FLUORANTHENE TOXICITY IN *Mytilus galloprovincialis* AT DIFFERENT STAGES OF GAMETOGENESIS MEASURED BY BIOCHEMICAL BIOMARKERS

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INTRODUCTION

Some endogenous factors as the mussel gametogenic cycle, seem to be related with enzymatic responses and could act as confounding factors in the use of this biomarker in large scale monitoring programs (González-Fernández *et al.*, 2015). The gonadal development of the wild mussel (*Mytilus galloprovincialis*), used as a sentinel species in the N-NW Spanish Marine Pollution Monitoring Program, takes place between late autumn and winter and spring when main spawning occurs.

OBJECTIVES

The aim of this study was to evaluate the effect of the reproductive status of the mussels on their response to the polycyclic aromatic hydrocarbon (PAH) fluoranthene (FLU) measured by several biochemical biomarkers of exposure (superoxide-dismutase -SOD-, catalase -CAT-, glutation reductase -GR-, glutation peroxidase -GPx-, glutation-s-transferase -GST-) and damage (lipid peroxidation -LPO-).

MATERIALS & METHODS

Experiment was designed to obtain mussels with the same condition in two different reproductive stages. Mussels were conditioned with *Isocrysis galbana* (T-ISO) for 1 week.

Mussels were exposed to two nominal concentrations of FLU (3 and 60 µg L^{-1}) for 3 weeks.

Exposure was carried out twice: during mussel resting time (Sept-Oct) and during mussels gametogenic cycle (March).

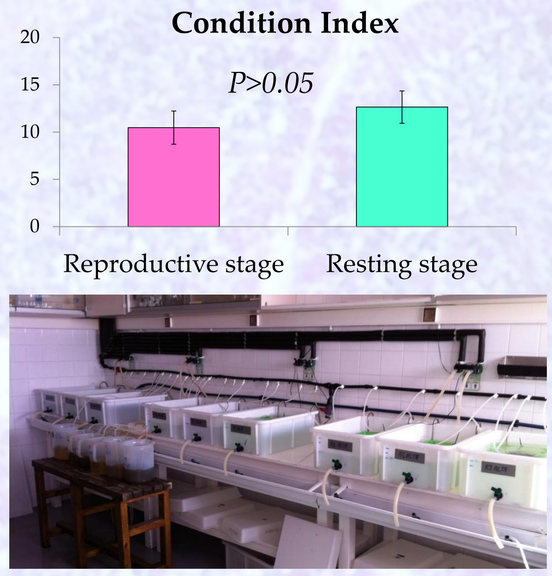
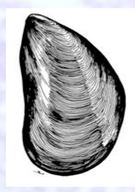


Figure 1. Mussel exposure to FLU.

POLLUTION BIOMARKERS

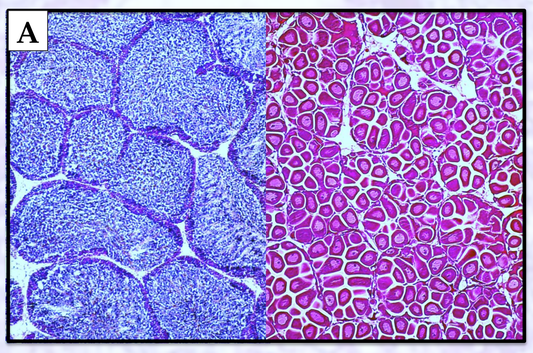


Determination proteins **(Lowry *et al.*1951)**
Determination enzymatic activities:
- **CAT: Claiborne (1985)**
- **SOD: Sigma-Aldrich SOD determination kit**
- **GPx: Halliwell and Gutteridge (1999)**
- **GR: Ramos-Martínez *et al.* (1983)**
- **GST: Habig *et al.* (1974)**
- **LPO: Buege and Aust (1978)**

STATISTICAL ANALYSES

Multifactorial ANOVA, SNK test (pos-hoc comparisons) between treatments (Toxic) and T-Student test between reproductive stages (RS).

RESULTS



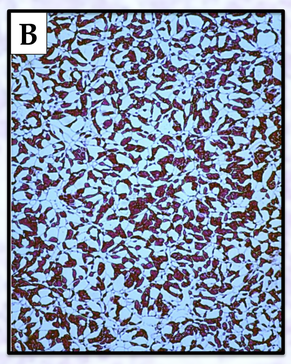


Figure 2. Control mussel histology:

(A) Gametogenic stage (March). Male (left) and female (right).

(B) Resting stage (Sept-Oct).

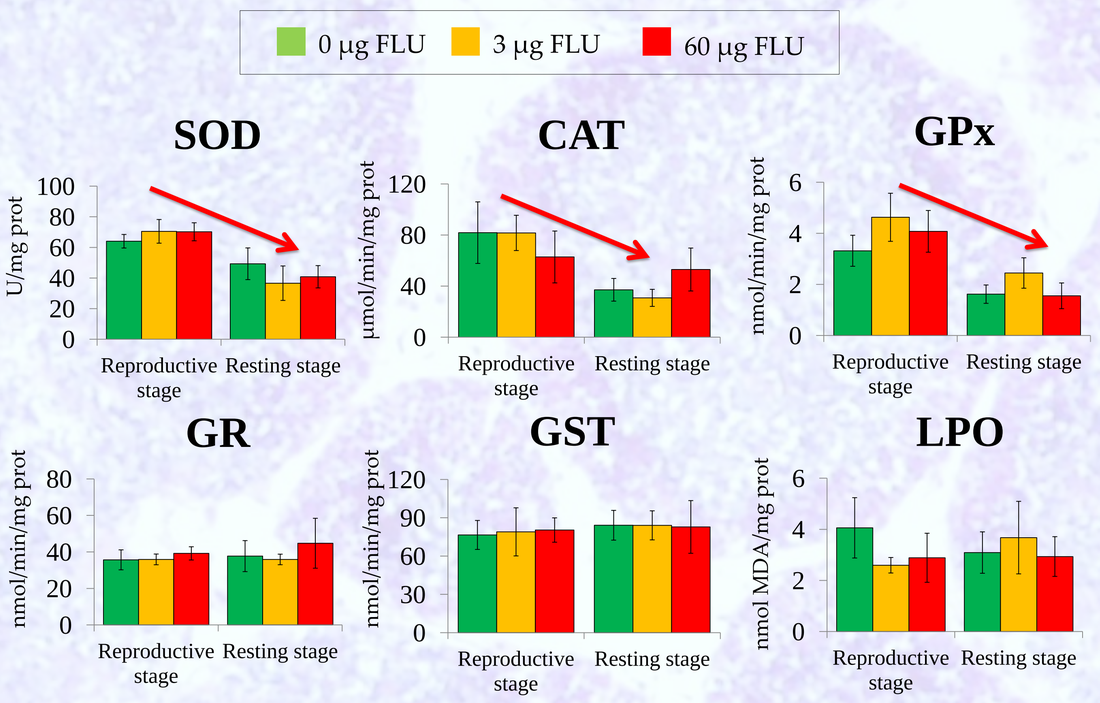


Figure 3. Summary of biochemical biomarker responses (means and standard deviations) of mussels, *Mytilus galloprovincialis*, at two different reproductive states (reproductive and resting stages) exposed to two nominal concentrations of Fluoranthene (Low and High). SOD: Superoxide-dismutase, CAT: Catalase, GPx: Glutathione peroxidase, GR: Glutathione Reductase, GST: Glutathione-S-transferase and LPO: Lipid Peroxidation

Table 1. Values of F-test from multifactorial ANOVA analysis being one factor the reproductive state (RS) and the other factor, the exposure treatment (Toxic). Interaction between both factors was also considered (RS*T).

Biomarker	F RS	F Toxic	F RS*T
SOD	65,44 **p<0.000**	0,49 p=0.62	3,11 **p=0.06**
CAT	29,42 **p<0.000**	0,09 p=0.91	4,63 **P<0.05**
GPx	69,26 **p<0.000**	6,22 p=0.01	0,84 p=0.45
GR	0,55 p=0.47	1,35 p=0.28	0,16 p=0.85
GST	1,15 p=0.29	0,08 p=0.92	0,04 p=0.97
LPO	0,25 p=0.62	0,57 p=0.58	1,87 p=0.18

Table 2. Means and p-values of T-Student analyses carried out on biochemical biomarkers considering the reproductive state.

Biomarker	Reproductive stage	Resting stage	p-value
SOD	**64,03**	49,31	**p<0.05**
CAT	**81,83**	37,11	**p<0.01**
GPx	**3,32**	1,62	**p<0.01**
GR	35,66	37,71	p=0.66
GST	76,52	84,10	p=0.33
LPO	4,06	3,09	p=0.17

CONCLUSIONS

- **The effect of *reproductive stage* (RS) was higher than the effect of *toxicant* on mussels. Higher SOD, CAT and GPx enzymatic activities were found at *reproductive stage*.**
- **The effect of toxicant was only evident at *resting stage*. Mussels exposed to high FLU concentration displayed the highest CAT values whereas mussels exposed to low FLU concentration showed the highest GPx values.**
- **No effect of *reproductive stage* or *toxicant* was detected on GR, GST and LPO activities.**
- **Mussel at *resting stage* seem to be more susceptible to pollution.**
- **Corrective strategies related with natural mussel gametogenesis cycle should be developed to enable the adequate use of biomarker s in monitoring programs.**

ACKNOWLEDGMENTS

This study has been founded by the BIOCOM project (CTM2012-30737, Spanish Ministry of Economy and Competitiveness) C. González-Fernández was supported by a pre-doctoral IEO grant.