* species complex): from lab testings to large-scale field validation

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The white-clawed crayfish *A. pallipes* & *A. fulcisianus* is species complex endemic of Western European rivers, threatened by extinction, due to the combined effect of habitat loss – or degradation – and competition with pests (American crayfish and crayfish plague). As a result, *A. pallipes* is submitted to protection measures and conservation programs, requiring robust monitoring data, which are often difficult to implement given the nocturnal lifestyle of this species, habitat difficult to survey and the often (very) low population densities. Environmental DNA (eDNA) analysis for monitoring this species is becoming widespread, with five different published assays, but with no comparison about their operational advantages and limitations. Here, we first compared these assays (both on crayfish tissues and eDNA field samples) and highlighted some trends: those from Atkinson et al. (2017) were better to detect *A. pallipes* compared with those from Troth et al. (2019) but failed to detect *A. fulcisianus*, what is possible with Troth et al. (2019) primers. Some additional comparisons about the efficiency of the different primers found in the literature have been done. These trials were applied on field, during a coordinated action through France. In total, from the 250 management structures involved in this project, 25 have actively participated, to select 60 study sites in rivers spread over 20 watersheds. The results of the first campaign (2023) validated the possibility of detecting the presence of white-clawed crayfish by eDNA, up to > 1km downstream of the population, and multiplex detection alert about potential dangers (co-occurring invasive *Faxonius limosus*, *Procambarus clarkii*, *Pacifastacus leniusculus* and the pathogen *Aphanomyces astaci*).

O122. Seeking for microbial bioindicators of river run-off inputs: new insights from eDNA data sets

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Estuarine ecosystems, where freshwater from rivers meets oceanic saline waters, form unique zones for coastal biodiversity dynamics and water quality assessment. This research focuses on identifying microbial bioindicators of fluvial influence in offshore environments, offering new insights into the continuum between riverine and marine ecosystems. Supported by the Horizon Europe OBAMA-NEXT project, this work is being carried out in close collaboration between three partners sharing data (Ifremer, AZTI and SZN). To represent a large variety of estuarine ecosystems, samples were collected in the English Channel (2 sites), Bay of Biscay (22), Tyrrhenian and Adriatic Seas (17) at estuary outlets and along an inshore-offshore gradient in areas impacted at different degrees by the river plume and urbanization.

By using amplicon sequencing of eDNA (V4 and V1-V2 18S rDNA; and V4-V5 16S rDNA), we studied differences in bacterial and protist communities of the same size fractions with the aim of identifying reliable indicators of river run-off impacts in marine waters. Specific bacterial families and fungi of potential terrestrial origin have been identified as some of the taxa that best describe the difference across inshore-offshore gradients, yet this difference is locally variable and dependent on river run-off importance. For example, bacteria belonging to the *Comamonadaceae* family seem to increase in relative abundance in riverine-impacted waters across all selected sites. Our project aims to define a European frame and strategy to use microbial indicators of river run-off, seeking common taxa and associated thresholds which could improve the monitoring of sensitive aquatic environments at the international level. This research advances our understanding of the microbial dynamics within the land-sea interface and highlights the potential use of microbial bioindicators, but also explores how eDNA can provide useful information for answering to stakeholders' needs.

O123. Environmental DNA metabarcoding for the assessment of vertebrate biodiversity along the estuarine gradient of the Rance river (Brittany, France)

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Estuaries are ecologically critical areas that serve as essential habitats for many species but are subjected to great anthropogenic pressures. The need to monitor these ecosystems is therefore particularly important but made difficult notably by their role as an ecotone between the sea and the continent, used by many species only in transit or following the rhythm of the tides. However, in Europe, the Water Framework Directive (WFD) monitor fish communities, among other parameters, to assess the health of estuaries. Environmental DNA (eDNA) metabarcoding offers a promising and non-invasive complement to existing methods for monitoring estuarine biodiversity. It notably provides reliable taxonomic information on the species present thus producing reliable snapshot of taxonomic assemblages and of their variation over time and space. This study explored fish-, and more generally, vertebrate-

diversity along the Rance estuary (Brittany, northwest of France) using eDNA metabarcoding and to compare results to monitoring using WFD method (beam trawl). It aimed to evaluate the relevance of eDNA approach both in terms of implementation and results, especially concerning fish diversity. Samples were collected from five stations along the estuarine gradient, ranging from marine to freshwater environments. A total of 124 distinct MOTUs were detected across all samples (n = 10). The taxonomic compositions varied between stations and clearly reflected the estuarine longitudinal gradient. Jaccard's dissimilarities increased with spatial distance and were primarily (88%) explained by taxonomic turnover. Several biodiversity indicators were computed on this data, revealing, among other findings, stable taxonomic richness and phylogenetic diversity along the estuarine gradient. The results, which align closely with WFD sampling, underscore the value of this approach which requires other test sites, but appears promising for future biomonitoring of estuarine ecosystems.

O124. eDNA outperforms traditional methods for detecting organic pollution in a non-perennial river

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Non-perennial rivers and streams (NPRs), which stop flowing or dry up periodically during the year, account for more than half of the world's rivers, a figure likely to increase due to global warming and growing demand for water. As perennial rivers, NPRs are increasingly threatened by human impacts. The Water Framework Directive in Europe mandates the monitoring of these impacts but it poses major challenges for NPRs. Traditional biomonitoring methods rely on the collection and morphological identification of organisms, followed by calculating quality indices based on taxa abundance and occurrence. This approach is often unsuitable for NPRs: the sampling protocol may not be applicable depending on the hydrological cycle, and NPR specific taxa are overlooked or described at a very coarse taxonomic grain while perennial rivers classical bioindicators are often absent in NPRs. As a result, the impact of human disturbance is often undetectable in an intermittent context. Molecular approaches based on high-throughput sequencing are a source of potential solutions for the biomonitoring of NPRs. One of these, called bulk metabarcoding, is based on the sequencing of DNA extracted from shredded specimens or even grounded kick-net samples. This approach allows a large diversity of organisms to be described at a much finer taxonomic level and for any life stage. Another molecular approach, environmental DNA (eDNA) metabarcoding bypasses specimen collection by sequencing DNA directly from environmental samples such as water samples. It is much less invasive, but the suitability of this method to detect local disturbances is still debated. In this study, we test whether eDNA and bulk metabarcoding can detect anthropogenic disturbance (wastewater treatment plant)

along an intermittence gradient on macroinvertebrate communities. By combining detailed taxonomic description and high rate of detection, we show that eDNA metabarcoding uniquely detects anthropogenic impacts on macroinvertebrate communities regardless of river intermittence, outperforming morphotaxonomy and bulk metabarcoding. Additionally, we identify chironomids as a promising and species rich group of potential bioindicators for NPRs.

O125. Innovating biodiversity monitoring: Translating eDNA research and democratizing data to empower stakeholders and the public

Ole Bjørn Brodnicke (DHI A/S, Denmark), Dora Szekely (DHI A/S, Denmark), Lars Mortensen (DHI A/S, Denmark), Jesper Dannisoee (DHI A/S, Denmark) & Katrina Povidisa-Delefosse (Total Energies, Denmark)

As biodiversity monitoring becomes increasingly critical to meet regulatory frameworks such as the EU Water Framework Directive (WFD), Marine Strategy Framework Directive (MSFD), and Corporate Sustainability Reporting Directive (CSRD), innovative omics methodologies offer transformative opportunities for compliance and conservation. This presentation explores the application of environmental DNA (eDNA) monitoring as a complementary approach to traditional compliance biodiversity survey methods with Total Energies as stakeholder. In this 2024 North Sea case study, we explore the transformative potential of eDNA-based monitoring by comparing its effectiveness, resolution, and efficiency against conventional survey methods. Our analysis includes biodiversity indices such as the novel Biodiversity State Indicator, which incorporates functional diversity and community interactions, derived from both eDNA and traditional data. To promote transparency and data accessibility, we will also showcase the North Sea Environmental Portal, a pioneering public-access platform integrating biodiversity, chemical, and omics data to empower decision-makers, industry stakeholders, and the broader public. This presentation underscores the practical applications of eDNA methodologies in advancing biodiversity monitoring and reporting frameworks. It is vital that we bridge innovative omic approaches with practical applications, this talk emphasizes the role of eDNA in fostering a nature-positive future while enhancing accountability in biodiversity monitoring and reporting. This case study provides an example of that bridge and delivers actionable insights into integrating eDNA-based methodologies into biodiversity reporting practices and leveraging advanced data tools to align with sustainability goals of stakeholders and regulatory requirements from governments.

O126. eDNA-based assessment of species diversity and its temporal development in a Dutch offshore wind farm

Daniël van Berkel (Wageningen University, the Netherlands), Reindert Nijland (Wageningen University, the Netherlands)

The growing need for sustainable energy has led to an expansion of offshore wind farms (OWFs) in Europe, including in the North Sea. While the construction negatively impacts marine life, OWFs could create opportunities once operational. The scour protection, rocks and boulders placed around turbine foundations, creates hard substrate on otherwise sandy seabeds, attracting species and enhancing biodiversity. Monitoring these artificial reefs is essential to assess opportunities for marine life. Traditional monitoring methods, including

scrape samples and photo/video imaging, are costly and often overlook cryptic species and those hidden in rock crevices. An effective alternative is species identification through environmental DNA (eDNA). Our study uses eDNA to monitor species diversity and composition on the scour protection of a new OWF in the Dutch North Sea. Shortly after commissioning, eDNA was collected from three turbine scour protections and two reference sites: one near the OWF to assess secondary effects like fishing bans, and one far from the OWF as a baseline. Samples will be collected three times per year for three years to track short- and medium-term effects. Invertebrates are identified with the COI gene and phylum-specific markers, while vertebrates are identified using a short amplicon (180 bp), and a long amplicon (2 kbp). Additionally, eDNA will be sequenced directly using long-read metagenomics, providing better insight in absolute species abundance and haplotypes of abundant species. Within three months of commissioning, over 55 marine species were detected in the OWF, including reef-associated species like rock gunnel (*Pholis gunnellus*). While biodiversity indices did not differ inside or outside the OWF, species composition and food web structures varied between scour protection sites and sandy seabeds. The upcoming long-term eDNA collection will provide more insight in spatial patterns, succession stages and key indicator species in the OWF.

O127. Monitoring Elasmobranch diversity using eDNA in Offshore Wind Farms

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Environmental DNA (eDNA) metabarcoding is a non-invasive alternative to traditional trawl surveys used for assessing biodiversity in offshore wind farms (OWFs). eDNA could be valuable for studying threatened elasmobranch species, which may face risks from offshore operations such as vessel traffic, chemical pollution, and electromagnetic fields (EMFs) from subsea cables. Conversely, OWFs may benefit elasmobranchs due to the absence of bottom trawling, thereby reducing fisheries pressure and habitat destruction. The overall effects of these factors on elasmobranch presence in OWFs are not yet fully understood. As OWF development expands in the Dutch North Sea, elasmobranch habitats and OWFs will increasingly overlap, intensifying spatial competition between fisheries, conservation, and energy sectors. This emphasises the need for informed marine spatial planning. The present study used eDNA to evaluate the presence of elasmobranchs in OWFs in the Dutch North Sea. Species were confirmed using both the universal primer pair MiFish and a genus-specific primer pair targeting the mitochondrial COI gene. Samples were sequenced using the Oxford Nanopore MinION and analysed using the Decona pipeline (Doorenspleet et al., 2024). Over two years, four OWFs were sampled, with one site sampled quarterly. Five elasmobranch species (two sharks, three rays) were identified in OWFs, with the seasonal presence of *Mustelus asterias* along subsea cables consistent with known migratory patterns. These findings confirmed that elasmobranchs use OWFs as a habitat in the Dutch North Sea. We

therefore recommend that bottom trawling, should not be trialled in OWFs. Additionally, we encourage further research into the reasons elasmobranchs use OWFs, such as for migration, foraging, or reproductive purposes. We also highlight the utility of eDNA for non-invasive, seasonal monitoring of elasmobranchs and demonstrate that eDNA could be widely applied in OWFs for routine biodiversity assessments.

POSTER PRESENTATIONS

P01. Monitoring biodiversity of an alpine watershed using eDNA metabarcoding and ecological surveys: a collaborative work between students, scientists and citizens

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During the new Environmental Genomics Master program in Lyon, students conducted a two-year research project in collaboration with the scientific NGO "Le CREA Mont Blanc". In their first year (2022), students collected environmental DNA (eDNA) from water and sediment along a river stream of the French Alps to determine the diversity of plants and mammals. They also visited the CREA observation sites, including camera traps and quadrats. Subsequently, DNA was extracted and amplified using primers targeting a chloroplastic gene for plants and 16S mitochondrial gene for mammals. In their second year, students developed an analysis pipeline to identify the taxa obtained by eDNA, in comparison with the ones detected by traditional methods: camera trap images analyzed by artificial intelligence, scientists and participatory science for mammal communities, identification by botanists for plant communities. Although the majority of the observed species were recovered by eDNA,

some taxa were specifically detected by only one of the two methods. Additionally, the students found a significant effect of the eDNA matrix (water or sediment) and the sampling altitude on the community description. As both a pedagogical exercise and a means of communicating their findings, the students authored a scientific article aimed at contributing to the exploration of the impact of climate change on Alpine biodiversity and sharing this knowledge with the public. Every year a next generation of students is repeating the same eDNA sampling, while enriching the eDNA data-set, improving or including new bioinformatic routines. In a few years, this collective work shall provide a data-set and tool to catalog alpine biodiversity using eDNA with recommendations based on its weaknesses and strengths.

P02. ProTecteDNA: portable solutions for eDNA-based biodiversity monitoring in protected areas

Vid Švara (Carinthia University of Applied Sciences, Austria), Joana Verissimo (CIBIO/InBIO, Portugal), Paolo Scariano (Carinthia University of Applied Sciences, Austria), Michael Jungmeier (Carinthia University of Applied Sciences, Austria), Filipa MS Martins (CIBIO/InBIO, Portugal)

In order to comprehensively monitor species diversity and optimally plan the management of protected areas (PAs), the acquisition of precise information on species communities is essential. The demand for biodiversity data is growing rapidly, especially in light of global change, biodiversity decline, and increased nature protection efforts. A molecular DNA-based approach, metabarcoding of species communities, has been shown to detect species effectively and accurately. To foster this approach, the project ProTecteDNA aims to develop and implement novel environmental DNA (eDNA) based metabarcoding for biodiversity assessment in different freshwater habitats in PAs, using a portable monitoring toolset A technology, a tabletop laboratory set and the pocket-size Oxford Nanopore MinION sequencing device, that makes DNA-based methods affordable and applicable for biodiversity monitoring and molecular biological research, is used for the method implementation. The project is being carried out in two stages: first, a comprehensive protocol, including equipment lists for the implementation of the novel approach, is being assembled. In the second stage, the proposed toolset is being applied in a real world setting with samples collected from selected protected areas in Austria (a high alpine stream in a national park, a protected bog in a biosphere reserve, and a pristine river). The laboratory test run on these freshwater samples are used for protocol optimization in a four-step workflow that optimizes sample processing and species identification. The workflow consists of eDNA sample collection using a scalable and transferable sampling kit, DNA extraction using a portable laboratory, DNA sequencing using the portable Oxford Nanopore MinION device, and sequence analysis using an open access bioinformatics pipeline. The project aims to make novel technology more available for biodiversity assessment of PAs locally and globally.

P03. On-site species detection based on eDNA: example of the Natterjack toad (*Epidalea calamita*)

Abigaël Chieux (ARGALY, France), Aurélie Bonin (ARGALY, France), Alessia Guerrieri (ARGALY, France), Eva Bellemain (ARGALY, France)

In recent years, the use of eDNA approaches for community characterization and species monitoring has experienced significant growth. In biodiversity conservation and management, successful applications of eDNA-based methods include for example the early detection of invasive species and the search for rare or elusive species. However, all eDNA approaches rely mostly on sample processing in the laboratory by skilled staff, which implies long processing times and high analysis costs. To tap the potential of eDNA for biodiversity survey and species detection and make it a pertinent and versatile tool for conservation and management, we are in urgent need of low-cost protocols that can be easily deployed in the field by non-specialists, and that can yield quick and robust results. Here, we will present the development of molecular biology techniques based on isothermal amplification for the in-situ detection of the Natterjack toad (*Epidalea calamita*) in aquatic environments using eDNA. Specifically, we will discuss the RPA (Recombinase Polymerase Amplification), LAMP (Loop-mediated Isothermal Amplification), and CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) methods. For each, we will explain the process and present the advances of our research. We will also develop the advantages and limitations of these methods to highlight their potential in a biodiversity conservation context.

P04. The noose is tightening on parasites: how environmental nucleic acids (RNA/DNA) help us identify the different forms of freshwater parasites

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Environmental DNA led to significant advances in the detection of pathogens, including those that are microscopic, endoparasitic, or present at low concentrations, as well as their hosts in aquatic environments. This has enabled the characterisation of community composition, species interactions, and distribution patterns. However, DNAs can persist in ecosystems and do not inform on the physiological status of the targeted organisms (i.e. death or living organism, parasitic life stage). Further investigation is thus required to ascertain the precise nature of the signals detected in water and what they can tell us about host/pathogen interactions. Environmental RNA (eRNA), due to its presumed rapid degradation, could serve as a valuable tool for identifying active organisms, while offering a unique potential for epidemiological monitoring of infectious life stages through the analysis of their specific transcripts. Here we focus on the development of ARNe markers targeting the miracidia and cercariae of *Schistosoma mansoni*, two infectious stages with distinct epidemiological roles in the complex life cycle of this trematode. We evaluated the specificity, sensitivity and quantification capacity of these markers, first in silico and then experimentally. We quantified ADNe/ARNe ratio and RNAs specific to miracidia and cercariae from water where these two *S. mansoni* life-stages were present at different ratios (10/90, 50/50, 90/10). We also

experimentally studied the dynamics of RNAs and DNAs degradation in aquaria in which the cercariae/miracidia ratio was set to 50/50 after 24h, 48h and 120h. Precise quantification of each of these life-stages would represent a significant step forward in the biomonitoring of complex-cycle pathogens in freshwater, going beyond simple species detection. Furthermore, this study provides new insights on longevity and our ability to quantify eRNAs. It also highlights the usefulness of eRNA in pathogen ecology and epidemiology.

P05. eRNAmaris - the use of environmental RNA to improve fish stock assessments in marine systems

Verena Trenkel (UMR DECOD Ifremer, France), Michael Traugott (Univ. Innsbruck, Austria), Robin Faillettaz (UMR DECOD Ifremer, France), Salomé Fabri-Ruiz (UMR DECOD Ifremer, France), Daniela Sint (Univ. Innsbruck, Austria)

Large-scale scientific bottom trawl surveys are still the method of choice to investigate fish-stock densities in marine systems, despite their negative impacts on the environment. The analysis of eDNA, which became a common biomonitoring tool in many aquatic habitats and might replace bottom trawling, fails in providing good population estimates for marine fish. A reason is the stability of eDNA over elevated timespans in the environment, facilitating diffusion of eDNA signals over long distances and conflicting with estimating the size of local populations. Recently eRNA analysis was proposed as a potential alternative due to its faster degradation which should enhance local population estimates. However, only little is known on the actual properties of eRNA. Therefore, we conduct this eRNA study on marine fish, to explore the potential of eRNA in overcoming the limitations of eDNA analysis in marine systems. Within this project we address three key questions: (1) How do shedding and decay rates of different eRNA types (mRNA, rRNA) compare to those of eDNA? (2) Can the analysis of eRNA help to overcome shortcomings of eDNA analysis in marine systems? (3) How can hydrodynamic drift models improve the ability of eDNA/eRNA analysis to deliver non-invasive spatio-temporally highly resolved information on marine fish population densities? The questions above will be targeted in a French-Austrian-collaboration by an experimental design following three consequential steps. First, we obtain base-line data on the abundance and stability of eRNA in marine environments from experiments with different fish species. Second, we use this data to develop and parametrise a hydrodynamic diffusion/advection model describing eDNA/eRNA transport in the ocean, aiming to increase the predictive power of eDNA/eRNA analysis for fish-stock estimates. Active fish movement will be integrated into the model to evaluate its relative importance compared to eDNA/eRNA transport. Third, we evaluate the new approaches using field data collected during field surveys in the Bay of Biscay by using bottom trawling and eDNA/eRNA sampling. We are convinced, that the findings from this project will help to design the next generation of non-invasive marine monitoring programs for biodiversity conservation and sustainable fisheries management.

P06. Wastewater treatment with an oxidizing agent: efficiency on pathogens and antimicrobial resistance

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Pathogens and antimicrobial resistance (AMR) in wastewater and effluents of wastewater treatment plants (WWTP) represent a global health and environmental challenge that needs urgent attention. AMR has emerged as a leading cause of global mortality. According to a World Health Organization (WHO) report, antimicrobial-resistant pathogens are the primary health threat, causing over 700,000 annual deaths. Alarming projections anticipate 10 million deaths by 2050. Given the pressing consequences of AMR, numerous disinfection processes have been explored in WWTP to mitigate the release of AMR in surface waters, including chlorination, UV disinfection, ozonation, and membrane bioreactors. However, in real-scale WWTPs, the reduction in AMR is generally limited, typically achieving 0 to 3-log reduction in antibiotic resistance genes (ARGs). In some cases, an increase in the availability of genetic material, including plasmids and ARGs, has even been noted in treated water. The continued discharge of untreated or inadequately treated wastewater containing AMR and AMR-acquired pathogens contributes to the spread of infections and the proliferation of AMR, posing significant risks to human health and ecosystem integrity. There is therefore an urgent need to improve treatment strategies and implement comprehensive monitoring programs in order to preserve water quality and mitigate the risks associated with wastewater discharges. The present project aims to tackle these challenges by assessing the effectiveness of a novel disinfection method using performic acid (PFA, HCO_3H). This emerging disinfectant has garnered increasing attention in wastewater treatment due to its effeciency against various pathogenic microorganisms, cost-effectiveness and greater environmental compatibility. The development of various high-throughput sequencing methods (metabarcoding, metagenomics, metatranscriptomics) based on genome (DNA, RNA) and protein analysis enables the identification of organism composition and function. Among these methods, the "Shotgun Metagenomic Sequencing" technique using "short-reads" (Illumina Mi/NetSeq) is currently the most widely used to study the composition of different microbial groups (eukaryotes, bacteria, archaea, and viruses). Metagenomic analysis methods will to be used in this study to evaluate the effectiveness of water treatment processes on pathogens and antimicrobial resistance. Our findings demonstrate that higher PFA concentrations and longer contact times result in increased efficiency in controlling microbiomes and antimicrobial resistance genes. These results underscore PFA's effectiveness in reducing microbial and antimicrobial resistance gene levels, even with minimal concentrations and short contact times, thus demonstrating its suitability for widespread application in WWTPs.

P07. eDNA as a tool for precision biodiversity reporting: aligning science and stakeholder needs

Emilie Delpuech (LDgenX, France), Maëva Leitwein (LDgenX, France)

Various anthropogenic pressures, such as the destruction of natural environments and the over-exploitation of resources, have led to a drastic decline in biodiversity at all scales, impacting the evolutionary potential of species. With biodiversity loss becoming a global concern, stakeholders from policy-makers to conservationists increasingly demand accurate, actionable data for decision-making. Through LDgenX, a genomic analysis and expertise consultancy run by two PhDs in genetics and bioinformatics, we study DNA to study, monitor, and protect biodiversity. Protecting ecosystems and the species that populate them without integrating the underlying evolutionary dynamics is no longer enough to protect biodiversity. Certain measures that have a directly positive effect on demography (repopulation, translocation) can, on the other hand, negatively affect the evolution of populations by causing losses of genetic diversity and/or local adaptations. This is why we are highlighting the use of genetic markers (SNPs) for better monitoring of populations and ecosystems. To address these issues, we are implementing several approaches: (i) population genetics via high-throughput sequencing and in-depth analysis of identified markers; (ii) selection of traits of interest and study of cross-breeding plans to improve aquaculture or restocking populations; and (iii) environmental DNA (eDNA) for a better description of biodiversity, via sequencing of long DNA fragments. This non-invasive technique provides a snapshot of ecosystem composition in real-time. By using genomic data, conservation managers can make more informed decisions and develop more effective management strategies. In this way, genomic expertise promises to play an increasingly important role in protecting populations and ecosystems.

P08. Can eDNA be a decision support for sea turtle nesting monitoring?

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Sea turtles, known to be "umbrella species", play a fundamental role in the conservation of marine ecosystems. However, they face multiple threats, including water pollution, illegal fishing, climate change and human impacts on their nesting and feeding sites, which can threat the natural life cycle of these species. In Martinique, two species reproduce regularly: the hawksbill turtle (*Eretmochelys imbricata*) and the leatherback turtle (*Dermochelys coriacea*). A research project initiated in 2020 by Aquasearch's teams aims to deepen knowledge of the populations of sea turtles that come to nest on the island. Our objective is to assess if eDNA could have been used as an indicator for monitoring sea turtle egg-laying at the sites. In order to do so, we took water samples (30L) every two weeks from June to September 2024 on the 3 beaches historically monitored and recognized as major nesting sites. We then analysed the presence of hawksbill and leatherback turtle DNA and compared this with the visual nesting monitoring data (night patrols). The primary aim of this study is to

validate the application of eDNA filters for monitoring the populations of these threatened species.

P09. Three converging eDNA approaches to track the reproductive dynamics of two invasive dreissenid mussels in lakes

Marine Vautier (INRAE Carrtel, France), Isabelle Domaizon (INRAE Carrtel, France)

Dreissena polymorpha, commonly known as the zebra mussel, and *Dreissena rostriformis bugensis*, also known as the quagga mussel, are two freshwater mussel species classified as invasive alien species (IAS). These species are notorious for their rapid spread, prolific reproductive capacity, and detrimental effects on native biodiversity and ecosystem functioning. Understanding the colonisation processes of quagga and zebra mussels in lakes requires an understanding of their reproductive dynamics. As the two species are morphologically indistinguishable during their larval stages, environmental DNA (eDNA) approaches are particularly relevant for characterising their reproduction in natural environments where they cohabit, which is often the case in lakes. Thus, this study aims to compare three eDNA approaches to evaluate their effectiveness in detecting the presence and abundance of veliger larvae, and consequently, the reproduction of dreissen in lakes. Two of the eDNA methods were based on water samples, while the third was based on plankton bulk samples obtained using plankton nets. The three eDNA methods were compared with counts of veliger larvae collected with plankton nets. Two sets of primers and probes specific to the two mussel species were developed and validated for multiplex use in digital droplet PCR (ddPCR), and absolute quantification of eDNA samples was then performed using ddPCR. eDNA methods were applied monthly for a year in four lakes located in the French Alps: Lake Geneva, Lake Bourget, Lake Annecy, and Lake Aiguebelette. The study showed a positive and significant correlation between all eDNA approaches and the number of veliger larvae. These eDNA approaches, when combined with ddPCR, are effective for characterising the reproductive dynamics of the two invasive mussel species in lakes. Reproduction was observed throughout the year in the two lakes dominated by the quagga mussel, Lake Geneva and Lake Bourget. In contrast, it was only observed during the warm season in the other two lakes dominated by the zebra mussel, Annecy and Aiguebelette. These observations confirm that the quagga mussel is more tolerant of cold temperatures than the zebra mussel, which explains, at least in part, why it outperforms it in the perialpine lakes.

P10. Diurnal and intratidal variation in microbial community structure and gene expression in a mudflat biofilm

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Intertidal zones are highly dynamic marine environments where the sediment surface is exposed to significant variations in light, salinity, temperature, nutrients, and oxygen levels throughout tidal and diurnal cycles. These zones are highly productive and can be important carbon sinks. The sediment surface is covered by microbial biofilms composed of highly diverse consortia of microbial organisms, embedded in a biogenic polymeric matrix, making it challenging to determine the specific metabolic networks involved in carbon recycling and storage. In this study, we used a multi-omic approach using metabarcoding, comparative metatranscriptomics and metabolomics as well as measurements of geochemical parameters, primary production and respiration to investigate microbial community dynamics during both day and night tidal emersions in an intertidal mudflat. Despite fluctuating nutrient availability, salinity and light conditions, the overall microbial functionality and primary productivity remained high. Genes linked to the stramenopile circadian clock showed robust expression oscillations, indicating that the diatom clock remains synchronized even under highly variable conditions found in a benthic biofilm. Related, diatom light-harvesting complex proteins exhibited increased expression before dawn, suggesting circadian clock regulation, revealing the coupling of these processes in natural intertidal sediment conditions. Additionally, we observed diurnal shifts in the diatom chrysolaminarin and lipid metabolism suggesting diel investments in storage of fixed carbon as high-energy molecules. These findings highlight the robustness of microbial functioning and the metabolic networks that support carbon recycling and energy storage in these highly variable environments.

P11. The culture collection of cyanobacteria at the MNHN (National Museum of Natural History)

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Established in the late 1920s, the culture collection of cyanobacteria at the French National Museum of Natural History (MNHN) now comprises over 1100 non-axenic live strains isolated mostly from freshwater ecosystems in France. As a research-oriented collection, it contributes to biodiversity, taxonomy, genomics, and bioactive compound research. Notably, the collection contains multiple strains of various genera of bloom-forming cyanobacteria that are of ecological concern, some of which produce cyanotoxins. Novel strategies for strain identification, conservation and accessibility are being implemented to provide an up-to-date resource to the community. Using a few recent examples, we here describe how developing a specific strategy for the enrichment of various clones from the same taxa and environments provides a new vista of the micro-diversity of cyanobacteria concerning their evolution and ecology. These enlarged perceptions of clonal diversity offer us new opportunities to better characterize the plasticity of cyanobacteria blooming populations in terms of local adaptation, physiology, ecotoxicology, chemical ecology and microbial interaction.

P12. Evaluating littoral zone restoration in lake through a multi-taxa eDNA ecological assessment

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Lakes are excellent indicators of environmental health, accumulating pollutants and nutrients from their surroundings. It is moreover imperative to preserve and restore these ecosystems, which face numerous stressors, to mitigate biodiversity loss and maintain their functionality which are essential for the neighbouring populations. In France, restoration efforts predominantly target aquatic hydromorphological components, focusing on reinstating water level fluctuations, re-establishing reedbeds and creating or rehabilitating islands within lakes. Conventional biodiversity monitoring methods are applied to measure the potential positive impact of restoration. These methods, which rely on morphological identification of algae, benthic macrofauna, ichthyofauna and macrophytes, are labor-intensive, need to have access to taxonomic experts, and can be invasive (capture of individual with nets). Consequently, there has been a growing interest in molecular identification techniques such as DNA metabarcoding, enabling rapid and cost-effective assessment of community taxonomic composition and revealing cryptic species, thereby enhancing the resolution of ecosystem assessments. Environmental DNA (eDNA), targeting DNA extracted from environmental samples, provides a comprehensive snapshot of biodiversity within aquatic environments. Lake Geneva (France/Switzerland), the largest lake in Western Europe, suffers from an extreme simplification of its shoreline, which has been almost completely rectified. To reverse this trend, some area were renatured in 2015 with the creation of reed beds, small islands and underwater dykes in compensation of the construction of a harbour in Geneva. Our study aims to elucidate the impacts of the different restoration interventions (namely, breakwater construction, reedbed establishment and vegetation enhancement) on freshwater biodiversity of Lake Geneva's shoreline. Every month for a year, we monitored changes in the benthic and planktonic communities of restored and non-restored shorelines. We sampled environmental DNA in water and in biofilms , targeting three biological compartments which are crucial to ecosystem functioning (algae, macroinvertebrates and fish). We conducted comparisons between restored and non-restored areas and analysed the seasonal changes along the year considering various biological metrics (from diversities to functionnal diversity analyses) to bring valuable insights into the efficacy of restoration strategies in fostering ecosystem resilience and sustaining biodiversity in freshwater ecosystems.

P13. Tracking aquatic biodiversity with eDNA: a study in quebec's mining regions

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Biodiversity is essential for the proper functioning of ecosystems. However, in Canada, it is particularly threatened in regions where mining activities fragment habitats and degrade aquatic ecosystems. Rigorous biodiversity assessment is therefore crucial to guide

conservation strategies, but it requires extensive data collection, which traditional methods, such as direct observation and trapping, often fail to provide effectively. In this context, environmental DNA (eDNA) has emerged as an innovative and promising tool. This technique involves analyzing DNA traces released into the environment by organisms through cells, excreta, scales, enabling the detection of even the rarest species in a non-invasive, faster, and more cost-effective manner than traditional methods. In this study, eDNA was used to assess the presence of several fish species around two mining sites in the James Bay area. Sampling campaigns were conducted in 2022 and 2023, and the collected eDNA was analyzed using qPCR with species-specific detection kits developed as part of the pan-Canadian iTrackDNA project. The results revealed the presence of walleye and brook trout, as well as yellow perch and Northern pike, both considered invasive species. Notably, the absence of sauger and the endangered lake sturgeon around the mining sites was also observed. Although mining activities did not seem to impact water quality, they may influence hydrology and alter habitats. Organisms are also subject to competitive pressures, and eDNA may help reveal certain ecological dynamics. It provides a concrete solution for better biodiversity management in environments subject to intense human pressures and can contribute to the protection of threatened species and to a deeper understanding of rapidly changing ecosystems.

P14. Identifying macrophytes using environmental DNA metabarcoding for biomonitoring and ecosystem management

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Mediterranean lagoons are subjected to multiple anthropic pressures and global change. This results in profound modifications of the ecosystem structure, functioning and provided services. Understanding the future response of lagoon biological communities is therefore of major interest for defining effective conservation actions to preserve or restore these ecosystems. Marine macrophytes represent a major biological compartment regarding their primary production (total net primary production of 2 Pg C/year) and their role in forming essential habitats for many species. The structure of benthic macrophyte communities vary spatially and temporally with environmental conditions. In terms of management, macrophyte species diversity and abundance are monitored based on morphological traits as defined by the Water Framework Directive. Although these regular field campaigns allow assessing the ecological status of water bodies and monitor long-term trends, they are costly, time-consuming and limited by the user's expertise. Metabarcoding is nowadays used as an integrative tool to characterize biodiversity of a chosen biological compartment from environmental DNA (eDNA). This tool represents a major asset for the fine detection and identification of species. Still, macrophytes represent a compartment for which there does not seem to be a consensus. In a context of anthropic pressure and global change, it is of main importance to obtain a macrophyte species inventory as exhaustive as possible and detect

early on rare or new species adapted to new environmental conditions. Therefore, the development of tools based on early markers may allow to anticipate the regime shifts of lagoon ecosystems and would help implement preventive management measures. Here, we tested molecular tools based on eDNA to assess the diversity of macrophytes to further be able to anticipate such regime shifts and help implement preventive management measures.

P15. Using OMICS methods to evaluate and monitor marine microbial responses to human in a management perspective - the MICROSURV project

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Clara Dignan

The planktonic microbiota is abundant, diverse and, thanks to its rapid multiplication, reacts very quickly to variations in its environment. In a complex environment, under multiple natural and human influences, the microbial community adapts first to disturbances that have the greatest impact on its growth and survival. These adaptive responses of the planktonic microbiota thus provide valuable indicators of the ecological state of the environment. The MICROSURV project draws on this adaptive capacity of the microbiota to develop biomonitoring tools for coastal environments subject to contrasting anthropogenic pressures. Three types of tools will be tested: (i) the structure of the planktonic microbial loop studied quantitatively by imaging flow cytometry, (ii) the taxonomic diversity of prokaryotic and eukaryotic microorganisms, studied qualitatively using long-read metabarcoding, (iii) the functional diversity of the microbial community (with a focus on macronutrients bacterial metabolic pathways and bacterial resistome), studied by shotgun long-read metagenomics. A sampling campaign in highly contrasting coastal port environments, on several spatio-temporal scales, will be carried out in 2025. It will assess the relevance of selected microbial parameters to indicate different sources of degradation in the ecological quality of coastal waters (dry and wet weather events, port activities). These degradations will be assessed by various physico-chemical measurements covering natural variations and chemical contamination of anthropogenic origin. Identifying the most relevant microbial responses for prioritizing natural and anthropogenic stress factors, as well as the required sampling frequency over time, will enable us to propose indicators of the state of coastal ecosystems, which can be integrated into regular monitoring networks.

P16. Decyphering the tolerance of leaf litter biofilms to the biofungicide Kasugamycin through the combination of metametabolomics with structural and functional descriptors

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In the context of agroecology practices, biofungicides have been increasingly used, yet their environmental impact remains poorly documented. In particular, there is a paucity of knowledge about tolerance and resilience of microbial communities to these chemicals while it is critically needed to assess the benefit/risk balance of using these new compounds. To tackle this challenge, meta-metabolomics is an innovative approach in ecotoxicology that provides a snapshot of the molecular phenotype and biochemical processes of a community in response to a contaminant. Additionally, biofilms are highly relevant models in ecotoxicology due to their significant taxonomic and functional diversity, which plays a key role in ecosystems (e.g., biogeochemical cycles). In this context, this study aims to determine the tolerance mechanisms (i.e., tolerance outcome pathway) of leaf litter biofilms to a biofungicide, the kasugamycin (kazu). To this end, biofilms were exposed in controlled conditions during 4 weeks to kazu at 10 mg/L and 100 mg/L. Samplings were performed after 1, 7, 14 and 28 days. Pollution-Induced Community Tolerance (PICT) was implemented at all sampling dates to evaluate the tolerance of the community to the kazu. In parallel, a combination of untargeted metametabolomics approach and structural descriptors (metabarcoding, biomass, ergosterol), as well as physiological/functional descriptors (respiration, β-glucosidase, laccase), was implemented. The results highlighted that kazu led to a decrease in leaf decomposition and bacterial density within the biofilm. A stimulation of β-glucosidase and a reduction in respiration were also observed. Finally, tolerance to kazu was acquired after 7 weeks of exposure, as indicated by changes in laccase activity. However, this acquired tolerance appears to diminish after 28 days of exposure. The results will soon be supplemented by metabolomic and metabarcoding analyses to further understand the biochemical disturbances and microbial biodiversity linked to kazu exposure. By advancing knowledge on the acquisition of tolerance in leaf litter to a biofungicide, molecular descriptors could be identified as specific biomarkers of tolerance, potentially applicable in efforts to preserve aquatic ecosystems.

P17. Microbial diversity baseline linked to hydrogeological conditions and anthropic pressures in the Beauce Aquifer, France.

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Groundwater accounts for nearly 99% of the liquid fresh water circulating on our planet. In the Water Framework Directive, groundwater quality assessment is carried out through targeted chemical analysis but does not consider its ecological state. Microorganisms are present up to great depths and contribute to the geochemical state of water and to pollution mitigation as well as harboring biodiversity, thus contributing to many above ground ecosystem services. However, we lack extensive knowledge of microbial diversity and activities in these environments and few significant studies are available although knowledge of groundwater microbial biodiversity and its drivers could lead to a better understanding of the biogeochemistry of aquifers, the impact of pollution and the identification of new indicators, thus assisting groundwater management. The work presented here aimed to analyze groundwater microbial diversity on a large scale to establish the existence of a

biological signature linked to the hydrogeochemistry with the hypothesis that differences could indicate disturbances. Sixty samples were collected from the Beauce aquifer (France). This aquifer, located between approximately 50 and 70 m depth, includes areas connected to the surface and confined zones and various anthropic and geogenic pressures (pesticides, nitrates, arsenic, etc.). Samples were collected in spring 2021, DNA was extracted from the filtered biomass and microbial diversity assessed through 16S rRNA gene metabarcoding (Illumina). Physico-chemical data highlighted three hydrogeochemical signatures, one with oxygenated conditions and high nitrate contents, one in the confined zone with anoxic conditions and high dissolved iron and manganese contents and one at the interface. The analysis of sequencing data alone and then in conjunction with hydrogeochemical data makes it possible to accentuate the differences between the three zones and highlight points whose signature does not correspond to that expected, highlighting possible dysfunctions linked to anthropogenic pressures and opening new possibilities for water quality assessment.

P18. The potential of eDNA metabarcoding for monitoring demersal fish communities in marine proteced areas of the Baltic Sea

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The integration of eDNA metabarcoding into surveys on benthic communities in marine protected areas is not only an important contribution to an efficient and exact inventory of species, but also serves the protection of vulnerable communities. The analysis of eDNA sequences could offer the possibility of investigating the composition and changes in demersal fish communities, largely or even completely avoiding catches by mobile bottom trawling. However, the future application of eDNA metabarcoding for non-invasive monitoring requires comprehensive validation and intercalibration with observational and catch-based abundance data. We investigated demersal fish communities using eDNA metabarcoding in bottom water samples of several coastal areas of the southern Baltic Sea that exhibit gradients in seawater salinity, water depth and physical and chemical sediment characteristics. Our results show that eDNA sequence analysis effectively identifies spatial and temporal patterns and differences in diversity in fish communities of the southern Baltic Sea. The comparison of eDNA-based community analysis with fishing data obtained from bottom-contacting gears sheds light on the strengths and weaknesses of both approaches. The implementation of eDNA metabarcoding in monitoring concepts for marine protected areas and recommendations for the development of site-specific databases for taxonomic annotations will be discussed.

P19. Evolution of genetic and phenotypic diversity in a marine microbial community exposed to pollutants: a microcosm study.

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Preserving the oceans is a major challenge of the 21st century. In 2000, the Water Framework Directive harmonized European regulations on water management to protect and restore the good ecological status of aquatic ecosystems, including marine and coastal areas. This study aims to investigate the impact of pollutants on marine microbial communities, which play a crucial role in ecosystem health. Indeed, this microbial fauna plays a major role in the ecosystemic equilibrium, notably through its significant contribution to the biogeochemical cycle of nutrients (carbon, nitrogen, phosphrous). By combining genetic and phenotypic approaches, we aimed to predict the long-term ecological effects of marine pollution and develop improved management strategies. We used microcosms to expose a wild marine microbial community (from Biological Station of Roscoff, France) to various toxicant (anthracene, benzene, chlorpyrifos, copper chloride, and PFOA) and combined phenotypic (Biolog® Phenotype Microarrays) and genetic (16S rRNA-encoding genes - V4-V5 regions) approaches to assess i) changes in community structure, ii) phenotypic responses to pollutant, and iii) the benefits of integrating these methods to better evaluate the impact of pollutants on ecosystem. The obtained results highlight a certain functional resilience despite a significant effect on genetic diversity. Moreover, only specific exposure conditions, such as higher pollutant concentrations, appear to significantly affect ecosystem functions. Leveraging this knowledge, the future challenge will be to develop biological indicator-based monitoring methods to estimate and predict the impact of pollutants on these ecosystems, in order to better protect them.

P20. Developing high throughput metametabolomics in freshwater Periphyton to enhance chemical risk assessment

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Facing increasing chemical pollution of aquatic ecosystems, one major challenge of environmental risk assessment is linking chemical exposure to impact at higher levels of biological organization. While various descriptors exist for effects at the individual and population levels, a gap remains in evaluating impacts at the microbiome community level. Aquatic periphyton, a complex assemblage of microorganisms, are increasingly used to study ecological impacts on microbiomes because of their diversity and role in key ecosystem functions. Among available techniques, metabolomics enables simultaneous characterization of exposure, associated effects and toxicity pathways, offering a promising approach to detect early, sensitive responses of microbiomes to chemical stress. However, metabolomics in chemical risk assessment for aquatic microbial communities (i.e. metametabolomics) is still in its early stages due to its relatively low throughput for screening purposes. Therefore, the aim of this work is to develop a high-throughput metametabolomics workflow (i.e., miniaturized exposure setup, sample preparation, data acquisition, data analysis, and

application) for periphyton to enhance chemical risk assessment in aquatic ecosystems. To determine the minimum periphyton quantity required for metabolomics, we first compared metabolome profiles at different quantities. Our results showed no marked differences between 1 to 10 mg, while 0.5 mg showed distinct differences from other quantities. By testing different microplates and glass dics (48, 24 and 12-well) for periphyton colonization, we found that different size of discs led to similar periphyton quantity after 14 days, averaging around 1 mg. Therefore, 48-well size slides and microplates will be further used for the exposure setup. Automated extraction is under development. The next step will consist in the establishment of a standardized data analysis pipeline towards the determination of community metabolism sensitivity threshold based on aggregated meta-metabolome dose response but also the annotation of this metametabolome. This workflow will subsequently be used to screen periphyton exposed to various organic and inorganic chemicals.

P21. Precision in river monitoring: key eDNA sampling sites unlock comprehensive fish biodiversity insights

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Effective conservation of river ecosystems hinges on accurate biodiversity assessments, particularly of fish communities. Environmental DNA (eDNA) metabarcoding has emerged as a powerful tool for such assessments, yet optimizing sampling strategies to accurately capture species diversity across heterogeneous river systems remains a challenge. Our recent study evaluates the number and placement of eDNA sampling sites required to achieve robust fish community characterization across three distinct river systems, comparing the results with traditional electrofishing techniques on an unprecedented resolution. Our findings show that to capture ≥95% of the estimated species richness, eDNA sampling required between one and nine sites per river system, spanning ten kilometres. In the most diverse river, a single eDNA site outperformed electrofishing, detecting more species with a fraction of the effort. Key environmental factors—such as substrate type, river discharge rate, and adjacent inflows—were crucial in identifying optimal sampling locations, ensuring the accurate reflection of species richness and habitat use. Moreover, key sampling sites were identified in both downstream and upstream sections of the studied rivers, often in congruence with water quality and environmental factors. This work moves beyond demonstrating the efficiency and precision of eDNA metabarcoding, offering practical insights into sampling strategies that can be directly tailored to different monitoring frameworks based on baseline knowledge of the study system. Different monitoring priorities, including capturing rare species or long-term ecosystem changes, require careful balancing between sampling efficiency and data precision. These challenges, along with our tested insights, will be further presented and discussed, offering a pathway to refine biodiversity assessment strategies in riverine ecosystems.

P22. Comparison of shot-gun sequencing and metabarcoding to assess alpine lakes phytoplankton diversity

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Phytoplankton diversity is a key parameter for monitoring Alpine lake ecosystems and their quality. For several years now, the study of this diversity has been revolutionized by the arrival of a new approach based on DNA sequencing, metabarcoding. Faster and less costly than the standardised approach based on microscopy, metabarcoding theoretically provides access to the full range of diversity, including nano-phytoplankton, which has been greatly underestimated until now. However, metabarcoding also has its limitations, particularly when it comes to primers and amplification. To assess the importance of these biases, we compared the diversity of phytoplankton in two large Alpine lakes (Annecy and Geneva), obtained using chloroplastic primers dedicated to cover the full diversity of phytoplankton and another DNA-based approach, shot-gun sequencing, which sequences all DNA whithout any selection and amplification. The results show that the abundances of most groups of phytoplankton are consistent between the two approaches, validating at this large scale the primers used. However, two groups, Chlorophyta and Ochrophyta showed more divergence, due to under-amplification or no amplification of some taxonomic group. This is likely due to the high diversity of these groups, not covered by the reference databases, as well as a possible presence of introns in their choloroplastic ribosomal genes. These limits are likely to be overcome with increasing reference database completion and the use of long-read metabarcoding. Overall, our study confirms the pertinence of using chloroplastic primers for assessing the phytoplankton diversity of Alpine lakes.

P23. Enhancing plankton monitoring: a comparison of short and long-read eDNA metabarcoding for characterizing plankton communities in the Belgian part of the North Sea

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In recent decades, environmental DNA (eDNA) metabarcoding has revolutionized ecological monitoring of plankton diversity, particularly using short-read sequencing techniques. While widely adopted, short-read (SR) sequencing, faces limitations in taxonomic resolution due to the typically short amplicon lengths. To overcome this challenge, we investigate the potential of long-read (LR) sequencing for improving the taxonomic resolution of plankton community analysis. Our study focuses on protist communities in the coastal and offshore waters of the Belgian part of the North Sea, sampled in 2022 and 2023. We compared the overall plankton and particularly protist taxonomic resolution, diversity, and community structure using two sequencing approaches: LR sequencing (V4-V5 region) with Oxford Nanopore Technology (ONT) and SR sequencing (V4 region) with Illumina MiSeq. We hypothesize that while LR

metabarcoding will provide finer taxonomic resolution, particularly in distinguishing closely related taxa, a high level of congruence in the identification of major taxonomic groups between both methods is anticipated. We also expect a higher diversity to be captured by LR metabarcoding. Our pilot investigation will provide valuable insights into the effectiveness of LR metabarcoding in plankton monitoring, with ONT's low cost, portability, and scalability showing great potential for future ecological studies and routine biodiversity assessments.

P24. Refining eDNA taxonomic assignments with a phylogenetic approach

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To monitor species in samples of soil, water or even air, environmental DNA (eDNA) methods such as metabarcoding or metagenomics are increasingly used. Recently such techniques have been employed commonly to assess the impact of climate change on marine species. However, the current bioinformatic tools used for species assignment are based on sequence similarity which is not the best metric for this purpose giving that two sequences might be highly similar but belong to different taxa, hence a different branch of a phylogenetic tree. Therefore, but also due to the lack of data for some biogeographic regions in databases, these bioinformatic tools tend to misassign species. Sometimes, they also fail to achieve taxonomic assignment at low taxonomic levels due to sequence resolution limitations. To address this problem, we developed a phylogenetic method which takes as input the identified amplicon sequence variants (ASVs) of multiple sampling locations and reference sequences from the NCBI database to infer a tree. This tool is flexible because it gives the possibility to choose between different genetic markers (12S, 16S or CO1) but it is also possible to use your own marker by giving its primer sequences, name and maximum length. The NCBI reference sequences are selected based on keywords and processed to retrieve the selected region of the marker to reduce potential biases during the alignment. Given that this approach yields large phylogenetic trees, we have developed two visualization strategies: (1) plotting a tree for multiple locations but focusing on one taxonomic group and (2) plotting a tree including all assigned taxa for a sampling location. With this phylogenetic approach we were able to detect with the first visualization strategy an average of 13.28% misassigned ASVs but also to refine the taxonomic assignment of 14.70% ASVs on average by at least one taxonomic level. Hence our approach considerably improves the identification of taxa in eDNA samples.

P25. Evaluating the impact of sampling strategies and bioinformatics on ethanol-based DNA metabarcoding

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Recent developments on ethanol-based DNA (etDNA) metabarcoding have shown that it is possible to extract meaningful information about macroinvertebrate community diversity and composition from the ethanol used to preserve bulk samples. The major advantages of

this molecular approach are the reduced processing time and costs, and the possibility to keep specimens intact for other experiments. Yet, organisms with highly sclerotised exoskeleton or that are rare in the sample have been found to release a lower amount of DNA into solution and tend to be consistently missed by etDNA metabarcoding, thereby compromising the viability of the method. Few studies have shown that the first steps of the metabarcoding workflow are crucial for the good performance of etDNA-based assays, such as the decision on storage time before sampling and the ethanol phase to be analysed, the inclusion of pre-treatment strategies (i.e., freezing), and the choice of the DNA extraction protocol. In this study, we aimed to evaluate the combined effect of various technical choices on the performance of etDNA metabarcoding, considering factors such as sample volume, sample type (ethanol phase vs sorting), pre-capture treatments (evaporation vs filtration) across four bioinformatic workflows. Our results revealed that, despite no impact on DNA concentration, the increase of sample volume leads to an increase of PCR amplification yields and proportion of families matching the morphological identification. This was particularly evident in the detection of hard-bodied and cased families. Significant differences in the community richness and composition were found when comparing ethanol phases either with or without a sorting step, as recovered by all bioinformatic workflows. Our results suggest that the higher performance (with lower observed variation) in taxonomic detection at higher volumes is likely a consequence of a higher availability of longer fragments of DNA in solution. This study highlights the importance of understanding the impact of technical choices to improve the efficiency of a DNA-based method and reinstates etDNA metabarcoding as a potential method in the context of biomonitoring.

P26. Building the diatom (Heterokontophyta) DNA library with rare species using traditional sequencing

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Finding and culturing rare species that exhibit k-selection growth can be challenging. The ability to obtain genetic results, when necessary, without culturing is an added tool in the quest to populate genetic libraries for taxonomic and environmental research. Under optimum conditions, both culturing and single cell genetic studies will be required. Single cell nested amplifications have been successful with the power of reproducibility and numbers, although specimen voucher validation creates some limitation. The single cell and limited culturing nested amplification approach has been used to examine the diversity of three genera in Canada (*Neidium Pfitzer*, *Frustulia Rabenhorst*, *Navicula Bory*) with 9 new species identified and described, along with many yet to be determined. The current traditional DNA project is to help build genetic biodiversity libraries of Canadian taxa across multiple genera at the gene-based level. At present over 500 single cells have been successfully amplified for at least one gene with more than 2000 single cells waiting to be tested. Selected genera, have more success than others (e.g. *Navicula* versus *Fragilaria*), but cell size is not necessarily restrictive with successful amplifications even with genera like *Acthanthidium* and *Staurosira*. Current limitations are with the number of species level taxonomically conserved genes and available primers to give taxonomically meaningful reads.

P27. Preliminary comparison of microscopy and molecular methods for determining cyanobacterial composition of a frequently blooming pond

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Cyanobacterial blooms in lakes, ponds and accumulations present a problem in Slovenia, especially in the north-eastern part, mostly because of the high nutrient concentrations. These kinds of blooms can be toxic and have an effect on the organisms living in the water as also people using this kind of waterbodies for recreation. Our aim was to compare traditional methods such as microscopy with molecular methods such as new generation sequencing (NGS). We did a comparison between six phytoplankton samples throughout the warmer season of the year 2022, where we compared taxon composition done with microscopy and NGS. In addition, we also compared phytobenthos taxon composition to phytoplankton samples. Once a month between april 2022 and september 2022 we sampled phytoplankton in the pond which is located in the north-east Slovenia in the village called Hotinja vas (46.46786, 15.67782). Microscopically cyanobacterial cell count of phytoplankton samples was performed with counting chambers. For molecular analysis we isolated DNA with commercial kit from all the samples and new generation sequencing (Illumina) was performed. Furthermore, toxin concentrations were measured. Our results show some similarities between microscopic and molecular analysis. With both methods we detected the same most abundant toxic genera – *Mycrocistis*, *Aphanizomenon* and *Dolichospermum*. With NGS we detected rare genera and pico-cyanobacteria. For example, non-toxic genus, such as *Cyanobium* weren't common when analysing the samples with microscope. This is probably due to the small size of certain genera which are harder to identify with light microscopy. We also noticed that with NGS some very abundant taxons were only determined to orders, which is probably due to lack of cyanobacterial genetic sequences in databases.

P28. Using environmental DNA to track the spread of invasive host-parasite complexes: a case study of the invasive freshwater fish *Pseudorasbora parva* and the cryptic fungal parasite *Sphaerothecum destruens*

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The spread of non-native species threatens biodiversity and exacerbates societal challenges like food security. To address this, effective conservation programs require detection methods that are easy to implement, accurate, and non-invasive. Over the past 15 years, environmental DNA (eDNA) techniques have gained popularity, surpassing traditional sampling methods. In this context, our study focused on tracking the invasive host-pathogen complex *Pseudorasbora parva* and *Sphaerothecum destruens* using eDNA metabarcoding. We collected water samples from freshwater canals over five months in the Camargue region and once in Corsica Island, both in southern of France. Total DNA was extracted from filtered water samples, and PCR-amplicons were sequenced using Illumina or Nanopore technologies. Our results revealed a high detection rate of *P. parva* in lentic ecosystems, aligning with habitat preferences of this small freshwater fish. Additionally, the detection rate in Camargue increased in May and June, likely due to the peak of the spawning season, which leads to more DNA being released into the environment (i.e. concentration and interaction of individuals). While eDNA successfully detected this invasive fish, we were unable to detect its cryptic fungal parasite, *S. destruens*, highlighting the challenges of identifying intracellular and cryptic fungal pathogens through eDNA methods.

P29. eDNA monitoring at a large scale to spot at-risk salmonid populations regarding an emerging infectious disease

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The epidemiological dynamics of an infectious disease result from the complex interaction between a parasite, its host, and the environment. The proliferative kidney disease (PKD), caused by *Tetracapsuloides bryosalmonae*, is responsible for major declines in salmonid fish, increasingly reported during the last two decades in Northern Europe and North America, both in wild and farmed populations. The life cycle of the parasite, involving a bryozoan as a main host, and the development of PKD in fish being temperature-dependent, the severity of epidemics and their geographical distribution is expected to increase with climate change impacting both the temperature and hydrology of the streams, therefore increasing the threat towards salmonid populations. Understanding the distribution of the parasite and its determinants, as well as its impacts on salmonid populations are therefore key elements for the development of management plans. Environmental DNA techniques prove to be very efficient and easy-to-use techniques to deploy for such monitoring. Here, we present a case study lead at the scale of the Auvergne-Rhône-Alpes region on 182 sampled sites, with the great contribution of local fishing federations.

P30. The eDNA as an interesting tool to protect endangered endemic fish species against restocking: an example with French graylings (Teleostei, Salmonidae)

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The Loire drainage is one of the largest French catchments which has preserved the most some wild aspects with a remarkable biodiversity including endemism. The Loire grayling *Thymallus ligericus* which was recently described in 2019 is one of these endemic species. It has a patrimonial value and the eight remaining populations in the Loire/Allier headwater system are threatened by habitat alteration, global warming and pollution, with an evaluation as Endangered in the last regional Red List IUCN Auvergne-Rhône-Alpes (AuRA). However, as graylings have a high interest for recreational fishing, some local angling associations have introduced the European grayling *T. thymallus* in the distribution area of *T. ligericus* conferring then a new threat: the hybridization. The aim of this study was to verify which populations are not impacted by the presence of *T. thymallus* using the eDNA approach. Thirteen water samples were collected during the grayling mating period in April 2023 in the different rivers where the Loire grayling is known to occur in AuRA. For both species, specific primers on a mitochondrial marker were developed using the locked nucleic acids method, an approach which increases the affinity for the complementary nucleotide and by consequence the difference of melting temperatures between a match and a mismatch during the ddPCR comparing to a classic probe. Our results allow the detection of graylings in 11 out of the 13 samples. And among them, *T. ligericus* was detected in every site, whereas *T. thymallus* was spotted in three locations such as the Allier river at Coudes and Langeac and also in the upper Loire river at Goudet. These are corroborated by data of restocking events. The other populations seem to be unimpacted in view of these results which should be useful for riverine managers in order to protect the Loire grayling. Finally, we sorely recommend to stop the restocking of European grayling in the Loire drainage and to use local strains instead.

P31. Does the Aquitanian pike (Teleostei, Esocidae) breed during the closed angling season? Elements of answer according to the eDNA approach.

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The Northern pike (*Esox lucius*) is an emblematic fish species with an important interest for recreational fishing. Its angling season is closed from February to the end of April to allow the breeding. However, a new pike species was described in Southwestern France in 2014: the Aquitanian pike (*Esox aquitanicus*). This endemic and heritage species is threatened, and yet very little is known about its biology and life traits, including its breeding season. According to historical literature, pike reproduction in Southwestern France begins in mid-February. In order to verify this, we employed the environmental DNA (eDNA) approach. Water samples, taken in duplicates, were collected from late November 2023 to late February 2024 from a known Aquitanian pike spawning ground, amounting to 31 samples. Pike eDNA concentrations were measured using ddPCR targeting the ITS1 marker. The results indicate an earlier breeding season compared to knowledges about the Northern pike, suggesting that this species could be the earliest reproducing pike species. This early reproduction might be an adaptation to the nutrient-poor environment in which the species lives. Further studies are required on other spawning grounds to confirm these results which will bring important knowledges for riverine managers for the conservation of this endemic and patrimonial pike species.

P32. Can eDNA metabarcoding be used to develop a biological quality index for disconnected pools in temporary rivers?

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Temporary rivers are fluvial ecosystems that alternate between different aquatic phases: flow, disconnected pools (DPs), and dry riverbeds. Rarely included in regular biomonitoring and conservation programs, DPs are important refugia for dry-phase aquatic biodiversity and contribute to rewetting communities through recolonization processes. Traditional macroinvertebrate-based biological indices developed for perennial rivers perform poorly in DPs, mainly because natural drought interferes with human disturbance. In addition, DPs tend to have small habitat sizes and high invertebrate densities. Consequently, local macroinvertebrate communities can be severely affected by traditional, highly invasive sampling methods. Given the high conservation interest of DPs, alternative sampling methods should be explored. Besides improving taxonomic resolution over traditional biomonitoring methods, eDNA-based methods allow sampling without invasive techniques. We aimed to investigate whether eDNA metabarcoding could represent macroinvertebrate changes along a gradient of human impact in the DPs, with a view to the eventual development of specific biomonitoring tools. We collected sediment eDNA samples from 55 DPs in Catalonia and processed them using fwhF2-EPTDr2N primers and Illumina high-throughput sequencing. We determined the anthropogenic impact gradient using the anthropogenic Human Influence Index (HII) (i.e. population pressure, land use, infrastructure, and access) and identified indicator species along this gradient. We used these indicator species to propose ecological limits across the HII and establish connections for the future development of a Multimetric Index of biological quality. Our study represents the first attempt to develop a biological index of DPs using molecular methods. This index is timely and useful as climate change and increasing water demand will make DPs the dominant habitat in many river systems worldwide.

P33. Assessment of the cyanobacteria risk, considering the invasive bryozoan *Pectinatella magnifica*

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Summer episodes of toxin-producing cyanobacterial blooms in lakes are becoming a growing concern for residents, users, and territorial managers. These blooms often led to bathing and/or nautical activities restrictions, while the increasing of heat waves draws continuously more people to lakes. The Devesset lake is affected by harmful cyanobacterial blooms and invaded, during summer, by *Pectinatella magnifica*. This invasive freshwater bryozoan forms colonies consisting in one layer of clusters-arranged zooids, attached to a bulky gelatinous matrix. The zooids produce very resistant statoblasts that remain dormant during winter and germinate in summer generating new colonies. Moreover, numerous algae and potentially toxinogenic species are hosted by *P. magnifica*, mainly, *Woronichinia sp*., a toxin-producing cyanobacteria. In this lake, *P. magnifica* is affected by numerous algae and potentially toxic cyanobacteria, mainly, *Woronichinia sp*. Microorganisms colonizing *P. magnifica* are poorly

studied. Therefore, our aim is to assess the microbial community structure by 16S metagenomics coupled with new generation sequencing method. As the presence of toxin-producing genes is not directly correlated to the toxin production, toxins assays are also carrying out. During 2024 summer, sampling campaigns were carried out at different invaded zones of the shore lake. *P. magnifica* biofilms and the surrounding water were harvested in order to the constitute a DNA collection and to measure cyanotoxins. Our result will allow us to determine if the presence of the invasive Pectinatella bryozoan creates an increased risk for lake users, which would impose a new strategy for the sanitary control of bathing.

P34. Guilt by association? The role of cyanobacteria-associated bacteria in harmful algal blooms.

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Cyanobacterial blooms, characterized as a rapid proliferation of cyanobacterial biomass, frequently occur in freshwater systems worldwide. During these blooms, Cyanobacteria could produce a large quantity and diversity of cyanotoxins, threatening aquatic systems and public health. Human-induced environmental changes, such as nutrient over-enrichment caused by industries and agriculture, have increased the frequency and intensity of these blooms. Monitoring, predicting and preventing blooms is therefore a priority – but also a challenge. Indeed, bloom dynamics are controlled by the interaction of abiotic (physico-chemical) factors with a complex biotic community composed of genetically diverse microbes, including the microbial community living around Cyanobacteria, also called phycosphere. A part of this community, have shown strong and intimate interactions with their cyanobacterial host. However, our knowledge in understanding cyanobacteria-microbiome associations, as well as the processes driving these relationships is still highly limited. It is for example unclear whether cyanobacteria actively control microbiome selection and if so, how these interactions evolves over times and with fluctuating ecological conditions. To address these unknowns, we plan to characterize the microbiome of different bloom-forming cyanobacterial genera, focusing on how its composition may varies across sites, times, environmental conditions, and whether there is a specific bacterial composition associated with each cyanobacteria genus. Multiple lakes across the world will be samples before, during and after bloom, using single colony strategy and environmental metagenomic. We will therefore characterize the cyanobacterial microbiome composition variation and potential phylosymbiosis signal. We will then evaluate the presence of co-evolution signal and finally, we will determine the functional complementarity between the microbiome and its cyanobacterial host using genome scale metabolic models.

P35. Chemical landscape of invasive aquatic plant exometabolomes

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Egeria densa and *Lagarosiphon major* are the main invasive hydrophytes in French Atlantic Lakes. These exotic species can modify trophic levels by producing large quantities of biomass in lakes, and have been studied and monitored for many years. Previous investigations revealed the occurrence of endogenous metabolites in these plants known to be able to influence epiphytic communities, phytoplankton, or other plants. Nevertheless, the actual excretion and environmental occurrence of these metabolites remain poorly documented. Thus, there is a need to gain knowledge of the actual occurrence and potential ecological impact of *E. densa* and *L. major* exometabolome, especially through their allelopathic potential. To this end, untargeted metabolomics using high-resolution mass spectrometer and chemometrics approaches is relevant through its ability to depict, as a first step, the exometabolome chemical landscape. In this context, this study aims to investigate, in these invasive plants, the excretion kinetic of biomolecules, especially those known for their allelopathic properties. To do so, plants and surrounding water were collected on Lacanau lake. After a period of acclimatization, photosynthetically active plant fragments of both species were grown separately under controlled conditions in inner mesocosms of 10 L of sterilized water for 10 days. Plant fragments were cultured under two different light conditions: 1) in the dark simulating the absence of irradiance of the lower part of plant stands, near the sediment; 2) nycthemeral cycle based on the seasonal photoperiod. Nutrients, temperature and density conditions were similar to those occurring within the field. Metabolomics was implemented on samples collected after 1, 2, 5 and 8 days and on plant surrounding waters from the lake. PCA and HCA showed significant discrepancies in exometabolome between the two species both in the field and in the mesocosms. ASCA analysis highlighted the significant effect of the time and the species and their interaction in the exometabolome fingerprint while the light regime did not have any effect. Further investigations are ongoing to characterize the endo- and the surface metabolome of both invasive species to unravel their actual contribution to the exometabolome chemical landscape, especially on the field.

P36. How the feeding regimes impact the waste metabolomes in aquaponics

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Aquaponics is a system that integrates aquaculture (fish farming) with hydroponics (growing plants in water), creating a symbiotic environment where fish waste provides nutrients for plants, and plants help purify the water for the fish. Synchronizing the circadian rhythms of both fish and plants in an aquaponics system may offer specific benefits related to metabolomes: maximizing nutrient uptake by plants when fish are most active (e.g., during feeding and waste production) and improving overall water quality by optimizing plant growth and nutrient cycling. The relationship between circadian rhythms in fish and plants within aquaponics systems, particularly regarding their integrated cultivation, has not been fully clarified. We hypothesize that varying fish feeding regimes throughout the day may influence metabolome excretion in aquaponics systems. The aim of the present study was to investigate the effect of fish feeding times on the concentration of oxygen, ammonium, nitrates, and suspended solids in an aquaponics system where common carp and lettuce were co-cultivated. The system consisted of 10 fish tanks, 8 plant tanks, and mechanical and biological filters. The experiment was conducted from March to May in two consecutive years,

2023 and 2024. Common carp, with an initial average weight of 248.52 ± 0.8g and 249.2 ± 0.9g, respectively, for both experimental years (without significant differences between the two), were stocked in the experimental aquaponics system at Trakia University, Stara Zagora, Bulgaria. Eighty lettuce plants (*Lactuca sativa*) were transferred from a greenhouse in Plovdiv to hydroponic pots at Trakia University in both years of the experiment. Hydrochemical parameters were measured online using appropriate probes connected to an SC1000 controller (Hach). Consistent lighting and climatic conditions were maintained during both experiments. In 2023, the tested feeding times for the fish were 9:00, 12:00, and 15:00. In 2024, the feeding times were adjusted to 11:00, 14:00, and 17:00. The present study provides an opportunity for optimizing the feeding cycle and water quality, synchronizing the biological rhythms of fish and plants, enhancing the sustainability and productivity of aquaponic systems, and expanding knowledge about the interaction of metabolomes in this innovative system.

P37. Quantifying an example of invasion dynamics and interaction with endemic species: the case of the red blood mysid *Hemimysis anomala* in Lake Geneva

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Alien species may constitute a major threat for aquatic systems due to their multiple potential impacts on biodiversity and ecosystem functioning. During the last decades, the red blood mysid *Hemimysis anomala* colonized a large variety of ecosystems in Europe and US, but it remains difficult to obtain information on its presence, abundance and distribution. An eDNA approach coupled with ddPCR has been developed for an efficient detection and monitoring of the dynamics of this species in Lake Geneva. This poster highlights and discusses the efficiency of the method, revealing the seasonal dynamics of *Hemimysis anomala* and its possible interaction (e.g. prey-predator relationship) with the European perch (*Perca fluviatilis*).

P38. Relative effect of physico-chemical parameters and contaminants on the diversity of diatom communities along the French Mediterranean coast

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Biofilms are diversified microbial community colonizing natural and artificial substrates in a dynamic process. Moreover, Diatoms represent the dominant autotrophic group in marine biofilms and community drivers are mostly environmental parameters more than substrates. The objective of this study was unraveling the relative importance of physico-chemicals vs pollutants as biofilm diatom community drivers in marine ecosystems. Diatom communities have been determined by a metabarcoding approach (rbcL sequences affiliated using the diatbarcode database) after the immersion of polyethylene plates at 49 locations during 3 months along the 1800 km of the French Mediterranean coasts including Corsica. The bioaccumulation of contaminants in biofilms was also investigated and compared with caged mussels for a wide range of both organic and metallic contaminants: 37 chemical elements

(including Cr, Mn, Co, Ni, Cu, Zn, As, Cd, Hg and Pb) and 57 organic compounds (i.e., 18 polycyclic aromatic hydrocarbons (PAHs), 8 dioxin-like and 6 non-dioxin-like polychlorinated biphenyls (PCBs) and 25 organochlorine pesticides (OCPs)). Results showed different multi-contaminated profiles in the different sites along the coast. Moreover, a remarkable correlation between concentrations in both biological matrices was observed for PAHs and PCBs, whereas OCP and metal bioaccumulation varied depending on each compound. Diatom communities were primarily shaped by major environmental parameters, i.e. temperature and salinity, leading to the definition of three ecoregions. However, at the eco-region scale, dbRDA and variance partitioning showed that cocktails of pollutants also clearly shaped diatom communities. These results highlighted the potential of biofilms as relevant bioindicators of the marine multicontamination.

P39. Combination of metabolomics and machine learning to unravel environmental drivers of spatial heterogeneity of microbial metabolome assemblage in aquatic periphyton: The COMBO project

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Freshwater periphyton, playing a key role in ecosystem functions and services, are increasingly used in ecotoxicology to better understand the link between chemical exposure and ecosystem disturbance. To this end, one key challenge is to understand better how environmental conditions interact and modulate the community dynamics in situ. In this context, this project aims to gain understanding of the spatial heterogeneity of the natural periphyton metabolome assembly under various environmental conditions through the implementation of statistic and predictive meta-metabolomics approach. This attempts to characterize the assembling rules (stockastic vs deterministic) of metabolomes in periphyton and to identify the main environmental driving forces of meta-metabolome assemblages that contributes to its spatial heterogeneity. To do so, autochthonous periphytons were collected in 100 sites widely distributed in France encompassing various water physico-chemistry and chemical/ecological status (water agency data). The photosynthetic yield of the periphyton was characterized in situ in parallel of the measurement of the water physico-chemistry (nutrients, micropollutants) by the water agency. Physico-chemical results confirmed the heterogeneity between the sites. The global biomass parameters (e.g. proteins) will be soon characterized while untargeted metametabolomics based on LC-HRMS is ongoing. Those

metametabolomics data will provide a comprehensive picture of the chemical landscape of the periphyton, potentially highlighting the existence of a core meta-metabolome. Then meta-community ecology metrics (α/β diversity) will be used to define assembling mechanisms. In parallel, statistics and machine-learning will be implemented in order to identify features or groups of features that can predict specific environmental conditions and water quality status. In addition, by linking metabolites to biomass or photosynthesis, COMBO would support the discovery of predictive effect biomarkers at the community level that may have impair ecosystem functions. Overall, this knowledge will support the translation of metametabolome responses of periphyton to chemical stress under controlled conditions into real applications for biomonitoring purposes.

P40. TrendDNA: Studying long-term biodiversity change using environmental DNA contained in the German Specimen Bank

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In the current era of rapid ecosystem change, long-term biodiversity data pose the basis for quantifying trends, predicting consequences, and supporting management actions. However, long-term biodiversity data are scarcely available. A long-term sample collection that has been little explored so far is the German Environmental Specimen Bank (ESB). The ultra-cold storage at -150 °C preserves environmental DNA (eDNA) present in the samples, which is an ideal source for holistic biodiversity assessments. One of the ESB sample types is suspended particulate matter (SPM), collected from 13 different sites in the Rhine, Saar, Danube, Elbe, Mulde, and Saale rivers, dating back to 2005. SPM is collected monthly using sedimentation traps, which are then pooled into yearly homogenates. In the project, we are analyzing 211 SPM samples from 6 rivers and 17 years using eDNA metabarcoding targeting fish and invertebrates. We highlight that eDNA data from the TrendDNA project provides comprehensive and plausible taxa lists, shows evidence of the introduction of invasive species, and unveils significant patterns of faunal community changes across years and sites. These results can aid in understanding biodiversity patterns, species turnover, tracking the invasion of species, assessing the progress of endangered species protection, and predicting future change. We provide an outlook on further project results and highlight the potential that the ESB can play in complementing long-term biodiversity monitoring in Germany.

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