Changes in several biomarkers after exposure of *Daphnia magna* to sublethal concentrations of Pyriproxyfen

Beatriz Salesa[1], Francisco Javier Torres-Gavilá[2], María Dolores Ferrando-Rodrigo[1], Encarnación Sancho[1]

[1]Departamento de Biología Celular, Biología Funcional y Antropología Física. Facultad de Biológicas. Universitat de València.

[2]Instituto de Investigación en Medio Ambiente y Ciencia Marina (IMEDMAR-UCV). Universidad Católica de Valencia San Vicente Mártir.

INTRODUCTION

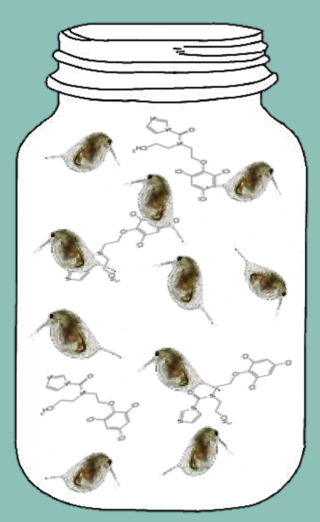
- ✓ Pyriproxyfen → potent insect disruptor
- ✓ Control pest → public health programs as an alternative to organophosphate and pyrethroid pesticides (aquatic medium)
- ✓ Aquatic animals could be affected at very low concentrations of toxicants in their medium
- ✓ Studies about several biochemicals and gene expression in exposed animals could be an early individual tool to alert about some genetic biomarkers dysfunctions and advertise a possible damage before the population level was affected

Could biochemical parameters and gene expression of metabolism predict the individual-level effect of pyriproxyfen in *Daphnia magna* individuals after 21 days of exposure?

MATERIALS AND METHODS

21-days Chronic assay

(Sublethal exposure to 3.14, 4.74, 6.12, 8.41, and 14.02 µg/L)



RNA extraction
cDNA synthesis
qRT-PCR

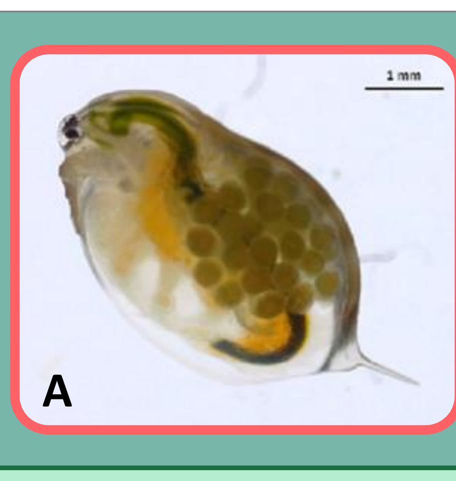
Biochemical metabolites

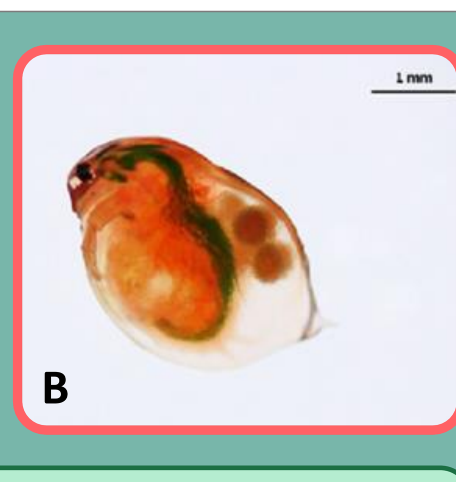
(6x replicates)

BIOMARKERS SELECTED: **cholesterol**, **triglycerides**, **glucose**, **lactate**, and **LDH** activity, expression of genes: related to lipid metabolism (***fabd***), oxidative stress (***cat*** and ***gst***), heat shock proteins synthesis (***hsp70*** and ***hsp90***), hemoglobin synthesis (***hgb1*** and ***hgb2***), metallotioneins synthesis (***mt-a***, ***mt-b*** and ***mt-c***), and vitellogenines synthesis (***vgt1*** y ***vgt2***), plus reference gene (*GAPDH*)

RESULTS

Morphological differences were observed between unexposed (**A**) and exposed (**B**) females: as development of gradual orange-red fashion colour. And this characteristic was encompassed by the overexpression of ***hgb 1*** and ***hgb 2*** genes



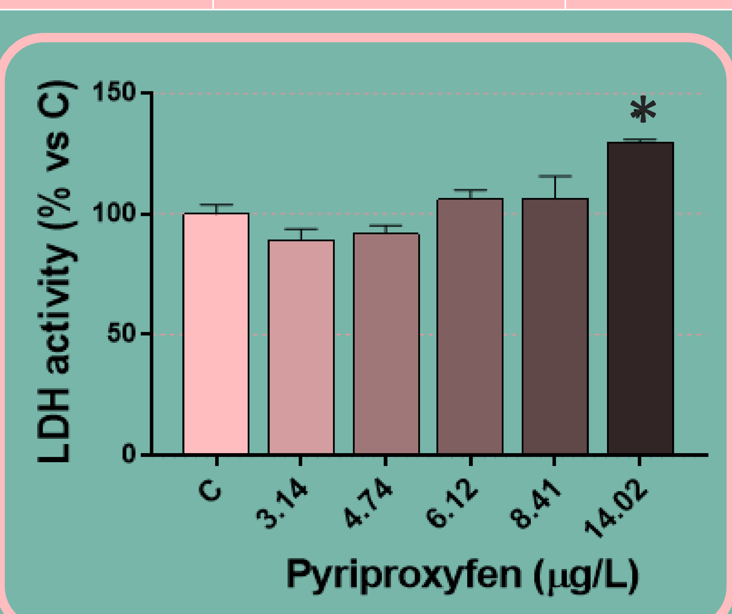


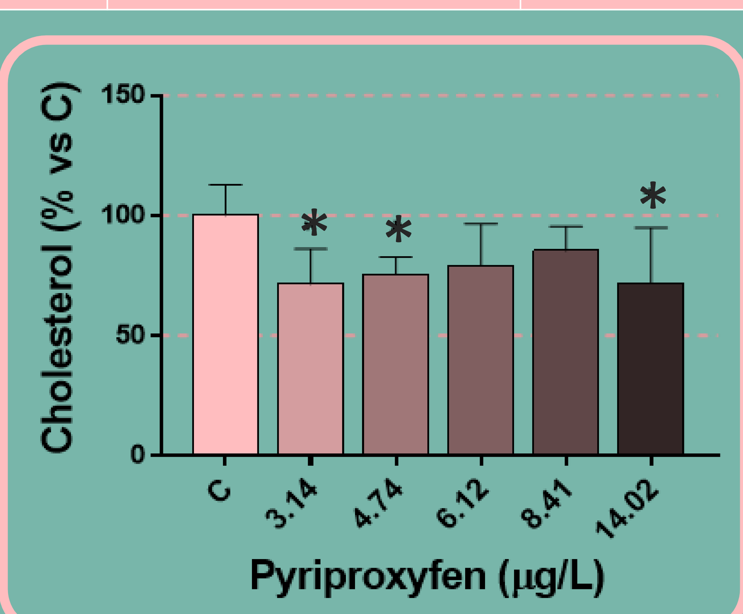
cat gene resulted underexpressed in the individuals as a consequence of the exposure to the insecticide, indicating a possible disruptive effect in the response to oxidative stress

	Control	3.14 µg/L	4.74 µg/L	6.12 µg/L	8.41 µg/L	14.02 µg/L
hgb1	1.00 ± 0.29	0.61 ± 0.09	2.31 ± 0.81	1.25 ± 0.22	3.23± 0.73*	3.59 ± 1.11*
hgb2	1.00 ± 0.24	2.61 ± 0.55	3.63 ± 0.66*	2.64 ± 0.26	3.74± 0.63*	3.37 ± 0.78*
hsp70	1.00 ± 0.12	0.97 ± 0.18	0.86 ± 0.06	0.97 ± 0.10	0.88± 0.11	0.76 ± 0.16
hsp90	1.00 ± 0.11	0.62 ± 0.11*	0.41 ± 0.03*	0.60 ± 0.05*	0.45± 0.07*	0.42 ± 0.09*
cat	1.00 ± 0.13	0.39 ± 0.04*	0.50 ± 0.08*	0.48 ± 0.08*	0.46± 0.11*	0.32 ± 0.03*
gst	1.00 ± 0.20	1.08 ± 0.08	0.80 ± 0.12	0.81 ± 0.12	0.94± 0.07	0.64 ± 0.13
vtg1	1.00 ± 0.22	0.16 ± 0.03*	0.23 ± 0.06*	0.14 ± 0.04*	0.21± 0.05*	0.17 ± 0.05*
vtg2	1.00 ± 0.22	0.15 ± 0.02*	0.21 ± 0.05*	0.16 ± 0.05*	0.20± 0.04*	0.16 ± 0.05*
mt-a	1.00 ± 0.17	1.14 ± 0.25	1.10 ± 0.13	1.17 ± 0.15	0.92± 0.10	1.14 ± 0.23
mt-b	1.00 ± 0.12	0.59 ± 0.08	0.73 ± 0.11	0.71 ± 0.04	0.70± 0.07	0.80 ± 0.20
mt-c	1.00 ± 0.26	1.56 ± 0.15	1.14 ± 0.18	1.27 ± 0.17	1.10± 0.11	1.24 ± 0.17
fabd	1.00 ± 0.11	0.66 ± 0.07*	0.54 ± 0.03*	0.63 ± 0.08*	0.56± 0.04*	0.53 ± 0.06*

hsp90 gene resulted underexpressed in the individuals as a consequence of the exposure to the insecticide

As a positive control genes related to oogenesis (***vtg1/vtg2***) confirmed the effect of pyriproxyfen as reproductor disruptor in aquatic invertebrates other than insects





fabd gene underexpression was reduced at all the assays. On the other hand, **cholesterol** levels were reduced at some of the concentrations tested and enzyme **LDH** activity increased significantly in the daphnids exposed to the highest pesticide concentration

CONCLUSIONS

Daphnia magna **metabolism was altered** after Pyriproxifen exposure as indicated by different gene expression, but only disturbances in both cholesterol and the LDH enzyme activity were observed.

Survival and **growth** of the individuals **could be compromised** as derived from the gene disturbances determined, especially those related to **hemoglobin**. Longer exposures as well as lower Pyriproxifen concentrations should be considered to obtain the minimal effects in aquatic invertebrates, such as daphnids.

Such assays will be useful in identifying disruptive effects at the individual level in the aquatic environments and then protecting invertebrate populations of species, as *D. magna*