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Physiological Responses of Daphnids to Pharmaceutical Mixtures

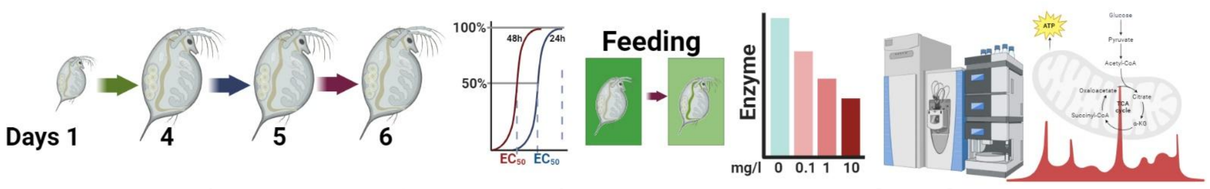
Emma Rowan, Anne Leung, Katie O'Rourke, Konstantinos Grintzalis

Dublin City University, School of Biotechnology, Republic of Ireland

INTRODUCTION & AIM

Pharmaceuticals are increasingly present in freshwater ecosystems due to their overgrowing use in industry, agriculture and healthcare. Antibiotics, notably raise concerns due to the generation of antimicrobial resistant strains, while complex mixtures of pharmaceuticals can lead to synergistic effects, thus intensifying environmental hazards. Current water monitoring methods have limitations, especially regarding pharmaceutical "cocktails", prompting the exploration of ***Novel Approach Methodologies*** (NAMs), which aim to identify toxicity mechanisms and provide molecular information for pollution assessment. Daphnids as sentinel species are sensitive to environmental stimuli and are often used as bioindicators. In this study daphnids were exposed to a pharmaceutical mixture of diclofenac, metformin, gabapentin, amoxicillin, trimethoprim, and erythromycin, all of which are pharmaceutical contaminants recognized of emerging concern in the EU. Responses were captured with phenotypic and molecular endpoints. Using the ingestion rate and enzyme activity as biomarkers for freshwater pollution can give an insight into the overall health of an ecosystem.

METHOD

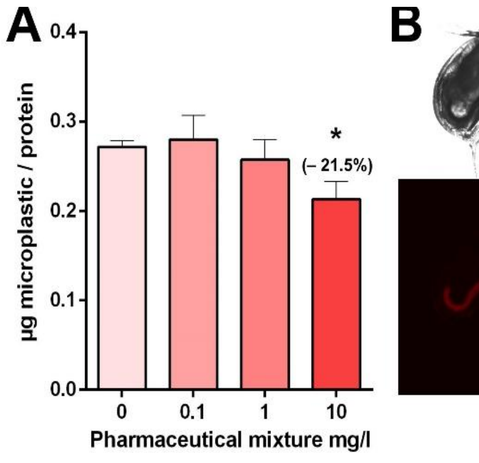


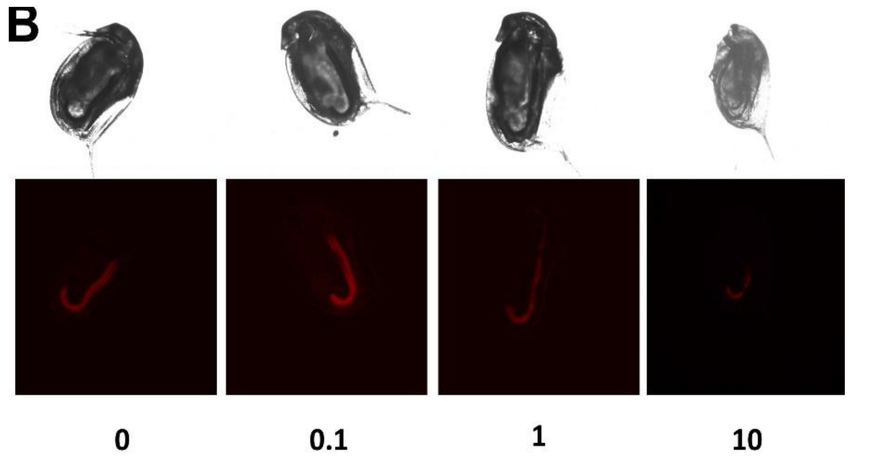
Daphnids (neonates<24 hours) were cultured for four days and exposed to non-lethal concentrations (0.1, 1 or 10 mg/l) of the pharmaceutical mixture for 24 and 48 hours. Following exposure, feeding rate of daphnids was assessed with a novel assay based on the ingestion of fluorescent microparticles. The presence of microparticles in the intestine of daphnids was verified with fluorescence microscopy. Additionally, activities of key enzymes such as acid (ACP) and alkaline (ALP) phosphatases, β-galactosidase (βGAL), lipase (LIP), peptidase (PEP), and glutathione-S-transferase (GST) were evaluated as markers of physiology. Finally, metabolites were extracted, and a targeted LC-MS analysis was performed to assess significant metabolic changes.

RESULTS & DISCUSSION

Feeding is a non-invasive method for assessing the impact of pollutants on freshwater organisms such as daphnids. It has been shown to be affected following exposure to chemicals. In this study, acute exposure to the pharmaceutical mixture resulted in a dose-dependent decrease in the ingestion rate with a notable decrease measured in the highest exposure.

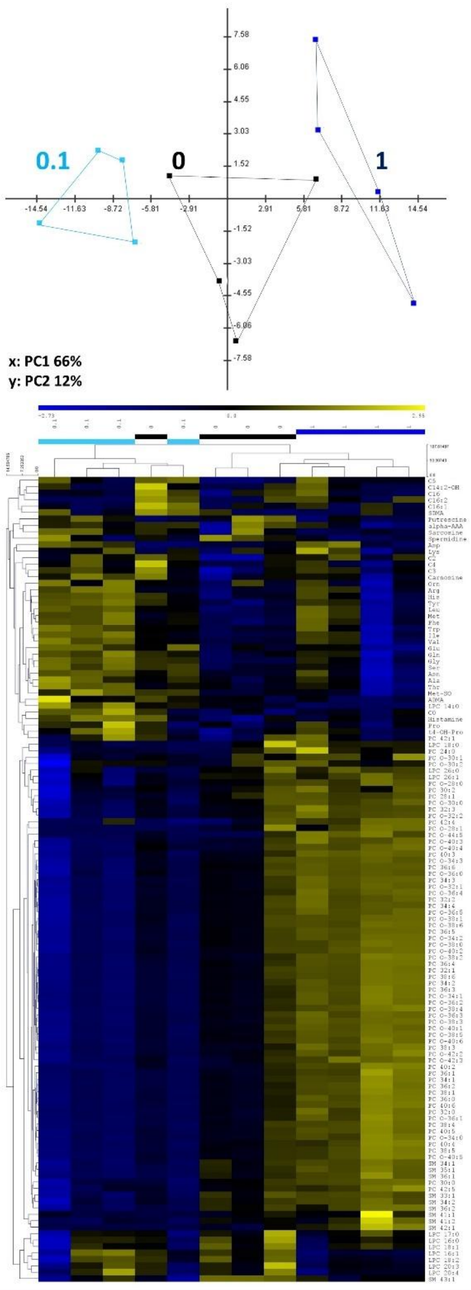
Exposure	Enzyme	Pharmaceutical Mixture (mg/l)		
		0.1	1	10
24 hours	ALP			
	ACP			
	βGAL			+25%
	LIP	-18%		-19%
	PEP			
	GST			
48 hours	ALP			
	ACP			
	βGAL		+17%	+49%
	LIP			
	PEP			
	GST		-14%	-20%

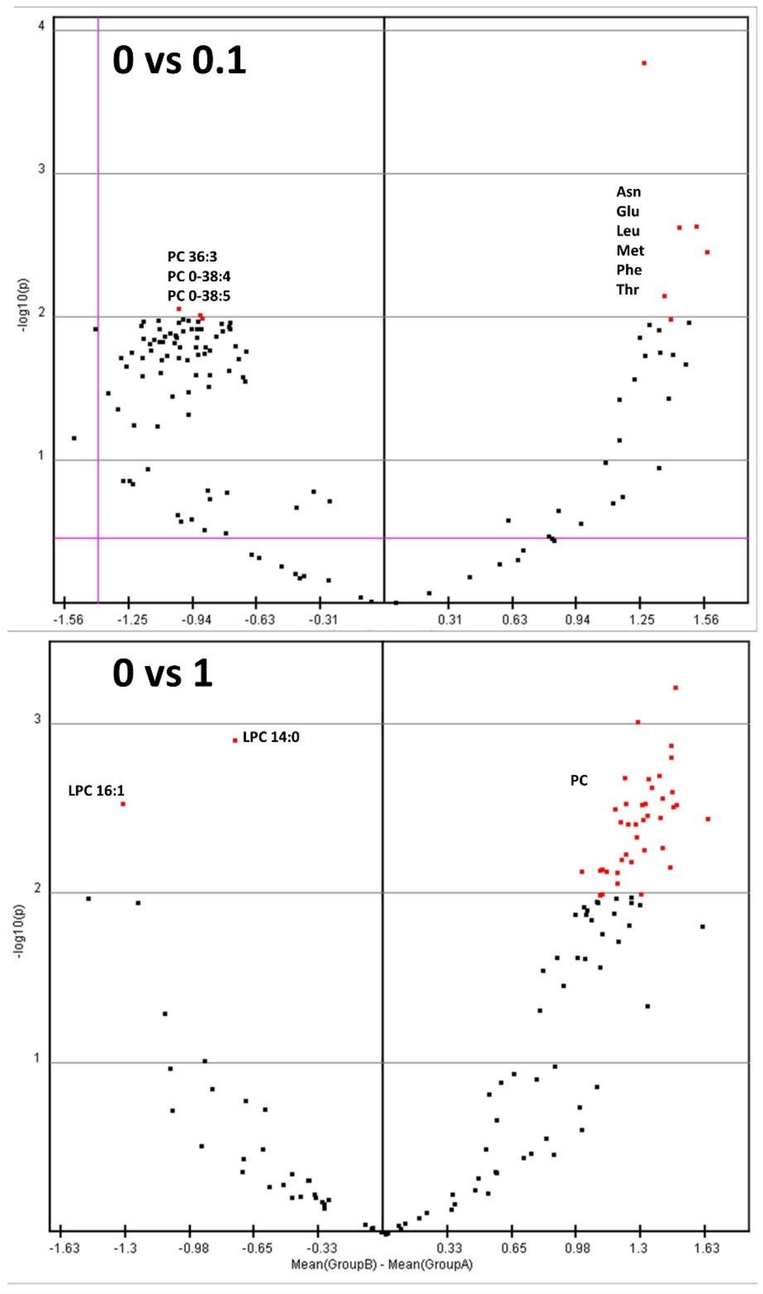




Furthermore, the time-dependent changes in enzyme activities revealed a decrease in lipase LIP. This decline in GST is attributed to diclofenac as part of the mixture to overburden the antioxidant defence system, leading to oxidative damage. The observed changes in enzyme activity show effects the pharmaceutical mixture is both concentration and duration-dependent.

Regarding metabolite analysis, two concentrations (0.1 and 1 mg/l) were assessed where different groups and clusters were identified using Principal Component Analysis (PCA) and hierarchical clustering with Manhattan distance metrics. Three phosphatidylcholines were downregulated and amino acids (Asn, Glu, Leu, Met, Phe, and THr) were upregulated with the lower exposure. Conversely, two lysophosphatidylcholines decreased but the phosphatidylcholines significantly increased upon exposure to 1 mg/l. This data shows that distinct metabolic alterations were induced by an increase in the concentration of pharmaceutical pollutants, indicating that distinct phenotypes are triggered by varying stress levels.





FUTURE WORK / REFERENCES

Regarding future research, the impact of this pharmaceutical mixture can be investigated with additional phenotypic endpoints. Another avenue is to culture daphnids in different combinations of the pharmaceuticals and vary the concentrations to assess potential antagonistic or additive effects.