Symposium Report

Rediscovering pelagic biodiversity: Progress, promise, and challenges of metabarcoding of microbes to mammals

Convenors: Ann Bucklin (University of Connecticut, USA) and
Bengt Karlson (Swedish Meteorological and Hydrological Institute, Sweden)
Sponsor: Scientific Committee for Ocean Research (SCOR); MetaZooGene Working Group (SCOR WG157)

Svenska Mässan, Swedish Exhibition & Congress Centre, Gothenburg, Sweden
September 13, 2019

Topics addressed: The Symposium was organized into three sessions, each focused on a topical area (see Symposium Program, Appendix I). Session I, *Diversity of the pelagic assemblage*, included 6 oral and 6 poster presentations. There were 4 talks and 4 posters for Session II, *Data and database resources*. Session III, *Exploring the potential of metabarcoding*, included 7 talks.

Discussion Session: The talks were followed by a discussion session organized and led by Anders Andersson and Katja Peijnenburg. Four groups were formed among the participants, with each group charged with addressing 5 questions. Outcomes and recommendations from the discussion are summarized briefly in Appendix II.

Participation: The SCOR Symposium was attended by 50 people, which was the maximum capacity allowed for the venue. Names, institutions, and email addresses are listed in Appendix III. Participants attending the Symposium came from 20 different countries. Countries with the most attendees included Sweden (10 participants), United States (6), Norway (5) and Germany (5).

Online documents available: The SCOR *Rediscovering Pelagic Biodiversity* Symposium program and abstracts can be found online at the SCOR WG157 MetaZooGene website (see https://metazoogene.org/).

Symposium sponsors: The Symposium was sponsored by the Scientific Committee on Oceanic Research (SCOR) and was also associated with UNESCO - Intergovernmental Oceanographic Commission (IOC) and the International Council for the Exploration of the Sea (ICES) 2019 Annual Science Conference (September 9-12, 2019; Gothenburg, Sweden).

Appendix I. Symposium Program

Rediscovering pelagic biodiversity:
Progress, promise, and challenges of metabarcoding of microbes to mammals

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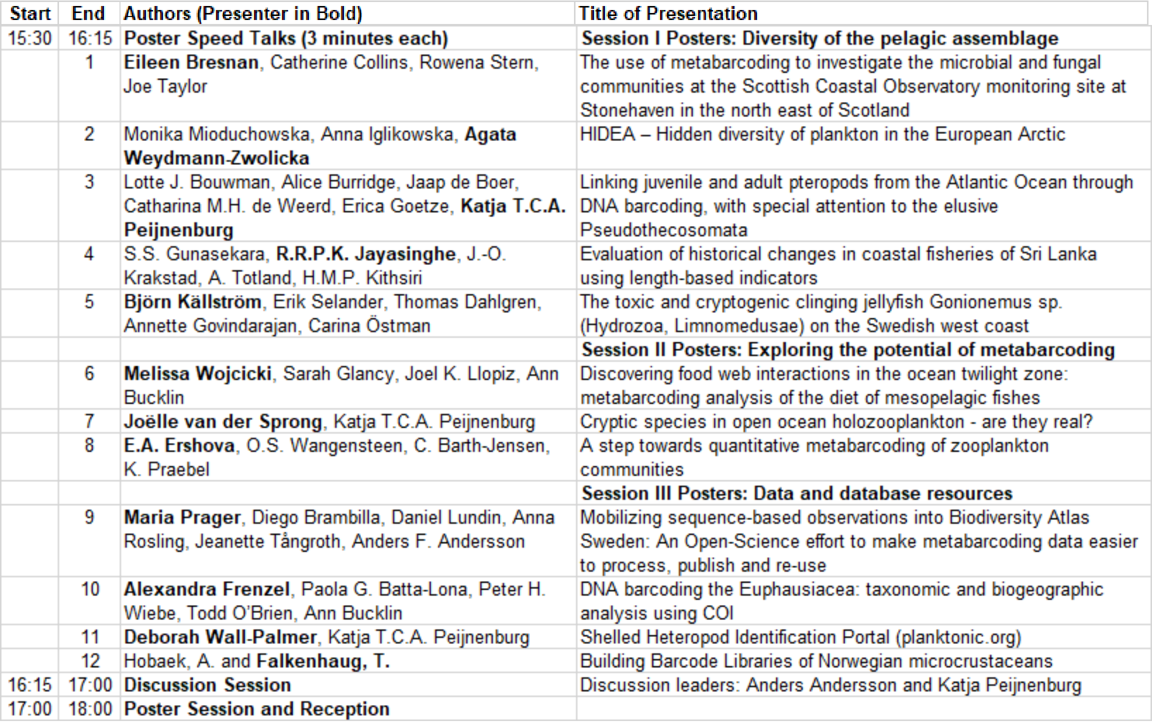
Start	End	Authors (Presenter in Bold)	Title of Presentation
8:00	8:45	**Registration & Coffee**	
8:45	9:00	**Welcome to the Symposium**	Ann Bucklin and Bengt Karlson
9:00	9:05	**Introduction to Session I**	**Session I: Diversity of the pelagic assemblage**
9:05	9:20	**Katja T.C.A. Peijnenburg**, Le Qin Choo, Alice Burridge, Lisette Mekkes, Deborah Wall-Palmer, Erica Goetze	Diversity and evolution of pteropods along latitudinal transects in the Atlantic Ocean
9:20	9:35	**Leocadio Blanco-Bercial**	Annual cycle of mesozooplankton in the Sargasso Sea
9:35	9:50	**Carolina E. González** , Erica Goetze, Rubén Escribano	Genetic structure and endemism in the cosmopolitan copepod *Pleuromamma abdominalis* in the Southeast Pacific
9:50	10:05	**Junya Hirai**, Kiyotaka Hidaka, Satoshi Nagai, Yugo Shimizu, Tadafumi Ichikawa	Metabarcoding analysis of planktonic copepods in the Kuroshio region off the southern coast of Japan
10:05	10:20	**Deborah Wall-Palmer**, Katja T.C.A. Peijnenburg	Raising awareness of the atlantid heteropods (Gastropoda, Pterotracheoidea) - calcifying holoplanktonic predators.
10:20	10:35	**Henna Savela**, Sanna Suikkanen, Anke Kremp	Seed banks as reservoirs of diversity driving evolutionary dynamics and persistence of Baltic phytoplankton in a changing environment – the RESERVOIR project
10:35	11:00	Coffee Break	
11:00	11:05	**Introduction to Session II**	**Session II: Data and database resources**
11:05	11:20	**Ryuji Machida**, Matthieu Leray, Nancy Knowlton	GenBank is a reliable resource for 21st century biodiversity research
11:20	11:35	**Li Chaolun**, Wang Minxiao, Cheng Fangping, Wang Xiaodong	Assessment of metabarcode as advanced tools
11:35	11:50	**Todd D. O'Brien**	MZGdb: The MetaZooGene database
11:50	12:05	Johannes Alneberg, Christin Bennke, Sara Beier, Carina Bunse, Christopher Quince, Karolina Ininbergs, Lasse Riemann, Martin Ekman, Klaus Jürgens, Matthias Labrenz, Jarone Pinhassi, **Anders F. Andersson**	A comprehensive catalogue of Baltic Sea bacterioplankton genomes
12:05	13:15	**Lunch on Site**	
13:15	13:20	Introduction to Session III	**Session III: Exploring the potential of metabarcoding**
13:20	13:35	**Sergio Stefanni**, Diana Catarino, Elio Biffali, Raimondo Pannone, Andrea Tarallo, Clara Loureiro, Ana Martins, David Stankovič	A "cold case" of metabarcoding analysis unravel hidden mesozooplankton diversity at two Azorean seamounts and neighboring island slopes (NE Atlantic)
13:35	13:50	**Tatiana Neretina,** Glafira Kolbasova, Anna Zhadan, Nikolai Neretin, Ksenia Kosobokova, Alexander Tzetlin	ITS sequences in marine invertebrates metabarcoding
13:50	14:05	**Paola G. Batta-Lona**, Joel K. Llopiz, Annette Govindarajan, Ann Bucklin	Metabarcoding analysis of salp diets and trophic relationships in mesopelagic food webs
14:05	14:20	**David Stanković,** Diego Borme, Valentina Tirelli, Alberto Pallavicini, Sergio Stefanni	A DNA metabarcoding approach to diet comparison in two sympatric pelagic fish in the Adriatic Sea – European sardine (*Sardina pilchardus*) and European anchovy (*Engraulis encrasicolus*)
14:20	14:35	**Masaki Miya**	Environmental DNA metabarcoding enables a data-driven approach for fish community research in large spatial and temporal scales
14:35	15:00	Coffee Break	
15:00	15:15	**Bengt Karlson**, Markus Lindh, Anders F. Andersson	Metabarcoding identifies previously unknown diversity of pelagic Cyanobacteria in the Baltic Sea and the Kattegat and the Skagerrak.
15:15	15:30	**Ann Bucklin**, Jennifer M. Questel, Bo Reese, Nancy J. Copley, Peter H. Wiebe	Time-series analysis of zooplankton diversity of the NW Atlantic continental shelf based on 18S rRNA metabarcodes

Appendix I. Symposium Program (continued)

Rediscovering pelagic biodiversity: Progress, promise, and challenges of metabarcoding of microbes to mammal



Start	End	Authors (Presenter in Bold)	Title of Presentation
15:30	16:15	**Poster Speed Talks (3 minutes each)**	**Session I Posters: Diversity of the pelagic assemblage**
	1	**Eileen Bresnan**, Catherine Collins, Rowena Stern, Joe Taylor	The use of metabarcoding to investigate the microbial and fungal communities at the Scottish Coastal Observatory monitoring site at Stonehaven in the north east of Scotland
	2	Monika Mioduchowska, Anna Iglikowska, **Agata Weydmann-Zwolicka**	HIDEA – Hidden diversity of plankton in the European Arctic
	3	Lotte J. Bouwman, Alice Burridge, Jaap de Boer, Catharina M.H. de Weerd, Erica Goetze, **Katja T.C.A. Peijnenburg**	Linking juvenile and adult pteropods from the Atlantic Ocean through DNA barcoding, with special attention to the elusive Pseudothecosomata
	4	S.S. Gunasekara, **R.R.P.K. Jayasinghe**, J.-O. Krakstad, A. Totland, H.M.P. Kithsiri	Evaluation of historical changes in coastal fisheries of Sri Lanka using length-based indicators
	5	**Björn Källström**, Erik Selander, Thomas Dahlgren, Annette Govindarajan, Carina Östman	The toxic and cryptogenic clinging jellyfish Gonionemus sp. (Hydrozoa, Limnomedusae) on the Swedish west coast
			Session II Posters: Exploring the potential of metabarcoding
	6	**Melissa Wojcicki**, Sarah Glancy, Joel K. Llopiz, Ann Bucklin	Discovering food web interactions in the ocean twilight zone: metabarcoding analysis of the diet of mesopelagic fishes
	7	**Joëlle van der Sprong**, Katja T.C.A. Peijnenburg	Cryptic species in open ocean holozooplankton - are they real?
	8	**E.A. Ershova**, O.S. Wangensteen, C. Barth-Jensen, K. Praebel	A step towards quantitative metabarcoding of zooplankton communities
			Session III Posters: Data and database resources
	9	**Maria Prager**, Diego Brambilla, Daniel Lundin, Anna Rosling, Jeanette Tångroth, Anders F. Andersson	Mobilizing sequence-based observations into Biodiversity Atlas Sweden: An Open-Science effort to make metabarcoding data easier to process, publish and re-use
	10	**Alexandra Frenzel**, Paola G. Batta-Lona, Peter H. Wiebe, Todd O'Brien, Ann Bucklin	DNA barcoding the Euphausiacea: taxonomic and biogeographic analysis using COI
	11	**Deborah Wall-Palmer**, Katja T.C.A. Peijnenburg	Shelled Heteropod Identification Portal (planktonic.org)
	12	Hobaek, A. and **Falkenhaug, T.**	Building Barcode Libraries of Norwegian microcrustaceans
16:15	17:00	**Discussion Session**	Discussion leaders: Anders Andersson and Katja Peijnenburg
17:00	18:00	**Poster Session and Reception**	

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2019 Annual Science Conference (*September 9-12, 2019 in Gothenburg, Sweden)*
See: http://www.ices.dk/news-and-events/asc/asc2019/Pages/default.aspx

Appendix II. Notes from SCOR Symposium Discussion Session

Discussion Organizers and Chairs: Anders Andersson and Katja Peijnenburg

During the Discussion Session, 5 questions were posed to 4 small groups of attendees

1) What are the biggest problems for implementing metabarcoding?

- Gaps in reference database; need complete data for all geographic regions and for all barcode gene regions
- Experts who can do metabarcoding analysis in each region (no shipping of samples).
- Reference databases for specific geographic regions, to allow better identifications?
- What is the meaning of OTUs? What is the relationship to species diversity?
- Quantitative analysis based on metabarcoding.
- need metadata and voucher specimens for all data
- open access to unpublished data
- lack of universal barcode region
- errors
- standardization of methods

2) Where are the biggest gaps in taxonomic reference data for plankton metabarcoding? What can we do about it?

- More gene regions – maybe whole mitochondrial genomes? Whole genomes?
- Technology may solve the problem, further development of long-read sequencing

3) How can we go from relative counts to more quantitative data? In spike-in controls of calls/specimens or DNA the solution?

- Who believes quantification from metabarcoding data will be possible (only a few) and those who believe this will require technical approaches that do not entail PCR (only a few).
- Another method will also need to be used in tandem: biomass, counts, etc
- Optimism that results will get better and better, in part due to accumulation of data
- Need additional analyses; beware of errors
- Calibration (correlation) curves will eventually make the case; but maybe not for fisheries management
- Need to work from first principles; describe and document what metabarcoding detects
- Target single-copy genes and shotgun sequences
- Spiking or target-enrichment (capture) probes not PCR
- Gene copy is not stable; phytoplankton copy number may be highly variable
- Species-by-species references for tying copy number to biomass

4) What new DNA based technologies could be useful to plankton ecologists?

- Portable sequencing devices
- Autonomous sequencing of DNA and plankton samples; no human intervention (NioION)
- Sequencing onboard ship by MinION
- Need to solve the high error rate with MinION
- FISH probes species; fluorescent labels that glow to identify specimens; cameras to photograph the sample and show spots of light
- Longer sequence reads with better quality
- eDNA analysis from water to sample fish biodiversity; many methodological concerns

Appendix II. Notes from SCOR Symposium Discussion Session (continued)

5) How can we move from descriptive research to hypothesis testing? What evolutionary or ecological questions can now be addressed with metabarcoding that could not before?

- hypotheses about basin scale patterns of biodiversity
- biodiversity hot spots
- what is the impact of overall effort – many people study the N Atlantic a particular region
- questions related to food web; who is eating who?
- are over 1M species yet to be discovered, in which groups are these new species to be found?
- many more to be found in deep ocean; one estimate: 3,000 new species
- how many species of marine zooplankton are there?
- in which species or groups should we expect regional differentiation and speciation, and which not? The answer depends on the habitat where you sample. E.g., likely in bentho-pelagic.
- species concept needs to be refined and updates
- what controls biodiversity, what are the environmental controls? can metabarcoding be used to address these questions?
- metagenomics brings whole new set of questions about which sequences to focus on?
- Is it possible to quantify and identify ecological impacts that can be measured in biodiversity; use metabarcoding to reveal impacts and recovery of mining and other events; use for large scale monitoring
- RNA metabarcoding may give insights into condition (dying) and activities
- when and how can we use DNA barcodes to identify new species; can we overturn the traditional approach requiring (Science editorial: 24 years for species discovered to be described)
- may need to return to species definitions; biological species concept may need to be revisited?
- we will always need morphological taxonomy! See Morard et al. (2016) Nomenclature for the nameless: a proposal for an integrative molecular taxonomy of cryptic diversity exemplified by planktonic Foraminifera. Syst. Biol.

Appendix III. SCOR Symposium Attendees

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