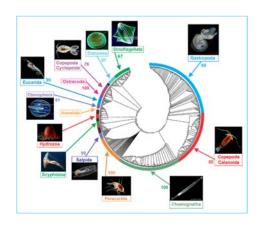




Toward a new global view of marine zooplankton biodiversity based on DNA metabarcoding and reference DNA sequence databases

Pennie Lindeque, Plymouth Marine Laboratory Ann Bucklin, University of Connecticut (USA) Ocean Biomolecular Observing Network Meeting Sept. 22









metazoogene.org/ and scor-int.org/group/157

SCOR WG157

MetaZooGene



2019 - Gothenburg Global Biodiversity Centre (Sweden)



2020 - Ocean Sciences Meeting, San Diego (USA)

Total of 23 members from 19 countries



Chair and Co-Vice-Chairs

- Ann Bucklin (Chair), Univ. Connecticut (USA)
- Katja Peijnenburg (Vice-Chair), Naturalis Biodiversity Centre (NL)
- Ksenia Kosobokova (Vice-Chair), Russian Acad. Sciences (RU)

SCOR WG157 Full Members:

- Leocadio Blanco-Bercial, Bermuda Inst. Ocean Sciences (BM)
- Tone Falkenhaug, Inst. Marine Research (NO)
- Junya Hirai, Univ. Tokyo (JP)
- Jenny Huggett, Dept. Environmental Affairs (SA)
- Chaolun Li, Chinese Acad. Sciences (CN)
- Ryuji Machida, Academia Sinica (TW)
- Todd O'Brien, NOAA Fisheries (USA)

SCOR WG157 Associate Members:

- Keun-Hyung Choi, Chungnam National Univ. (KR)
- · Astrid Cornils, Alfred Wegener Inst. (DE)
- Bruce Deagle, Australian Antarctic Div. (AU)
- Ruben Escribano, Univ. Concepción (CL)
- Erica Goetze, Univ. Hawaii at Manoa (USA)
- Tamar Guy-Haim, National Inst. Oceanography (IL)
- Aino Hosia, Univ. Museum of Bergen (NO)
- Silke Laakmann, Univ. Oldenburg (DE)
- Pennie Lindeque, Plymouth Marine Lab (UK)
- Maria Grazia Mazzochi, Stazione Zool. Anton Dohrn (IT)
- Mary Mar Noblezada, Univ. Philippines (PH)
- Naiara Rodriguez-Ezpeleta, AZTI (ES)
- Agata Weydmann, Univ. Gdańsk (PL)





Primary focus:

~8,000 species of holozooplankton (15 phyla, 32 classes) of animals that drift with ocean currents

Primary goal:

Integrative molecular – morphological taxonomic analysis of marine zooplankton biodiversity throughout global ocean

Terms of Reference

- 1) Create an open-access web portal for DNA barcodes for marine zooplankton
- 2) Design an optimal DNA barcoding pipeline for marine zooplankton
- 3) Develop best practices for DNA metabarcoding of marine zooplankton biodiversity

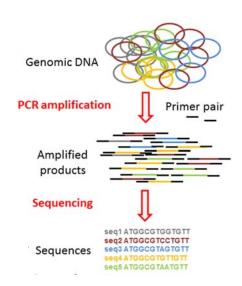


Metabarcoding of Marine Zooplankton







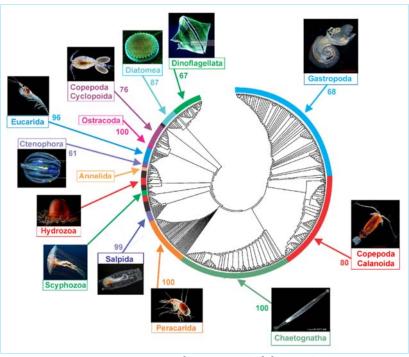


- Extraction of genomic DNA from environmental samples
- High-throughput DNA sequencing (Illumina MiSeq)
- PCR of short gene regions (mtCOI, 18S rRNA, others)
- Bioinformatic pipelines (Mothur, DADA2) for sequence QC and analysis
- Statistical analysis of biodiversity (Mothur, R, MatLab)

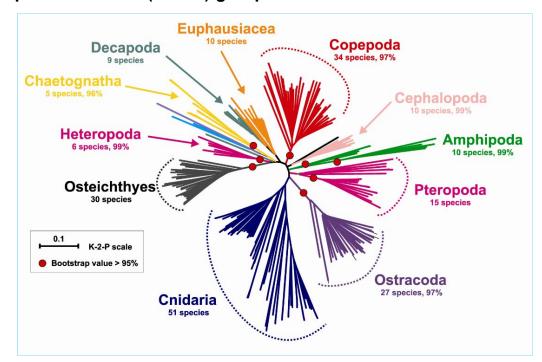


Metabarcoding of Zooplankton Diversity

- 18S rRNA "Tree of Life" gene resolves groups (not species) of pelagic assemblage
- MtCOI barcode region identifies species of some (not all) groups



18S rRNA V9 hypervariable region Figure: Bucklin et al. (2019)



MtCOI barcode region Figure: Bucklin et al. (2010)



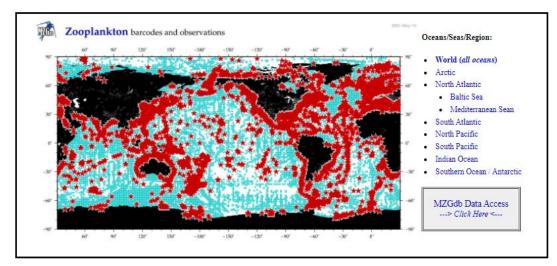


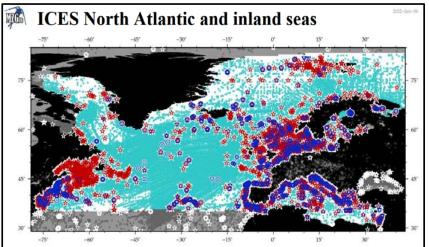




MetaZooGene Barcode Atlas & Database (MZGdb) Todd O'Brien, NOAA Fisheries (USA)

- Over 218,299 COI sequences for 11,356 species of marine zooplankton
- MZGdb expanded to include 18S rRNA & other gene regions; adding fish & protists
- Data from NCBI GenBank & BOLD (duplicates removed)
- Searchable by taxonomic group and ocean region











Exploring Marine Zooplankton with the MetaZooGene Barcode Atlas and Database (MZGdb) Todd O'Brien*, NOAA Fisheries (USA)



"What we know, where we know it, and what we still need to discover"

Barcoding/Biodiversity Question	GenBank / BOLD	COPEPOD / OBIS	MZGdb
How many zooplankton species have already been barcoded?	1	n/a	√ ,
Which species are found in my ocean or region of interest?	×	-	1
Which species (globally or in my focus region) have <u>not</u> been barcoded?	×	n/a	1
Which taxonomic groups are "under-barcoded" in my region?	×	n/a	1
Which oceans and taxa have the greatest need for new barcoding?	×	n/a	1

ICES CIEM







http://metazoogene.org/atlas

COPEPOD: Coastal and Oceanic Plankton Ecology, Production, and Observation Database



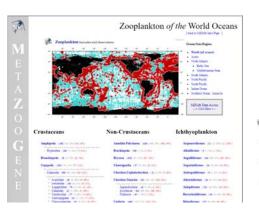
The MetaZooGene Barcode Atlas and Database http://metazoogene.org/mzgdb

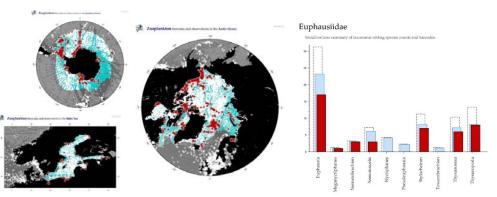
an **ATLAS** with *thousands* of **maps** and figures and summary tables that detail coverage by taxonomic group, and geographic region, and barcode type.

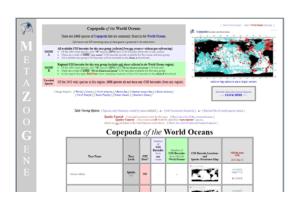
- Which taxa groups and ocean regions have the best coverage?
- Which taxa groups and regions need work (e.g., ideal for expanded/future sampling focus)?

a **DATABASE** with **data** divided by taxa group, geographic region, and barcode type.

- Reduce data processing by limiting barcodes to the taxa and geographic regions relevant to your work.
- The Mediterranean Sea has 1/3 of the species found in the North Atlantic, and 1/9 of "global" species.









What we discovered ...

- Best barcoding **geographic (spatial) coverage** was usually along the coastlines, and densest in the North Atlantic and North Pacific.
 - The Indian Ocean and South Pacific need more barcode sampling.
- Best barcoding taxonomic (species) coverage was found in the larger (easier to identify?), dominant/common species and taxa groups.
 - Barcodes for rarer species will be needed for accurate biodiversity applications.
- The original MZGdb focus was "marine zooplankton" ... but ...
 - *benthic* species ("*meroplankton*") are often present (especially nearshore)
 - our Baltic Sea and estuarine colleagues/users have non-marine species
 - zooplankton nets also capture various phytoplankton and larval fish
 - eDNA wants "everything else" (marine mammals and sea turtles)

Toward a global reference database of COI barcodes for marine zooplankton. Marine Biology (2021)



https://doi.org/10.1007/s00227-021-03887-y

Bucklin, A.*, K.T.C.A. Peijnenburg*, K.N. Kosobokova*, T.D. O'Brien*, L. Blanco-Bercial*, A. Cornils*, T. Falkenhaug*, R.R. Hopcroft, A. Hosia*, S. Laakmann*, C. Li*, L. Martell, J.M. Questel^{EC}, D. Wall-Palmer^{EC}, M. Wang, P.H. Wiebe, A. Weydmann-Zwolicka^{EC}



- Introduce, explain and promote the MetaZooGene Barcode Atlas and Database (MZGdb, https://metazoogene.org/MZGdb)
- Acknowledgements: MetaZooGene (SCOR WG157) and NSF



Patterns of Biodiversity of Marine Zooplankton Based on Molecular Analysis

- Special issue will be published in 2021; 14 papers available online
- Howard I. Browman ICES JMS Editor-in-Chief
- Ann Bucklin*, Katja Peijnenburg*, Ksenia Kosobokova*, Ryuji Machida* Themed Set motivators

Of 15 Publications: 7 papers by co-authors from MetaZooGene WG157:

- Bucklin, A.*, Peijnenburg, K.T.C.A.*, Kosobokova, K.*, Machida, R.J.* (2021) New insights into biodiversity, biogeography, ecology, and evolution of marine zooplankton based on molecular approaches. Introduction to the Themed Set. ICES JMS https://doi.org/10.1093/icesjms/fsab198
- Bucklin, A.*, Questel, J.M.^{EC}, Blanco-Bercial, L.*, Frenzel, A.^{EC}, Smolenack, S.^{EC}, and Wiebe, P.H. (2021) Population connectivity of the euphausiid, *Stylocheiron elongatum*, in the Gulf Stream (NW Atlantic Ocean) in relation to COI barcode diversity of *Stylocheiron* species. ICES JMS, https://doi.org/10.1093/icesjms/fsab158
- Di Capua, I. EC, Piredda, R., Mazzocchi, M.G.*, and Zingone, A. (2021) Metazoan diversity and seasonality through eDNA metabarcoding at a Mediterranean long-term ecological research site. ICES JMS, https://doi.org/10.1093/icesjms/fsab059
- Hirai, J.* EC, Hidaka, K., Nagai, S., and Shimizu, Y. (2020) DNA/RNA metabarcoding and morphological analysis of epipelagic copepod communities in the Izu Ridge off the southern coast of Japan. ICES JMS, https://doi.org/10.1093/icesjms/fsab064
- Machida, R.J.*, Kurihara, H., Nakajima, R., Sakamaki, T., Lin, Y.-Y., and Furusawa, K. (2021) Comparative analysis of zooplankton diversities and compositions estimated from complement DNA and genomic DNA amplicons, metatranscriptomics, and morphological identifications. ICES JMS, https://doi.org/10.1093/icesjms/fsab084
- Matthews, S.A. ^{EC}, Goetze, E.*, and Ohman, M.D. (2021) Recommendations for interpreting zooplankton metabarcoding and integrating molecular methods with morphological analyses. ICES JMS, https://doi.org/10.1093/icesjms/fsab107
- Parry, H.E., Atkinson, A., Somerfield, P.J., and Lindeque, P.K.* (2021) A metabarcoding comparison of taxonomic richness and composition between the water column and the benthic boundary layer. ICES JMS, https://doi.org/10.1093/icesjms/fsaa228











ASLO 2021

Aquatic Sciences Meeting 22-27 June 2021



SS32 Session Co-Chairs: Silke Laakmann*, Ann Bucklin*, Katja Peijnenburg*, Leocadio Blanco-Bercial*





















SS32 - Name that species: Toward a new global view of species diversity of marine zooplankton

Silke Laakmann^{1,2}, Ann Bucklin³, Katja T.C.A. Peijnenburg^{4,5}, Leocadio Blanco-Bercial⁶

1 Helmholtz Institute for Functional Marine Biodiversity at the University of Oldenburg (HIFMB), 2 Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, 3 University of Connecticut, 4 Naturalis Biodiversity Center, 5 University of Amsterdam, 6 Bermuda Institute of Ocean Science

















- 7 Talks, 2 Posters
- **Invited talks by WG157** members: Astrid Cornils* & Todd O'Brien*
- Good audience turnout (>40 people)
- **Discussion of best practices** for molecular analysis of zooplankton species diversity



ICES 2021 Annual Science Conference



6-10 September 2021 (Virtual)

Theme Session D:

Past, present, and future of marine plankton assemblages and communities Conveners: Dafne Eerkes-Medrano (UK), Piotr Margoński (Poland) Todd D. O'Brien* (USA)



Topics and approaches:

- empirical analysis of time series observations
- numerical and statistical modelling
- molecular genetic analysis of diversity or function
- new techniques to observe community change

Talks by MetaZooGene SCOR WG157 members*:

- * O'Brien, T.D., Spatiotemporal visualization of the North Atlantic copepod community change
- * Bucklin, A., et al. *Time-series COI metabarcoding of zooplankton species diversity*



OSM 2022 27 Feb – 4 Mar 2022 Hawaii, USA (Hybrid & Virtual)



Zooplankton diversity through space and time (ME20)

Co-Chairs: Katja Peijnenburg* (NL), Erica Goetze* (USA), Galice Hoarau (NO), Matthew Miller (CA)

This session will explore new insights into zooplankton, their diversity and roles in the ecosystem, which are being revealed through emergent approaches, such as 'omics and/or environmental DNA methods, imaging techniques combined with machine learning, and/or trait-based or distribution modelling, while also being inclusive of studies using more conventional methods.

This session is co-organised by SCOR WG157 MetaZooGene and is open to all members of the ocean science community. See: https://metazoogene.org



MetaZooGene Symposium

New Insights into Biodiversity, Biogeography, Ecology and Evolution of Marine Zooplankton Based on Molecular Approaches



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



Symposium in-person participants, including 11 Early Career Scientists.

Presenters & participants (~25 people) also joined virtually.

Sponsor: Scientific Committee for Ocean Research (SCOR); MetaZooGene Working Group (SCOR WG157)

MetaZooGene Symposium associated with ICES 2022 Annual Science Conference

Aviva Stadium, Dublin, Ireland - September 23, 2022

https://metazoogene.org/symposium2022







Focus on Early Career Scientists

Early Career colleagues & students have joined all WG157 activities.

- Participants at MetaZooGene meetings
- Presenters at special sessions
- Co-authors of WG157 publications
- Receive regular updates via WG157 email list

Capacity Building Workshops planned by WG157 for training in molecular & bioinformatics methods not yet possible.

MetaZooGene Early Career Symposium
September 10, 2022
ICES Annual Science Conference
Dublin, Ireland (In-person)



Metabarcoding Zooplankton Diversity

Ocean Decade Action No. 102.2













- MetaZooGene: Metabarcoding Zooplankton Diversity is endorsed as a new UN Ocean Decade Action (No. 102.2; https://www.oceandecade.org/)
- The project is attached to the Ocean Decade Programme, *Marine Life 2030* (https://marinelife2030.org/)

