



MetaZooGene Symposium



Presentation Abstracts

New insights into biodiversity, biogeography, ecology, and evolution of marine zooplankton based on molecular approaches

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Convenors: Ann Bucklin (University of Connecticut, USA); Katja Peijnenburg (Naturalis Biodiversity Center, NL); Leocadio Blanco-Bercial (Bermuda Institute of Ocean Sciences, BM); Silke Laakmann (Helmholtz Institute for Functional Marine Biodiversity at the University of Oldenburg, DE)

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Batta-Lona, Paola G., Joel K. Llopiz, Peter H. Wiebe, Ann Bucklin. DNA metabarcoding of diet diversity of *Salpa aspera* in the mesopelagic food web of the NW Atlantic

Gelatinous zooplankton are important grazers in mesopelagic food webs, although their diet and trophic impacts are unclear. One group, salps (Tunicata), includes species that are efficient filter feeders with extensive vertical migration behaviors that transport energy through the mesopelagic zone. We report results from field expeditions of the Ocean Twilight Zone program in the NW Atlantic Ocean. Diet composition was determined with DNA metabarcoding analysis of gut contents of *Salpa aspera* collected at different depths. Metabarcoding allows accurate detection of a broad taxonomic range of consumed prey and is thus providing new insights into trophic pathways and dynamics of pelagic food webs. Salps for this study were collected in vertically-stratified tows of a 1-m² MOCNESS in the NW Atlantic Slope Water during March, 2020. DNA was extracted from salp gut contents and sequenced for V4 and V9 hypervariable regions of the 18S rRNA gene. Taxonomic assignment of V4 and V9 sequences were resolved and classified using custom scripts and reference databases. Multivariate statistical analysis was used to compare prey composition for salps collected from 4 depth strata at 5 stations. Results of the metabarcoding analysis of the salp diet was used to summarize the trophic relationships of *S. aspera*, infer sources of productivity, and evaluate the impacts of salp vertical migration on particle and energy transfer in the mesopelagic ecosystem.

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Bucklin, Ann, Paola-Batta-Lona, Jennifer M. Questel, Mary Reid, Vickie You, Peter H. Wiebe. Population genetic diversity, structure, and connectivity of Euphausiacea based on COI barcodes: Implications for biodiversity and biogeography

Euphausiacea (krill) are ecologically important and abundant marine zooplankton that are key links between primary producers and consumers in pelagic food webs throughout the world ocean. Many euphausiid species have extensive biogeographical distributions spanning multiple ocean basins, creating the possibility of population genetic differentiation and (cryptic) speciation. Portions of mitochondrial cytochrome oxidase I (COI) have been used as DNA barcodes to identify and discriminate euphausiid species. Analysis of COI sequence variation has provided evidence of selection, geographic patterns of genetic diversity and structure, and pathways of population connectivity. We provide a global overview of COI barcoding of euphausiids based on the MetaZooGene Atlas and Database (MZGdb; <https://metazoogene.org/database>), which now includes ~3,000 barcodes for 65 species, with 18 accepted species with no barcodes. Analysis of COI sequences focuses on two genera, *Stylocheiron* and *Thysanoessa*, to examine the taxonomic significance of geographic variation and possible cryptic speciation. The results of euphausiid barcoding efforts to date are examined further to explore possible implications for the biodiversity and biogeography of euphausiids across the global ocean.

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Choo, Le Qin, G. Spaggiardi, E. Goetze, Milan Malinsky, Darshika Manral, Erik van Sebille, Galice Hoarau, Jef Huisman, Katja Peijnenburg. Genome-wide SNPs reveal distinct population structure in the holoplanktonic snail *Limacina bulimoides*

Many studies on population genomics have focused on terrestrial organisms. However, the seascape is a vast, interconnected three-dimensional habitat, which presents challenges for understanding the drivers of biodiversity in the open ocean. This is especially pressing given the threats of ocean warming, acidification and deoxygenation facing marine systems. We studied the distribution of genetic variation in the holoplanktonic pteropod *Limacina bulimoides* to gain insight into their population structure and adaptive potential. This species is found in (sub)tropical oceans globally, and is susceptible to ocean acidification due to their thin aragonitic shell. We developed a genome-wide target capture approach for 2,900 coding regions based on a draft transcriptome and genome of this non-model species. Based on the resulting 100,000 SNPs, we found that *L. bulimoides* is split into three populations within the Atlantic Ocean: North, Equatorial, and South, with two narrow dispersal barriers at 14-15°N and 15-18°S that coincide with regions of low abundance for this species. Despite the potential for basin-scale dispersal, physical connectivity modelling with particle release at sampling stations and subsequent tracking shows that limited mixing of populations is possible only at boundary regions within their estimated generation time of one year. To determine the nature of the dispersal barriers, we genotyped a comprehensive dataset of juveniles and adults found in the boundary regions. Selection, in addition to oceanography, are the likely drivers of divergence in this oceanic species, with functional variants identified across outlier loci in coding regions among the three populations.

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Dénes, Marcell, Gábor Endre Tóth, Rade Garič, Marijana Hure. Building the Adriatic Calanoid Database with the help of Oxford's MinION system.

Metabarcoding seems to be the future of biodiversity research. Simplifying time-consuming taxonomical methods, molecular approaches attract more and more ecology labs to join the new way of ecosystem evaluation each year. To achieve creditable results, reliable reference databases are needed. In response to this need we started building our own Adriatic multi-marker genetic database of calanoid copepods for our ongoing project. In this study we wanted to test the capabilities of the MinION sequencer for our purposes. All our samples were taken from our South Adriatic and North Adriatic coastal sampling stations through 2021. For the test, we used 42 specimens of calanoids with 4 markers (COI, 18S, 28S, ITS1-5.8S- ITS2) with fragment lengths ranging between 900-1300 BPs. We used previously acquired Sanger sequencing data to compare and verify our results. In the sequencing run 92.5% of the sequences were recovered with 99.95% final accuracy. 48% of the errors turned out to be indels with 1:2 deletion, insertion ratio. The most common errors were related to cytosine and guanine bases. The comparison of the two datasets also revealed, that sites with nucleotide repeats can cause ambiguous bases in the Sanger final product, while Nanopore resolved these sites at ease. The only drawback of the method seems to be its sensitivity for alien contamination, while using universal primers. In conclusion: with appropriate pre- and post-processing, MinION sequencing, with its ability to generate long barcodes is a viable alternative for Sanger sequencing in building genetic databases.

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Ershova, Elizaveta A., Tone Falkenhaus. Resolving biases in diversity estimates and quantitative interpretation of zooplankton metabarcoding data

Metabarcoding is a rapidly developing tool in marine zooplankton ecology, although most zooplankton surveys continue to rely on visual identification for monitoring purposes. We attempted to resolve some of the biases associated with both metabarcoding and traditional zooplankton sorting by sequencing a 313 b.p. fragment of the COI gene in 34 “mock” samples from the North Sea which were pre-sorted to species level, with biomass and abundance estimates obtained for each taxonomic group. The mock samples were prepared from live environmental sub-samples collected with a WP11 net and modified by adding/removing taxa to maximize diversity and varied contribution of different taxonomic groups between samples. After counting, the samples were preserved either in 97% ethanol or dried for 24 hours in a drying oven at 65°C (the routine way of preserving samples for dry weight measurements). The visual identification yielded a total of 59 unique holoplanktonic and 16 meroplanktonic species/taxonomic categories. Metabarcoding identified 76 holoplanktonic and 107 meroplanktonic species/taxa, which included all but 3 of the species identified visually. The additional species within the holoplankton belonged mainly to the hard-to-identify copepods, ostracods and cnidarians. We demonstrate robust correlations of relative sequence abundances to relative biomass for most taxonomic groups, with the strongest correlations observed for the crustaceans, and suggest conversion factors for different taxa to account for sequencing biases. We also suggest a list of semi-quantitative parameters that can be calculated from a combination of metabarcoding and bulk biomass data and used as “indicators” of marine pelagic ecosystems for routine monitoring purposes. We also discuss the limitations of metabarcoding, and emphasize the continued need for an integrated methodological approach when assessing marine zooplankton communities.

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Fitzgerald, Catherine L., James J. Pierson, Louis V. Plough. How methods influence results: comparing morphological vs molecular identification of zooplankton in the water column and in the stomachs of fish

Zooplankton in the water column and in the stomachs of their predators can be identified with both morphological and molecular techniques. However, there are benefits and drawbacks to each method, and the data from each may even produce differing conclusions about the ecology of the system. Here, we sampled zooplankton and zooplanktivorous larval fish from the Choptank river, a brackish tributary of the Chesapeake Bay in Maryland, USA. Our samples were collected from May – September in 2018 and 2019, along a salinity gradient from 0.1 to 14 ppt. Zooplankton samples from the water column were identified using traditional morphological methods and metabarcoding with marker cytochrome oxidase 1 (CO1); the results were compared for taxonomic resolution, quantitative accuracy, and common measures of community composition and diversity, as well as for time investment and cost of generating the data. We also compared the results of CO1 metabarcoding of the stomach contents of larval fish from this study to previous studies using morphological techniques with respect to the fishes’ dietary composition, breadth, and selectivity. Our findings suggest benefits and challenges to using these different methods for monitoring and ecological process studies.

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Garić, Rade, Mirna Batistić, Marcell Dénes. New species of Doliolida (Tunicata, Thaliacea) from the Adriatic Sea

New species of Doliolida is found in the South Adriatic in autumn 2021. Blastozooids of this species are similar to *Dolioletta gegenbauri* in endostyl position (from MII 1/2 to MIV 1/3), overall body consistency (both species are fragile and easily damaged in net tows), position and length of the testis and ovary and the position of the branchial bar. The main difference in *Dolioletta* sp. nov. and *Dolioletta gegenbauri* is in that intestine does not form a tight coil and after forming a right loop as in *Doliolum nationalis*, it sharply turns left so that anal aperture is facing left body wall. The other difference between these two species is in that the ganglion is positioned between MIII and MIV, but closer to MIV in *Dolioletta* sp. nov., while in *Dolioletta gegenbauri* it is positioned closer to MIII. COI phylogenetic analysis showed that *Dolioletta* sp. nov. forms a clade with *Dolioletta gegenbauri* (100% bootstrap support), while the COI sequence difference between these two species is 16.8%. The finding of a new doliolid species in the Adriatic Sea continues the worrisome trend in recent decades of new gelatinous species being described every couple of years in this well investigated area. The geographic origin of this species is still unknown.

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Hirai, Junya, Seiji Katakura, Satoshi Nagai. Interactions between marine viruses and planktonic copepod *Pseudocalanus newmani*

Both zooplankton and viruses play key roles in marine ecosystems; however, their interactions have been overlooked. Here, we introduce a case study focusing on the copepod *Pseudocalanus newmani*, which is one of the dominant zooplankton during winter-spring in the western subarctic Pacific, and diversity and ecological roles of zooplankton were investigated. Two major viruses belonging to Picornavirales (PSNE-Pico1 and PSNE-Pico2) were detected with almost full genome sequences from *P. newmani* using transcriptome analysis with rRNA depletion. The RT-qPCR analyses revealed high viral copies and prevalence of PSNE-Pico1 in *P. newmani* from late June to July when the abundance of *P. newmani* decreased with increasing water temperature. On the other hand, high copy numbers and prevalence of PSNE-Pico2 were observed during spring, mainly in early April, corresponding to the period of *P. newmani* population decline. Transcriptome analysis with Poly-A selection revealed large physiological changes in *P. newmani* with high viral loads of PSNE-Pico1, and differentially-expressed genes associated with innate immunity were detected. These results suggest that marine viruses might be associated with population dynamics and physiological changes of *P. newmani*. Although we still need further analyses, including transmission systems of viruses to copepods and evaluation of mortality due to viruses, investigation of zooplankton-virus interactions provides a new view of marine ecosystems.

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**Kamchev, Pane, Le Qin Choo, Milan Malinsky, Erica Goetze and Katja Peijnenburg.
Using a targeted SNP panel to assess species boundaries in a pteropod from the Atlantic Ocean**

How do new species arise and persist in the open ocean? Holoplanktonic organisms, such as shelled pteropods, pose an appealing subject to address this intriguing question. As major consumers and calcifiers in the open ocean, pteropods fulfill indispensable biogeochemical and ecological roles. Yet, little is known about species boundaries in the seemingly homogenous habitat they occupy. Previous research characterized genomic variation across >100,000 Single Nucleotide Polymorphisms (SNPs) in 142 individuals from the Atlantic Ocean and identified three distinct populations separated by two narrow dispersal barriers in the subtropical species *Limacina bulimoides*. To gain more insight into the nature and possible permeability of these dispersal barriers, a denser sampling of both adults and juveniles is necessary, especially in the boundary regions. Moreover, with larger sample size we may identify rare hybrids. We designed a targeted SNP panel of 30 SNPs to efficiently screen a large number of individuals and assign them to one of the three populations while estimating levels of genetic mixing. The SNP panel offered the same resolution of population structure as the previous dataset of >100,000 SNPs. At the same time, we could determine the spatial partitioning of the boundary regions, their temporal stability, and the extent of migration of juveniles and adults. We found convincing evidence that dispersal barriers can be crossed but do not lead to genetic mixing. Hence, we conclude there are three reproductively isolated species of *Limacina bulimoides* in the Atlantic Ocean that are morphologically indistinguishable.

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Koh, Marc, Ilha Byrne, Iva Popovic, Sharon Appleyard, Stephanie Venables, Cynthia Riginos, and Julian Uribe-Palomino. First Barcoding of copepod Monstrilloids from two locations in Australian coastal waters (Coral Bay WA and Southern Great Barrier Reef QLD).

Monstrilloida is an understudied and underappreciated parasitic order of marine copepods. Not much is known about these organisms due to the rarity of the adult stages in plankton samples and lack of expertise in their identification and classification, which is mostly based on morphological characters. The taxonomic relationships among genera proposed for the Monstrilloida order, their diversity, their geographical distribution and population connectivity in Australian waters and around the globe, are some of the several issues that have not been well resolved for these microcrustaceans. Our research presents the preliminary results of genetic barcoding (COI) monstrilloid specimens from two geographically separated coastal areas of Australia: Coral Bay -WA (Indian Ocean) and the Southern part of the Great Barrier Reef (GBR) (Pacific Ocean). Consensus sequences (from Sanger sequencing) were compared to publicly available sequences in GenBank and BOLD and showed all Southern GBR and Coral Bay specimens are genetically unique when compared to all other previously sequenced Monstrilloids from elsewhere around the world. This work supports the importance of molecular evidence as a tool towards the validation of monstrilloid species and demonstrates the need for a suitable reference library that can be used for future biodiversity and environmental assessments.

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Laakmann, Silke, Astrid Cornils, Katja Metfies, Julian Koplín, Stefan Neuhaus, Carina Bunse, Barbara Niehoff, Hauke Flores. Integrative approach to study diversity and quantity of zooplankton in the Nansen Basin

To better understand the role of zooplankton in the Arctic ecosystem, we have studied their diversity and quantity at different taxonomic levels using molecular genetic and optical approaches. We analysed RMT1 net samples (300 µm mesh size) collected from twelve stations along a transect from the shelf to the Nansen Basin during Polarstern cruise PS106. The net samples were divided in subsamples for quantitative and molecular genetic multi-species analyses. Abundance and biovolume were obtained via ZooScan image analysis. Biomass was estimated by converting biovolume (equivalent for wet mass) to dry weight using established conversion factors for Arctic zooplankton. Metabarcoding of mitochondrial COI and nuclear ribosomal 18S variable regions 4 and 9 identified specimens on different taxonomic levels from more than 10 phyla and sequence assignment confirmed the accurate identification of Arctic zooplankton species using the MetaZooGene database v3.0 both for COI and 18S sequence data. In both diversity and quantitative analyses, Arthropoda predominate and copepods, in particular *Calanus* species, dominated the zooplankton community. As expected, on species level the metabarcoding analysis revealed a higher number of taxa compared to the quantitative analysis, in particular for Cnidaria and Ctenophora. It also allows a new view on distribution on morphologically similar, conspecific species. In our presentation we will discuss, how the number of reads on different taxonomic levels from phylum to species relates to abundance and biomass measured by image analysis and we will explore the potential of this integrative approach for a deeper insight in the zooplankton community structure.

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Lavrador, Ana S., Fábio G. Amaral, Jorge Moutinho, Pedro E. Vieira, Filipe O. Costa, and Sofia Duarte. Detection and monitoring of non-indigenous invertebrate species in recreational marinas through DNA metabarcoding of zooplankton communities in the North of Portugal

DNA metabarcoding on zooplankton communities can be an efficient approach to detect early the presence of non-indigenous species (NIS) in coastal ecosystems, as it allows the detection of species regardless of life stage or density, providing a quicker response to a potential biological invasion. We surveyed zooplankton communities (55 µm mesh net) to detect marine invertebrate NIS in two marinas in the North of Portugal - Viana do Castelo (VC) and Leixões (L) - in three seasons (summer, autumn and winter) (2020/2021), by using DNA metabarcoding of the mitochondrial cytochrome C oxidase subunit I gene (COI) and the variable region V4 of the nuclear small subunit ribosomal gene (18S). Overall, 18S recovered the highest number of species and NIS (413, 8 NIS), compared to COI (172, 6 NIS). Annelida was the most well represented phylum in the COI dataset, while Nematoda (VC) and Arthropoda (L), in the 18S dataset. Twelve NIS were recovered through all the study, but only 3 were detected in both marinas, and only one NIS was simultaneously detected with both markers. Maximum NIS were found in autumn in L (3) and in winter in VC (3). In both L and VC no NIS was detected in all seasons. These results show the efficiency of DNA metabarcoding for early detection of NIS in zooplankton, but also reveal the need to employ different molecular markers and sampling different seasons to guarantee a more thorough detection of NIS in these environments.

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Lian, Xiping, Jiaying Liu, Chaolun Li, Yehui Tan. Effects of coastal current and upwelling on the distributions of *Calanus sinicus* in the northern South China Sea and its response to the environment

Calanus sinicus, the dominant species of planktonic copepod in the continental shelf waters of China. And it is also the key species in the structural and functional research on ecological system in China's offshore. Previous research showed that the *C. sinicus* is carried into the Taiwan Strait from population centers in the East China Sea by the China Coastal Current during the northeast monsoon period in winter. This species disappeared due to high temperatures and did not begin to enter into the northwest continental shelf of South China Sea from the East China Sea during the period of investigation in winter. However, recent study found that neither population recruitment from the East China Sea nor the provided protection from cold eddy and upwelling, there are still having *C. sinicus* in the northwest continental shelf of the South China Sea. We use traditional taxonomy method of morphological research and molecular phylogenetic methods analyzed the genetic diversity of *C. sinicus* in China coastal waters after compared with *C. sinicus* in the Yellow Sea and East China Sea. It was found that there was no obvious population segregation between these areas, and most of the sequences measured in these regions were indistinguishable, but the samples collected in the northern South China Sea were more variation. Although there may be local replenishment in the northern South China Sea, due to the transportation of China's coastal currents, there should also be a large number of external supplements. ... This study explored the survival strategy and complementary mechanism of *C. sinicus* in the northern South China Sea, and provided a scientific basis for the study of the population dynamics of *C. sinicus* in the northern South China Sea.

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Liu, Weiwei, Xiaofeng Lin, Yehui Tan. Biodiversity of aloricate oligotrich ciliates in typical coastal habitats of southern China and phylogenetic evolution

Aloricate oligotrich ciliates are important components of marine microplankton. The biodiversity studies on this group of species in typical coastal habitats of southern China were carried out in the last decade and their phylogeny relationships were discussed based on molecular data. The main results of these studies include that, 1) a total of 39 species belonging to 17 genera of seven families and four orders of aloricate oligotrich ciliates were identified, among which 16 new species, four new genera, one new family and order were reported; 2) the order Oligotrichida exhibited highest species richness among all taxa and was widely distributed in all coastal habitats, and the wetland area represented the hotspot habitat in the coast of southern China with highest richness of oligotrich species; 3) the phylogeny study based on 18srDNA confirmed the monophyly of subclasses Oligotrichia and Choreotrichia; 4) based on molecular phylogeny analyses and morphological comparison, the hypothesis on the evolution in the family Strombidiidae is updated. Novistrombidium is suggested representing the evolutionary base, which gives rise to two main groups composed of Strombidium-like and non Strombidium-like genera, respectively.

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Maathuis, M.A.M., Polling M., Nijhuis E.H., Voorhuijzen M.M., Keur M.C., Vorst, H.V. van der, Thijssen M.M.A., Sakinan, S, Jak R.G. Development of zooplankton monitoring in the shallow and turbid Dutch Wadden Sea integrating DNA metabarcoding and automatic image analysis

The Wadden Sea is a shallow and turbid intertidal estuary, housing a variety of protected habitat types and protected species, including sea mammals, birds and fish. Although the importance of zooplankton in the food web is recognized in relation to nature values and conservation targets, no monitoring is currently present. Hence, information on zooplankton biodiversity, distribution and biomass is very scarce. Therefore, from March 2021, a year-round monitoring study was undertaken to study zooplankton dynamics and to develop a zooplankton monitoring plan based on DNA analysis combined with image analysis. Monthly zooplankton samples were taken from a boat at two locations by pumping and filtering 100 L water over a 200 µm net. In addition, stomach contents of Atlantic herring (*Clupea harengus*) and European sprat (*Sprattus sprattus*) were collected to elucidate zooplankton in their diet. Protocols for MinION DNA metabarcoding analysis and bioinformatic pipelines were developed and tested and combined with Zooscan automatic image analysis. Zooscan data provides information on abundance and size distribution of zooplankton groups at higher taxonomic level, which is proposed as an indicator for food availability for fish. DNA metabarcoding provides information on a much more refined taxonomic level achieved by using several markers, including COI, 18SV9 and 18SV4. The first results will be discussed, including the plans moving forward to combine all data to show seasonal dynamics in abundance and biodiversity for a first in-detail image of zooplankton dynamics in the Wadden Sea.

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Maud, Jacqueline L., Kevin Xu Zhong, Colleen Kellogg and Brian P.V. Hunt. Disentangling food webs: combining CRISPR with metabarcoding to understand zooplankton diets

DNA metabarcoding to elucidate zooplankton diets has become the go-to molecular technique in the field of marine ecology and food webs. However, where eukaryotes feed on other eukaryotes, the use of 18S rRNA elicits the preferential amplification of predator DNA, whereby “fresh” predator DNA completely overwhelms partially-digested prey DNA. Existing methods to reduce predator DNA amplification include gut dissection, PCR clamps, blocking primers and restriction enzymes. Gut dissections are intensely time-consuming, and predator DNA may still dominate sequences. Blocking primers may coblock nontarget species and therefore conceal information. CRISPR technology was unveiled in 2012 and together with Cas9 nuclease, has been hailed as the “simplest, most versatile and precise method of genetic manipulation”, and a revolutionary way to edit genes and DNA. Here we present a pilot study, applying a recent CRISPR-based methodology, CRISPR-Cas9 Selective Amplicon Sequencing (CCSAS) to zooplankton diet analyses. Single-guide RNAs (sgRNA) from 18S V4 were designed for key mesozooplankton species from the Strait of Georgia, British Columbia, Canada to direct Cas9 to digest zooplankton predator DNA, and therefore maximize non-predator reads. Additionally, we employed an 18S non-metazoan primer and COI for comparison. Results indicate that the CCSAS method yielded a higher proportion of diet reads than previous tests without CRISPR. CCSAS liberated degraded DNA fragments from predator amplification pressure and generated an increased diversity of prey DNA. In addition, CCSAS resulted in an increased proportion of symbiont, fungal and terrestrial DNA, allowing questions to be answered on other facets of the microbiome.

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McCartin, Luke, Annette F. Govindarajan, Jill McDermott, Santiago Herrera.
Zooplankton environmental DNA community composition is structured by depth in the epipelagic Gulf of Mexico

Environmental DNA (eDNA) metabarcoding complements other techniques for marine biodiversity assessment. In order to appropriately sample for eDNA and infer ecological information from eDNA sequencing, the spatial resolution of eDNA metabarcoding data must be defined. We conducted extensive sampling for eDNA at Bright Bank: a federally-protected offshore reef in the Gulf of Mexico that supports economically important pelagic and benthopelagic fish species. Our goal was to determine the spatial resolution of eDNA at this productive habitat. In total, 47 distinct combinations of location and depth ranging from 20 to 100 meters were sampled at the bank using a CTD-Niskin bottle rosette. Metabarcoding was conducted by sequencing the V9 barcode region of 18S rRNA using primers that amplify a broad range of invertebrate taxa. We found that copepod and ostracod eDNA comprised the majority of sequence variants in 45 of 47 samples. Hydrozoan, annelid and tunicate eDNA was also abundant (at least 10% of animal sequences in any given sample). eDNA community similarity across samples was most significantly correlated with sample depth...Community turnover at the Class and lower taxonomic levels support these differences. Among arthropod eDNA, the relative abundance of ostracods was significantly higher in samples collected below the thermocline (T-test, $P < 0.01$). Trachymedusae eDNA exhibited the opposite pattern within cnidarians. Our findings support model predictions that stratification in the water column limits the vertical mixing of environmental DNA. Our results suggest that eDNA metabarcoding sequencing is an effective tool to determine zooplankton community composition at a given depth.

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O'Brien, Todd D., on behalf of SCOR WG157 members and participants. The MetaZooGene Database (MZGdb): What we did, what we discovered, and where we are going next

The first Term of Reference for the MetaZooGene SCOR working group is to create a reference sequence database and atlas of DNA barcodes for marine zooplankton, with unique enhancements allowing targeted searches by ocean region, taxonomic group, and multiple genes. This new resource adds functionality, discovery, and access to content from multiple molecular databases and archives, including GenBank, BOLD, MIDORI, and SILVA. By accessing and cross-referencing molecular data and identified species collection records from the OBIS and COPEPOD databases, barcoding coverage and summary statistics have been generated for more than 80 taxonomic groups of zooplankton for multiple ocean basins and regions. For example, of the 500+ species of calanoid copepods commonly found in the North Atlantic, <40% (212 species) have available sequences for the cytochrome oxidase I (COI) barcode region. Within the copepod order Calanoida, family-level barcoding coverage ranges from 100% (e.g., Calanidae) to less than 15% (e.g., Scolecitrichidae), with most families having less than 65% of their species barcoded. Unique to the MZGdb, these barcode coverage statistics make it possible to determine priorities for future barcoding efforts, based on taxonomic groups and geographic regions with the smallest number records. This presentation will review the goals for the MetaZooGene Atlas and Database (MZGdb), explain challenges successfully addressed, describe a few remaining ones, and provide a glimpse into the future, with highlights of our planned work both for MetaZooGene WG157 and in partnership with other groups and programs with shared goals. MetaZooGene MZGdb is available: <https://metazoogene.org/MZGdb>

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Ohnesorge, Alica, Stefan Neuhaus, Lucie Kuczynski, Sarah Taudien, Uwe John, Bernd Krock, Silke Laakmann. A view on zooplankton diversity in temperate waters inferred from organismal and environmental DNA metabarcoding

The application of molecular genetic multi-species identification tools for biodiversity assessments is considered to be a great complement to traditional monitoring methods. In order to assess the marine metazoan biodiversity in temperate regions (North and Baltic Sea) and to evaluate multi-species approaches, we analyzed COI sequence data from metabarcoding of zooplankton-net samples and environmental DNA (eDNA). We assigned sequences to species level based on the MetaZooGene sequence reference database (MZGdb) with more than a third of the invertebrate species barcoded for the study area being available in this data base. Our aim was to evaluate the sequence assignments based on MZGdb with emphasis on species of our study area in the context of methodology and sequence reference database entries. Over the entire COI dataset, the reference database has been shown to cover a broad range of taxa as we were able to assign and identify more than 180 invertebrate species from in total 11 different phyla. Moreover, the overall reliability of species assignment has been shown to be very high as > 90 % of these identified species are known to occur in the North and Baltic Sea or adjacent waters. Our approach paints a comprehensive picture of the pelagic and benthic metazoan diversity and gives insights in ongoing recruitment processes in the zooplankton community. We conclude that the MZGdb reference database is a valuable tool to identify marine zooplankton as well as benthic invertebrates from both zooplankton net samples and eDNA.

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Rauch, Cessa, Francisca C. Carvalho, Jon A. Kongsrud, Anders Hobæk and Tone Falkenhaus. Hyperbenthic Copepods (HYPCOP) in Norwegian waters

Copepoda are an extremely diverse group that is found all over the world in both marine and freshwater habitats. Species can be planktonic or parasitic, and a large group of them live in the hyperbenthic zone. Copepods are a very important food source for many organisms and an important link between primary producers and higher trophic levels. Copepods also have a significant role in vertical flux of carbon and may contribute to the benthopelagic coupling. Despite being so significant, we have limited knowledge about the biodiversity and taxonomy of these animals, especially the species that live in the hyperbenthic zone in Norway. In order to unravel the biodiversity and taxonomy of hyperbenthic copepods, the Norwegian Biodiversity Information Center (Artsdatabanken) is funding the project HYPCOP (Copepods in hyperbenthic habitats), with special focus on the species in the group of Harpacticoida. Copepods were collected in shallow waters in the coastal areas and deeper waters in the fjords and the continental shelf. When possible, they were photographed live and barcoded with 16S & COI in order to build up a reference library. The end goal is to survey the diversity of marine copepods in Norwegian waters and we expect to find and describe species that are new to science and new for Norway. Currently, some taxonomic competence in Norway is lacking, but through collaboration with foreign experts this knowledge will increase among Norwegian researchers and students.

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Schouten, Bo A., Lotte J. Bouwman, Catharina de Weerd, Jaap de Boer, Erica Goetze, Katja T.C.A. Peijnenburg. Integrative taxonomic approach uncovers hidden diversity within the elusive *Peracle* genus (Pteropoda)

Pteropods are a group of holoplanktonic gastropods, which are regarded as bioindicators of the effects of ocean acidification because of their delicate aragonite shells. However, identification of species based on their shell morphology alone can be problematic, particularly for lesser-known groups such as the deeper dwelling *Peracle* genus (Pseudothecosomata). Most species belonging to this genus are characterised by unique calcareous reticulate mesh patterns on the top coil of their shell, but their taxonomy is not well-resolved. The *Peracle* genus is circumglobally distributed and can be locally abundant, however, it has been largely overlooked in ocean acidification research and is excluded from e.g. global estimates of CaCO₃ export to the deep sea. Currently, eight *Peracle* species are described across all oceans (World Register of Marine Species). We applied an integrative approach combining state-of-the-art imaging (including SEM, Micro-CT) and DNA barcoding (CO1, 28S) to assess species boundaries in 194 *Peracle* specimens sampled from the Atlantic and Pacific Oceans. Preliminary phylogenetic analyses uncovered multiple evolutionary independent groups, some of which could not be linked to existing species descriptions. However, most groups could be distinguished by distinctive shell characters such as the type of reticulate patterns or crests. Hence, the number of *Peracle* species in the world's oceans needs to be revised.

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Spaggiardi, Giada, Le Qin Choo, Erica Goetze, Katja Peijnenburg. Species boundaries in the *Limacina* species complex

Holoplanktonic pteropods are widely regarded as bioindicators of ocean acidification due to their thin aragonitic shells vulnerable to dissolution. The *Limacina* genus is most frequently used for this purpose owing to its high abundance and widespread distribution across all oceans. Different species and populations can exhibit different sensitivities to environmental stressors, depending on their genetic backgrounds and evolutionary histories. Therefore, a detailed assessment of the current species boundaries and distribution ranges is needed to better predict their species-specific potential to respond to ocean changes. Here, we assessed diversity across the five nominal species in the *Limacina* genus with an integrative approach combining morphology with DNA barcoding (cytochrome c oxidase subunit I, 28S rDNA). Shell shape was studied for 302 individuals based on linear and geometric morphometric analyses on stacking microscopy images and micro-CT scans. We found that shell thickness and volume varied significantly across species, and cold-water species were larger. All five extant species could be identified based on shell shape except for *L. lesueurii* and *L. helicina*, which showed overlapping shell shape distributions. We found evidence for unrecognized diversity in the Arctic and Antarctic populations of *L. helicina* and *L. retroversa*. Our analyses also suggest that there may be hidden diversity within the warm-water species, considering they occur in distinct ocean basins. The levels of divergence may imply substantial differences in physiology and adaptive potential to climate change. We conclude that a taxonomic revision of the *Limacina* genus is a prerequisite to their use as bioindicators in acidification research.

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Stedman, Gabrielle, Oliver Kersten, Craig R. Smith, Jeffrey C. Drazen, Erica Goetze.
Community ecology and spatial patterning of abyssal benthopelagic plankton in the CCZ.

Plankton densities are greater within the abyssal benthic boundary layer (BBL) than in the overlying abyssopelagic zone because increased turbulence and particle concentration enhance food availability and biomass in a food-poor habitat. The benthopelagic community is comprised of both holoplankton, which may be specialized to the BBL, and the larval phases of many benthic species that are critical to the dispersal, colonization, and recovery of benthic populations. Characterization of the BBL community is needed to better understand its importance to abyssal ecosystem function, and to assess the potential extinction risks associated with deep-sea mining. We conducted the first regional-scale assessment (100-5,000 km) of abyssal plankton communities by comparing the eastern and western regions of the Clarion Clipperton Zone (CCZ). Twenty-eight in-situ pump deployments within the BBL (3 mab) yielded 24,542 individuals (>63 μm) that were identified and enumerated. Zooplankton densities exhibited both N/S and E/W spatial gradients in the CCZ and covaried with estimated POC flux, with nearly 2X higher total density in the east (~103 ind./1000m³) than in the west (~59 ind./1000m³). Holoplankton were dominated by copepods and their nauplii (97% plankton abundance) with subdominant taxa including chaetognaths, ostracods, and pelagic polychaetes. Meroplankton were patchy in abundance (0.65 – 6.83 ind/1000m³, 0 – 1.4 in/1000m³ in the east and west respectively) with taxa including polychaetes, echinoderms, bivalves, gastropods, barnacles, and bryozoans. Our results quantify baseline BBL communities over large areas of the deep seafloor mining and highlight future research directions to develop a richer understanding of this BBL community.

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van der Pouw Kraan, Dennis, Conor Graham, Fiona Kavanagh, Luca Mirimin.
Magnifying the resolution of (photo)microscopy with high-throughput molecular screening of important shellfish species in Irish coastal waters

Shellfish play essential roles in ecosystems and represent a significant component of the aquaculture industry. Understanding patterns in habitat preference and recruitment is key to appropriate management strategies but requires effective monitoring tools. Photo-microscopy provides a more time-efficient alternative to conventional microscopy but has a lower taxonomic resolution for cryptic taxa and early life stages (e.g., larvae), which can be resolved with DNA-based approaches. In this study, we used (photo)microscopy in combination with a new high-throughput (HT)-qPCR tool (consisting of 24 species-specific assays) and HT Sequencing to estimate the presence/absence and (relative) abundance of important shellfish species in heterogeneous environmental (plankton) samples. Known numbers of D-stage pacific oyster larvae have been spiked in additional plankton samples to be directly correlated with DNA copies, hence enabling larval abundance estimations per detected species. The genetic tools successfully detected the presence of mussel, queen scallop, common cockle, razor, soft-shell, and surf clams, velvet, brown and green shore crabs, and European lobsters, amongst other species. The number of larvae counted by microscopy correlated with copy numbers and larval estimates obtained with HT-qPCR screening. This study highlighted the invaluable potential of high-throughput molecular tools to enhance photomicroscopy by substantially increasing the taxonomic resolution and providing unprecedented data. Such multidisciplinary methods allow for more effective monitoring, which can improve the management of natural resources and aid national sustainable growth of the seafood sector.

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van Wijk, Danique, Iliana Bista, Daniëlle van der Burg, Geert-Jan Brummer, Katja T.C.A. Peijnenburg. Identifying pteropod diversity through metabarcoding of different types of environmental samples

Shelled pteropods are holoplanktonic gastropods which are regarded as bio-indicators of ocean acidification because their thin aragonitic shells are susceptible to dissolution in acidified conditions. However, characterizing their capacity for species-specific responses to environmental stressors is hindered by continued uncertainty of their geographic distributions in space and time. The need for efficient and accurate methodologies for monitoring pteropod diversity are therefore urgently needed. Metabarcoding provides a relatively quick and inexpensive way of categorizing species diversity in complex environmental samples. This study aims to design and test new primers targeting short barcode regions in the CO1 and 18S genes, that can recover pteropod diversity in environmental samples. Different types of samples to be tested include ancient sediments, long-term time-series samples, and sediment trap samples. Preliminary results reveal several promising primer pairs that work well across the diversity of pteropod species. The results from the metabarcoding analysis will be compared to species counts from the same sediment trap samples.

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Yebra, Lidia, Isabel Ferrera, Ana María Cabello, Miriam Domínguez, Jessica Vannini, Genuario Belmonte, Nerea Valcárcel-Pérez, Candela García-Gómez, Francisco Gómez-Jakobsen, Rowena Stern, Jesús M. Mercado. Characterization of microzooplankton communities in a polluted coastal area integrating high-throughput sequencing and microscopy

The Mediterranean Sea is subjected to strong anthropogenic pressures that may be causing important ecosystem changes, particularly in coastal areas under high anthropogenic pressure. We characterized the composition of the microzooplankton community in a coastal area in the N Alboran Sea (SW Mediterranean) highly impacted by urban wastewater pollution. Two offshore outfalls release urban wastewater to the sea at a 40 m bottom depth, from a nearby town. We applied an integrative taxonomic approach, combining metabarcoding of the mitochondrial COI and the 18S rRNA genes with morphological microscopic identification of organisms, collected with a CalVET net (50 µm mesh). Hydrology was notably affected near the bottom at the vicinity of the submarine emissaries exit, presenting increased temperature and turbidity, and decreased salinity due to the urban freshwater discharge. Nutrient concentrations exceeded the Water Framework Directive limits; however, chlorophyll a concentrations were not very high, due to strong water column stratification. Microzooplankton communities (50-200 µm) were dominated by dinoflagellates (50-80% relative abundance), followed by copepods (copepodites and nauplii), eggs and cysts. We found significant differences in communities' composition between the coastal shallow area and the offshore waters, driven by pollution and stratification.

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Zhou, Fanyu, Junya Hirai, Koji Hamasaki, Atsushi Tsuda. Feeding ecology of three euphausiid species in the low-latitude Eastern Indian and South Pacific Oceans inferred from 18S V9 metabarcoding

Euphausiids are important links between lower and higher trophic levels in marine ecosystems, yet their detailed diets have not been fully understood via conventional methods such as microscopical observation and stable isotope analysis, especially in tropical/subtropical water areas. Here we used 18S V9 metabarcoding to reveal the gut content compositions of three dominant euphausiid species in the low-latitude Eastern Indian and South Pacific Oceans (*Euphausia diomedea*, *Nematoscelis gracilis*, and *Thysanopoda tricuspidata*) and compare their feeding habits. An evident omnivorous dietary mainly including Dinoflagellata, Stramenopiles (Diatomea and Ochrophyta), Chlorophyta, and Copepoda was confirmed for all three species; however, *N. gracilis* displayed a more carnivorous feeding habit suggested by a significantly lower proportion of Dinoflagellata sequence reads (average 28.97%; 42.01% in *E. diomedea* and 54.09% in *T. tricuspidata*) and a higher Copepoda percentage (average 10.97%; 8.02% in *E. diomedea* and 5.65% in *T. tricuspidata*) in the gut. Furthermore, different feeding patterns across species were verified based on the dominant prey operational taxonomic units (OTUs) observed in each species ... A remarkable regional difference in feeding pattern was discovered between the Eastern Indian *E. diomedea* and the South Pacific ones, which was consistent with the environmental community pattern. These results indicate that the feeding habits of three euphausiid species are flexible and shaped by the prey availability. Additionally, the potential of dominant euphausiid species switching prey would promote their wide distribution across different environments, and the different prey preference of the studied species is likely to support the co-existence of the studied species.

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