Temporal effects of chemical and physical stressors on marine zooplankton: A molecular approach

Ilias Semmouri

Department of Animal Sciences and Aquatic Ecology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653 building F, 9000 Gent, Belgium
E-mail: ilias.semmouri@ugent.be

Oceans and seas are often perceived as the last wilderness on the planet. However, anthropogenic actions are already impacting these ecosystems, ranging from the coasts and the sea surface to the open ocean and the deep sea floor. Marine ecosystems are currently affected by multiple human activities, such as eutrophication, overfishing, the introduction of non-indigenous species, the contamination by hazardous chemicals and (micro)plastics, etc., in addition to climate change, leading to impaired environmental conditions. Evidence is growing that these changing environmental conditions have negative effects on the biodiversity and functioning of marine food webs. Due to their rapid responses to environmental variation, planktonic organisms are used as bio-indicators of ecosystem changes. With the need for better understanding the impact of a changing environment on zooplankton communities, zooplankton monitoring programs have been carried out in the marine environment globally since the early 20th century. Most zooplankton monitoring studies focus mainly on variability in biodiversity and biomass. However, this approach is hindered by challenges in the identification, which is time-consuming, complicated and requires biological expertise. A combination of new technologies and techniques, together with classical in situ and laboratory studies, could improve our understanding of such biodiversity patterns by assessing the health and physiology of marine plankton. In this thesis, we aimed to apply molecular methods to investigate spatiotemporal patterns in zooplankton dynamics, as well as to investigate the influence of environmental variation and stressors on these dynamics.

In chapter 2 of the thesis, we examined the spatial and temporal distribution of the zooplankton assemblage of the Belgian Part of the North Sea (BPNS) during a one-year period using both a metabarcoding approach as well as the traditional (microscopy) approach. A 650 bp fragment of the V4 and V5 region of the 18S rDNA barcode was characterized using the MinION™, a nanopore-based DNA/RNA sequencing platform (Oxford Nanopore Technologies). Metabarcoding allowed for comparisons of diversity and community composition, but not all groups (cumaceans, harpacticoid copepods) were successfully recovered. Additionally, some disparities existed between relative abundances of the most abundant taxa based on traditional counts and those based on sequence reads. Overall, we conclude that for zooplankton samples, metabarcoding is capable of detecting taxa with a higher resolution compared to microscopy, regardless of the developmental stage of the organism. A combination of molecular and morphological methods results in the highest detection and identification levels of zooplankton. The majority of the sequenced reads could be assigned to five taxa, i.e. the calanoid copepods Temora longicornis, Acartia clausi, Centropages sp., Calanus helgolandicus and Paracalanus parvus.

A more comprehensive molecular data set would be able to identify and assess the impact of the main drivers of changes in the marine ecosystem, rather than only determining species richness. Studying the functional activities of a community - in situ and without a priori knowledge of genes - has been facilitated by metatranscriptomics, i.e. the study of community gene transcription. Therefore, in chapter 3, we describe and evaluate the construction of a metatranscriptome dataset from the pelagic crustacean zooplankton community, sampled in one marine station in both winter and summer. We generated transcripts using the MinION, a portable, real-time DNA and RNA sequencing device. We found that metatranscriptomics is also capable of species detection, mainly identifying calanoid copepods, particularly Temora longicornis and Acartia clausi. GO term annotation revealed that genes involved in glycolytic and translation-related processes were most expressed in the community.

Based on the results of the previous chapters, T. longicornis appears to be the most dominant zooplankton species in the BPNS. Despite its economic and ecological importance, molecular data is still very limited for this species. Using HiSeq Illumina sequencing, we sequenced the whole transcriptome of T. longicornis, after being exposed to realistic temperatures of 14 and 17 °C, as described in chapter 4. After both an acute (1 day) and a more sustained (5 days) thermal exposure to 17 °C, we investigated gene expression differences compared to animals exposed to 14 °C. Temora only showed a mild response to both the temperature and the duration of the exposure. We found that the expression of 27 transcripts varied significantly with an increase in temperature of 3 °C, of which eight transcripts were differentially

expressed after acute exposure only. Gene set enrichment analysis revealed that, overall, T. longicornis was more impacted by a sustained thermal exposure, rather than an immediate (acute) exposure, with two times as many enriched GO terms in the sustained treatment. We also identified several general stress responses independent of exposure time, such as modified protein synthesis, energy mobilisation, cuticle and chaperone proteins.

Given the uncertainties regarding the molecular mechanisms involved in physiological and behavioural adaptation, the goal of chapter 5 was to explore shifts in gene expression in a population of adult T. longicornis in the BPNS, collected at different time points within 24 hours and within one week. Using Direct RNA Sequencing (dRNA seq), we generated approximately 2.5 million high quality reads with the MinION™. Differentially expressed gene (DEG) analysis of field collected adults identified up to 254 significant differences in gene expression, when comparing samples taken in the evening and later at night. Our results indicate that copepods use cuticular and metabolic transcripts as a molecular mechanism to compensate for alternating conditions. We also found that biological processes such as regulation of the plasma membrane, translation, transport and signal transduction were significantly different represented in our dataset, as confirmed by enrichment and network analyses. We did not find any significant differences in gene expression in transcripts involved in the core circadian machinery of T. longicornis, probably to limitations in the sequencing depth.

In chapter 6, we explored variation in population gene transcription across time and space using T. longicornis samples, collected at four different locations in the BPNS on three different time points (April, June, October) in 2018. RNA-seq analysis of field collected adults identified large seasonal differences in gene expression, mainly between spring-summer and autumn samples. The largest log-fold changes were in a set of genes encoding for ribosomal and myosin (heavy chain) transcripts. Enrichment analysis revealed a strong seasonal pattern in vitellogenin, cuticle and glycolytic gene expression as well. No clear spatial variation in expression patterns was found based on this dataset.

In chapter 7, we investigated the relative contribution of environmental variables to the densities, biomass and gene expression of Temora longicornis, based on a 4 year sampling campaign. We found spatial variation in the population density, as well as in body size, comparing copepods collected in the nearshore station as compared to the more offshore sampling stations. We applied generalized additive models to quantify the relative contribution of temperature, nutrients, salinity, turbidity, photosynthetic pigment concentrations and chemical pollution (i.e. polychlorinated biphenyls and polycyclic aromatic hydrocarbons) to the density and biomass dynamics of this species. Comparing both GAM and molecular methods, the same environmental parameters emerge influencing T.

longicornis densities, biomass and gene expression (i.e. Temperature, salinity, turbidity, summed PAH concentrations). Temperature was the most important environmental variable predicting the abundances and biomass of T. longicornis. The relative contributions of turbidity, salinity and summed PAH concentrations were rather modest. Studying the gene expression of field collected adults, we identified significant differences in expression of genes involved in metabolic processes and response to stressors. We found significant correlations between temperature and genes involved in vitellogenin production, proteolytic activities, heat shock proteins. The measured anthropogenic chemical concentrations did not induce significant differences in the gene expression of typical stress related genes, such as glutathione transferases or cytochrome P450. This study underlines the potential of field gene expression studies for biomonitoring purposes and the significance of considering seasonal variation in future studies.

In chapter 8, we combined and compared copepod abundance data of four dominant calanoid and the dominant harpacticoid copepod species collected in the BPNS during 2018–2021 with previously collected (2009–2010, 2015-2016) datasets for the same study area. The time series revealed a significant decrease in calanoid copepod abundance (Temora longicornis, Acartia clausi, Centropages sp., Calanus helgolandicus), while this was not the case for the studied harpacticoid copepod species, Euterpina acutifrons. We applied generalized additive models to quantify the relative contribution of temperature, nutrients, salinity, turbidity and anthropogenic chemicals (i.e. polychlorinated biphenyls and polycyclic aromatic hydrocarbons) to the dynamics of these species. Temperature was the only predictor consistently showing a high importance in all models predicting the abundances of the selected species. The various heat waves during the summer periods of these years are considered potential causes for these copepod decreases, since they corresponded to the physiological thermal limit of some of the studied copepod species. The results from this study illustrate the changes affecting this essential trophic level and highlights the value and relevance of biomonitoring and the collection of long-term data series in the context of climate change and water quality.