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

[metazoogen.org](http://metazoogen.org) and [scor-int.org/group/157](http://scor-int.org/group/157)
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- Keun-Hyung Choi, Chungnam National Univ. (KR)
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- Bruce Deagle, Australian Antarctic Div. (AU)
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- Pennie Lindeque, Plymouth Marine Lab (UK)
- Maria Grazia Mazzochi, Stazione Zool. Anton Dohrn (IT)
- Mary Mar Nobleza, Univ. Philippines (PH)
- Naiara Rodriguez-Ezpeleta, AZTI (ES)
- Agata Weydmann, Univ. Gdańsk (PL)

Total of 23 members from 19 countries
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

http://metazoogene.org/atlas & https://wgimt.net/molecular/atlas
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”

<table>
<thead>
<tr>
<th>Barcoding/Biodiversity Question</th>
<th>GenBank / BOLD</th>
<th>COPEPOD / OBIS</th>
<th>MZGdb</th>
</tr>
</thead>
<tbody>
<tr>
<td>How many zooplankton species have already been barcoded?</td>
<td>✔</td>
<td>n/a</td>
<td>✔</td>
</tr>
<tr>
<td>Which species are found in my ocean or region of interest?</td>
<td>✗</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Which species (globally or in my focus region) have not been barcoded?</td>
<td>✗</td>
<td>n/a</td>
<td>✔</td>
</tr>
<tr>
<td>Which taxonomic groups are “under-barcoded” in my region?</td>
<td>✗</td>
<td>n/a</td>
<td>✔</td>
</tr>
<tr>
<td>Which oceans and taxa have the greatest need for new barcoding?</td>
<td>✗</td>
<td>n/a</td>
<td>✔</td>
</tr>
</tbody>
</table>

http://metazoogene.org/atlas

COPEPOD: Coastal and Oceanic Plankton Ecology, Production, and Observation Database
The MetaZooGene Barcode **Atlas and Database**

[http://metazoogene.org/mzgdb](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*)
https://doi.org/10.1007/s00227-021-03887-y


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

Photos by R.R. Hopcroft and C. Clarke (UAF) and L.P. Madin (WHOI); see http://www.cmarz.org/galleries.html
MetaZooGene

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:

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

Silke Laakmann1,2, Ann Bucklin3, Katja T.C.A. Peijnenburg4,5, 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
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
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)

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)
MetaZooGene: 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/)
Thank You

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