Freshwater ecosystems under natural and anthropogenic stressors

The impact of ultraviolet radiation and nanoplastics on plankton and the ecosystem

Franca Stábile Ferreira

LUND UNIVERSITY

DOCTORAL DISSERTATION

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Faculty opponent
Professor Justyna Wolinska
Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB) Freie Universität Berlin (FU)

Popular science summary

Since life emerged on Earth, organisms have had to handle threats and stressors of different kinds. In freshwater ecosystems, one of these ancient, and very harmful stressors is the solar ultraviolet (UV) radiation. The first forms of life had to cope with high levels of UV radiation, until an oxygenated atmosphere was formed and reduced the levels of UV radiation that reached our planet. Therefore, life has been continuously exposed to this stressor, and various adaptations have evolved that allowed organisms to cope with it. On the other hand, human activities have introduced novel stressors to the Earth system, for example, different pollutants. Plastics are pollutants with widespread global distribution, and novel stressors in the history of Earth. When plastic material breaks down, smaller plastics called micro- and nanoplastics are formed. Nanoplastics, the smallest size fraction (as small as most virus, or even smaller!), harm a variety of freshwater organisms, but a comprehensive understanding of the effects of nanoplastics on natural freshwater ecosystems is still lacking.

The aim of this thesis is to address how UV radiation and nanoplastics affect freshwater organisms and their ecosystem. To explore this, I performed laboratory experiments which included small aquatic crustaceans, known as zooplankton, or micro algae and cyanobacteria, called phytoplankton. I also worked with wetland mesocosms, which are closer to real natural conditions. A mesocosm is a small ecosystem, set in an enclosure, where it is possible to manipulate some variables, and which can be replicated. This allowed me to explore how nanoplastics are distributed in the freshwater wetlands and the organisms that inhabit it, how a direct toxic effect on one organism may have indirect effects on others, and to test if nanoplastics affect organic matter decomposition.

Solar UV radiation fluctuates during the year, but also daily, and over short time scales with the position of the sun and rapidly occurring variations in cloudiness. Despite its variable nature, most studies on organisms' responses to UV radiation have assessed the effects of a constant exposure instead of fluctuating. In this thesis, I experimentally investigated how the survival, reproduction, and behaviour of the zooplankton species *Daphnia magna* is affected when exposed to constant or fluctuating UV radiation. I found that this species changes its behaviour depending on if it is exposed to a constant exposure or a repeatedly fluctuating UV radiation. I also found that the organisms exposed to the fluctuating UV radiation reproduced less, which indicates a cost to the organism due to the response to the fluctuating environment. This shows that the repeated vertical movements that *D. magna* performs daily imply a cost, something that has been debated.

All plastic items polluting the environment will inevitably break down into smaller pieces, and most will pass through the nanometre size scale before being completely degraded. Compared to the larger original plastic, these smaller plastic particles

have a much higher surface in relation to their volume. This is the reason why nanoplastics can interact differently with the environment. Further, because of their tiny size, they can enter cells and cause harm to cellular processes. Therefore, it is very important to also investigate these smallest versions of plastic pollution and not only micro-sized plastic particles as previously often focused on. However, nanoplastics are too small to detect for most analytical methods and are therefore difficult to study. Therefore, we still know very little about their effects on organisms and ecosystems, and how they move and accumulate.

I navigated the challenge of detecting these tiny particles by using artificial plastic particles that had a metal (gold) core in their centre, which is possible to detect with certain analytical methods. When I added these artificial nanoplastics to the freshwater wetland mesocosms, I found that most of the nanoplastics were retained in the wetlands. The nanoplastics mostly accumulated in the sediments of the aquatic compartment, but also in organisms such as the zooplankton *D. magna*, a benthic invertebrate called *Asellus aquaticus*, and aquatic plants. Later, in a similar study, I added artificial nanoplastics to the wetland mesocosms but this time they were made of full plastic. We knew these tiny plastics were harmful for *D. magna* in laboratory tests, and now I wanted to investigate what happened when these particles were added to a complex ecosystem, more like the natural environment. I found that *D. magna* went extinct when the wetland mesocosms were exposed to nanoplastic concentrations above 2 mg of plastic per litre. *D. magna*, feeds by filtering water and trapping the phytoplankton that is floating in the water with its filtering apparatus. The extinction of *D. magna* likely favoured the abundance of cyanobacteria, that did not have efficient predators. On the other hand, other phytoplankton species were negatively affected by the nanoplastics, because they reduced their abundances when exposed to increasing concentrations of nanoplastics. This made me wonder why the nanoplastics affected the different members of the phytoplankton community differently. I found, in a laboratory experiment, that nanoplastics interact differently with two phytoplankton species: where one had nanoplastic particles sticking to its cells while the other didn't. In addition, nanoplastics caused differential effects on phytoplankton growth and group formation, where fewer groups of cells were formed in the first, while nanoplastics induced cells to team up in groups in the other. These changes in group formation will likely impact where these species are in the aquatic system (either the bottom or suspended in the water column), and which species of zooplankton can eat them.

Collectively, this thesis analysed the responses of different organisms to both natural and human-introduced stressors. Despite long-standing adaptations, organisms still face costs associated with their response to natural stressors, as UV radiation. The impacts that humans are causing on natural ecosystems through plastic pollution also press organisms' natural populations. Further, these pollutants can cause shifts on the ecosystem that might be irreversible and harmful to the whole freshwater ecosystem, and to us humans. Therefore, the current trend of increasing plastic pollution is problematic, and new regulations but also major social changes are needed to change this path.