Influence of changing water temperature and plastics leachates on an individual and molecular level of the harpacticoid copepod *Nitokra spinipes*

Plijnaar Lucille[1,2], Niu Zhiyue[2], Anglada Segura Sol[2,3], De Schamphelaere Karel[4], Hablützel Pascal[2], Everaert Gert[2] and Catarino Ana Isabel[2]

[1] Hogeschool Utrecht, Princetonlaan 8, 3584 CB Utrecht, The Netherlands
E-mail: lucille.plijnaar@student.hu.nl
[2] Flanders Marine Institute (VLIZ), Wandelaarkaai 7, 8400 Oostende, Belgium
[3] Vrije Universiteit Brussel (VUB), Pleinlaan 2, 1050 Brussel, Belgium
[4] Department of Animal Sciences and Aquatic Ecology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, Building F, 9000 Gent, Belgium

In the last decades human activities have affected the ocean environment. Due to anthropogenic release of greenhouse gasses, atmospheric CO_2 levels have been rising to a current concentration of over 400 ppm, which has led to an average increase in the Earth's sea surface temperature of 1.5 °C compared to pre-industrial times. The increasing seawater temperatures can impact marine biodiversity and the functioning of the ocean. Besides the potential effects of climate change, plastic debris has the potential to cause adverse effects on marine life. An indirect effect of plastics is related to associated chemicals that leak from polymers. These chemicals have the potential to cause adverse effects to the base of the marine food web. To date, these effects have not been tested yet in a relevant and realistic scenario in combination with potential climate change effects. Indeed, so far, research lacks knowledge about the combined effects of increasing water temperature and plastic leachates. The aim of this project is to assess the effect of plastic leachates and increasing water temperature of the copepod *Nitokra spinipes* on an individual and molecular level. The harpacticoid copepod *N. spinipes* is an ecologically important group of crustaceans with a well-studied larval development. Larval development tests were conducted to track the development from the larvae stage to the copepodite stage while the organisms are exposed to leachates at two different temperatures (22 ºC and 24 ºC). In our work, newly hatched *N. spinipes* larvae were exposed to a dilution series of leachates from a bio-based following polylactide (PLA) following the ISO/TS 18220:2016 protocol in combination with an increased water temperature (+2 ºC). To test the effect of PLA leachates and an increased temperature on a molecular level, we will look at biomarkers, since they act as an early warning indicator for toxicity. We will assess the expression of multiple chaperoning genes, reproduction genes and oxidative stress genes by conducting quantitative reverse transcription polymerase chain reactions (RT-qPCR) and comparing expression levels of target genes with that of housekeeping genes ($2^{-\Delta\Delta Ct}$ method). Experimental work on the combined effects of temperature and leachates are currently ongoing. We anticipate that our results will contribute to assessing the effect of leachates and increasing water temperature on molecular and individual organism levels.

Keywords: Plastic pollution; Plastic leachates; Global warming; Nitokra spinipes; Gene expression; Larval survival rate; Larval development rate