

Workshop on “Advances in Genomic and Molecular Studies of Zooplankton”

by Katja Peijnenburg, Erica Goetze and Ryuji Machida

A number of crucial questions in zooplankton ecology could benefit from a molecular or genomic approach as one component of an interdisciplinary oceanographic research program. Yet the community of zooplankton ecologists adopting molecular techniques is still small and scattered around the world. At the same time, new genetic and genomic techniques are becoming available at an unprecedented pace (Fig. 1) and with declining sequencing costs. This will open up genome-enabled science on non-model species and will provide major new research avenues in biological oceanography. Assessing the current state-of-the-field in zooplankton molecular ecology and discussing future avenues for research were the main goals of a half-day workshop held on March 16, 2011, at the 5th International Zooplankton Production Symposium in Pucón, Chile. This workshop, co-convened by the authors of this article, hosted a diverse array of topics presented as both oral (12) and poster (6) contributions, followed by an informal discussion after lunch.

One of the important central messages that emerged in both talks and discussions during the workshop is that zooplankton populations can and do evolve, in some cases quite rapidly. Our invited speaker Carol Lee (University of Wisconsin, USA) pointed out that evolutionary shifts (adaptation from ancestral marine to invasive freshwater populations) for the copepod *Eurytemora affinis* have occurred within approximately 50 years in the wild, and only 12 generations in the lab. Katja Peijnenburg (University of Amsterdam, The Netherlands) also highlighted that marine zooplankton may be particularly likely to show rapid evolution due to the larger effect of natural selection relative to genetic drift in large populations. She and her

collaborators presented evidence of genetic differentiation of planktonic chaetognath and copepod populations isolated in marine lakes in Croatia over a relatively short time frame (4000–7000 years). Erica Goetze (University of Hawaii at Manoa, USA) stressed that many oceanic zooplankton species do show substantial genetic structure across their geographic range (an example is presented in Fig. 2), and that the extent of this genetic isolation varies across species. The spatial pattern of gene flow among zooplankton populations will be an important trait influencing the species capacity to adapt to environmental change. Her current work aims to test the overall hypothesis that depth habitat is a primary trait determining gene flow in the open ocean.

Another important observation from our workshop is that everywhere we look we find new species. Examples were presented by Jaime Gómez-Gutiérrez (CICIMAR, Mexico) *et al.* for ciliates, which are an important parasitoid of krill. The results from Hiroomi Miyamoto’s and Erica Goetze’s work also suggested cryptic and genetically divergent lineages in chaetognaths and copepods, respectively. An extreme case in chaetognaths was presented by Ryuji Machida on behalf of Hiroomi Miyamoto, who could not attend the workshop due to the unfortunate disasters that had just taken place in Japan. Miyamoto (University of Tokyo, Japan) *et al.* presented a mitochondrial phylogeny of 29 chaetognath species based on the barcoding gene Cytochrome Oxidase subunit I, showing that 14 morphological species were comprised of two or more highly divergent clades (>11%). If every mitochondrial clade is considered a phylogenetic species, then the number of species of chaetognaths may be twice that described from morphological character variation.

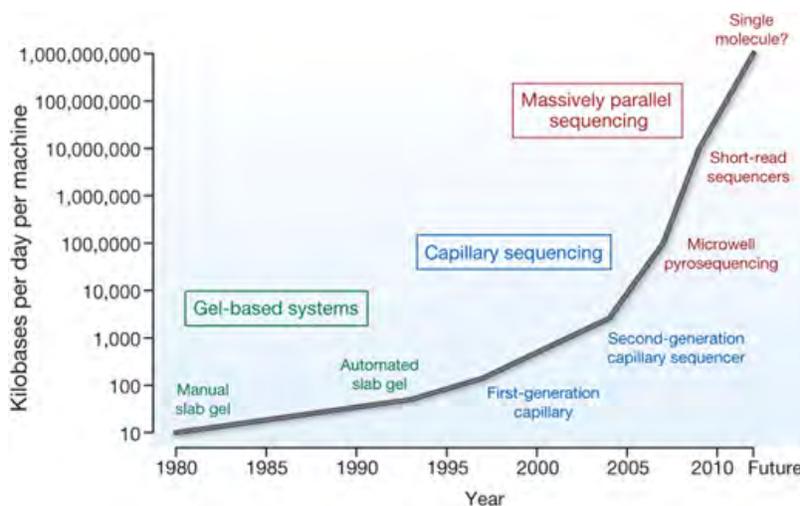


Fig. 1 Improvements in the rate of DNA sequencing over the past 30 years (from Stratton *et al.*, 2009, *Nature*, 458, 719–724).

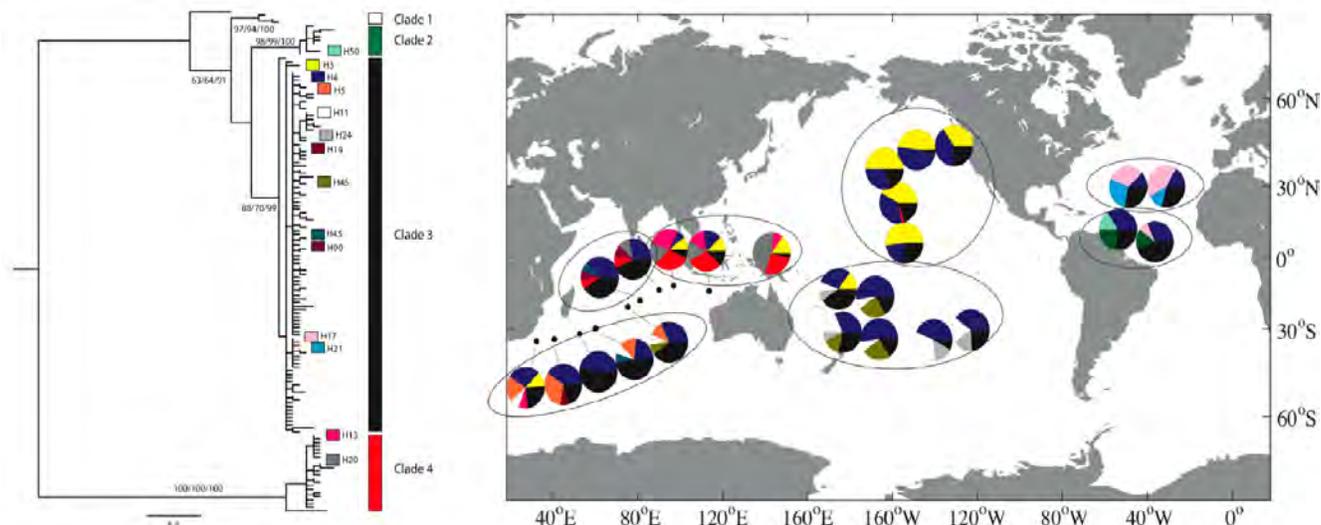


Fig. 2 Global population genetic structure in *Pleuromamma xiphias*, one example of an oceanic copepod species showing extensive genetic differentiation among populations in distinct pelagic provinces (sensu Longhurst, 2007). Parsimony gene tree (left) based on mitochondrial cytochrome oxidase I, with non-singleton haplotypes labelled by color. Global population structure (right) inferred from population graph, principal components, and Monmonier algorithm analyses, with pie charts illustrating haplotype frequencies (color as in gene tree), (from Goetze, unpublished).

A dynamic research area that generated a lot of interest was the new food web insights gained through application of molecular tools. Tatiana Rynearson (University of Rhode Island, USA) *et al.* identified a new trophic link in the diet of *Meganyctiphanes norvegica*, with up to 50% of the diet of this euphausiid consisting of an as yet completely unknown sediment microeukaryote. They used a new technique based on a blocking probe (PNA) and PCR with universal 18S primers in order to selectively amplify prey DNA (as opposed to krill DNA), and then combined this technique with qPCR to assess the prey spectrum of this pelagic euphausiid. Paolo Simonelli (University of Bergen, Norway) *et al.* presented data from qPCR assays of feeding rates in *Calanus* sp. and, rather surprisingly, found that experiments in the field seemed to be much more accurate than laboratory studies. qPCR-based estimates of feeding rates were compared to those estimated from conventional bottle incubation experiments. It may be that copepod digestion is more efficient in the lab or that prey cells are more resistant in the field.

Another research area well represented at the workshop was the development of new molecular tools to assess population or community-level responses to climate change. Community metagenetics is one emerging approach to studying genetic and specific diversity in bulk zooplankton samples, and could be a useful tool to rapidly assess changing zooplankton community composition. Here, nucleic acids are extracted from bulk zooplankton assemblages, and a target gene fragment is then amplified and sequenced on a massively parallel sequencer. A number of labs in Europe, North America and Asia are working to develop (or considering) this approach, but a number of methodological considerations need to be addressed before consensus can be reached on best practice techniques (*e.g.*, RNA *versus*

DNA, selection of the target gene or genes (Fig. 3), importance of DNA barcoding databases to be generated in parallel). During the workshop, we discussed the importance of reaching community consensus on metagenetic methods such that data generated from various research programs will be comparable on a global scale (Fig. 3).

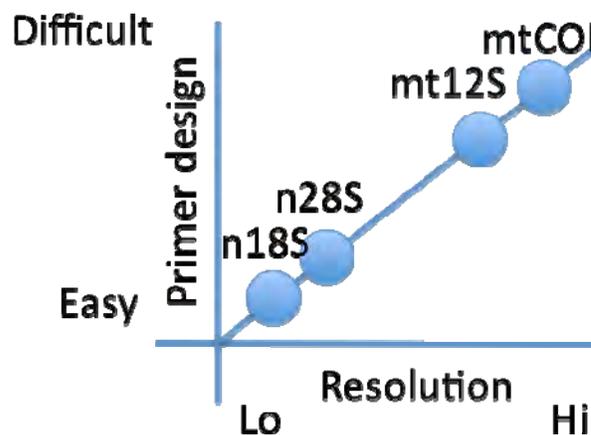


Fig. 3 The choice of a target gene for metagenetic surveys of zooplankton diversity is one important methodological consideration. There is an inherent trade-off between the taxonomic resolution possible using each gene, and the facility with which we can design truly universal primers. At the workshop it was noted that standardizing on one target gene for metagenetics would provide globally comparable datasets, but might not be possible given different research goals in programs worldwide. Four of the most likely candidate genes are included in this figure (from Machida, unpublished).

Ryuji Machida (Smithsonian Institution, USA) agreed to facilitate communication among labs conducting research in this area, and a number of scientists expressed interest in having an open policy about their method development

efforts in order to accelerate advances in this field. Studies of gene expression provide another avenue for investigating population-level responses to environmental variation (or change). Current research in this area, highlighted at the workshop by Petra Lenz (University of Hawaii at Manoa, USA) and Ebru Unal (University of Connecticut, USA) and collaborators, is focused on the development and use of a microarray in *Calanus finmarchicus*. Such a microarray allows for the simultaneous screening of many physiological processes, and identified suites of genes up- or down-regulated in response to particular types of stress, such as starvation. Mattias Johansson (Hatfield Marine Science Center, USA) also discussed the development of new molecular tools (whole mitochondrial genomes, microsatellites, reference transcriptomes) to examine euphausiid ecology and population structure in the North Pacific and beyond.

The field of zooplankton molecular ecology is on the cusp of entering a new era. Dramatically powerful new sequencing technologies now enable unprecedented access to the genome of non-model species and make possible research on a range of questions of global importance. For example, given that the distributions of marine plankton species are currently undergoing substantial change due to climate forcing (e.g., Beaugrand, G., Luczak, C., and M. Edwards, 2009. Rapid biogeographical shifts in the North Atlantic Ocean. *Global Change Biology*, 15, 1790–1803),

one topical research area is to understand the biological and oceanographic factors that constrain species distributions, and the ecological and evolutionary mechanisms that enable species to shift their range. What is the ‘evolvability’ of zooplankton species ranges, as we project into future ocean states (to 2100)? An interdisciplinary approach that integrates ecological research and genomics has the potential to determine the relative importance of natural selection *versus* neutral processes in determining zooplankton responses to climate change. Other research areas that could be advanced using new genomic techniques include looking at physiological responses to change in ocean pH (ocean acidification) and temperature, population genomic responses to environmental variation, and assessing changing genetic and specific diversity of zooplankton assemblages using metagenetic community surveys. Quantitative PCR is also likely to play an increasing role in how we assess grazing rates *in situ*, and possibly also in the enumeration of early life stages of planktonic organisms.

The creative use of new genomic technologies will be an important goal for our research community over the next 5 years. In order to be able to do this, it is very important for our community to meet regularly, share ideas, and stay up to date on what new methods work (or do not work) for addressing long-standing problems in biological oceanography.



Left photo: Erica Goetze (left) and Katja Peijnenburg (right) on the crater rim of the Villarica volcano in Pucón, Chile. Right photo: (from left to right) Ryuji Machida together with Ebru Unal, Georgina Cepeda, and Claudia Castellani in front of the Villarica volcano.

Dr. Katja Peijnenburg (K.T.C.A.Peijnenburg@uva.nl) is a molecular marine ecologist at the Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, The Netherlands. Her present postdoctoral research aims to unravel the tempo and mode of marine zooplankton evolution by examining genetic and morphometric variation in chaetognaths, calanoid copepods and pteropods.

Dr. Erica Goetze (egoetze@hawaii.edu) is a biological oceanographer in the School of Ocean and Earth Sciences and Technology at the University of Hawaii at Manoa, USA. In her current research, Erica is using molecular tools to address both population genetic and oceanographic questions in zooplankton ecology.

Dr. Ryuji Machida (ryujimachida@gmail.com) is a postdoctoral fellow at the Smithsonian National Museum of Natural History, USA. He is currently working on the application of meta-genetic, genomic, and transcriptomic analyses in marine metazoan communities, including both zooplankton and coral reef associated assemblages.