DNA of crayfish spermatozoa as a target of waterborne pesticides – an *ex vivo* screening to predict the impact on progeny fitness

R. Marçal[1,2,*], M. Pacheco[1,2] and S. Guilherme[1,2]

[1] *Department of Biology, University of Aveiro, Aveiro, Portugal*
[2] *CESAM – Centre for Environmental and Marine Studies, University of Aveiro, Aveiro, Portugal*
E-mail: armarcal@ua.pt

Germ cells have a central role in life, being the integrity of their genetic material vital to the offspring fitness. Male gametes, in particular, have lower antioxidant defences and a poor capacity of repairing DNA, wherefore they are more vulnerable to genotoxic agents, including in comparison with the oocytes. The DNA damage in spermatozoa cells might be the cause of low fertilization rate, troubles on embryonic development and a consequent decrease prolificacy, impairing the population success. It was already demonstrated that the huge amounts of pesticides released into the environment affect biota, even the non-target species which, in theory, would be protected or unaffected. The invasive crayfish, *Procambarus clarkii*, is a useful model organism for ecotoxicological studies since it can be considered a non-target specie for several pesticides. Bear this in mind, the aim of this study was to clarify if the spermatozoa DNA of *P. clarkii* are affected by waterborne pesticides at environmentally relevant concentrations. Thus, an ex vivo assay was performed and the genotoxic effect of six pesticides on male gametes of *P. clarkii* was assessed. The spermatozoa were exposed to (i) post-emergence herbicides: glyphosate (9 and 90 µg L-1) and penoxsulam (2.3 and 23 µg L-1), (ii) insecticides: dimethoate (2.4 and 24 µg L-1) and imidacloprid (13.1 and 131 µg L-1); (iii) fungicides: pyrimethanil (2.2 and 22 µg L-1) and imazalil (16 and 160 µg L-1). DNA damage was assessed using the alkaline comet assay. Genotoxicity (non-specific damage), measured by GDI (genetic damage indicator) values, was observed in the higher concentrations of glyphosate, penoxsulam, dimethoate, pyrimethanil, and imazalil. Penoxsulam and dimethoate revealed to be genotoxic even at lower concentrations, 2.3 and 2.4 µg L-1 respectively.

Penoxsulam was the pesticide with higher GDI. On the other hand, imidacloprid was the only pesticide that did not cause DNA damage in crayfish spermatozoa. The improved version of comet assay, using DNA lesion-specific repair enzymes (FPG and EndoIII), showed no significant oxidative damage considering all the tested pesticides. Overall, this work demonstrates that the ex vivo assay is an effective tool in ecotoxicological screening assays and, moreover, that environmental concentrations of waterbone pesticides could be genotoxic to spermatozoa of non-target species.