

Vitellogenin-like proteins in the freshwater amphipod *Gammarus fossarum* (Koch, 1835): Functional characterization throughout reproductive process, potential for use as an indicator of oocyte quality and endocrine disruption biomarker in males

This work focused on the validation of biological specificity of the quantitative LC-MS/MS assay by checking the natural variability of Vg levels during the reproductive cycle in *Gammarus fossarum* (i.e., including oogenesis and embryogenesis). Laboratory tests were performed for 21 days under controlled conditions to assess Vg changes in male and female gammarids after exposure to chemical stress. Females were exposed to two crustacean hormones, 20-hydroxyecdysone (0.01, 1 and 100 g L⁻¹) and methylfarnesoate (0.01, 1 and 100 g L⁻¹). No effect was recorded for 20-hydroxyecdysone, whereas in females exposed to methyl-farnesoate a deleterious impact on Vg production was observed. Males were exposed to crustacean hormones 20-hydroxyecdysone (0.01, 1 and 100 g L⁻¹) and methyl-farnesoate (0.01, 1 and 100 g L⁻¹), the insecticide methoxyfenozide (0.001, 0.1 and 10 g L⁻¹), the fungicide propiconazole (0.001, 0.1, 10 and 1000 g L⁻¹), and the pharmaceutical products benzophenone, carbamazepine, cyproterone, and R-propranolol (0.001, 0.1, 10 and 1000 g L⁻¹). Induction of Vg synthesis was recorded in males exposed to cyproterone, methoxyfenozide, methyl-farnesoate, and propiconazole. Finally, we validated the function of the ILIPGVGK peptide used to track vitellogenin in *G. fossarum* across reproductive processes (vitellogenesis and embryogenesis), and results confirmed the energy reserve role of Vg during embryo development. We show that oocyte surface measurement is directly related to Vg levels in the oocyte, constituting a reliable indicator of egg quality in *G. fossarum*. Consequently, it could be used as a reliable tool for biomonitoring programs. We recorded induction of Vg in male *G. fossarum*; however, the possible use of this tool as a specific biomarker of exposure to endocrine disruption should be confirmed in further studies.

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