

Does mussel nutritional status act as a confounding factor on biomarkers responses to pollution?

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1. Introduction

Large spatial scale mussel monitoring programs are characterized by a great variability of environmental conditions which cause important differences in animal physiology^{[1][2]}. One of the main differences encountered is the trophic condition which is highly variable when different habitats are monitored. Mussels, such as *Mytilus galloprovincialis*, are extensively used as sentinel species for pollution in marine coastal monitoring programs. From such studies, it has been stated that biological responses caused by pollutants seem to be masked by biological variables as mussel condition.

In this context, a laboratory experiment was designed to determine the relative importance of two stressors on some biochemical biomarkers: food availability and the presence of toxicants. Three experimental trophic conditions were simulated by regulating daily food ration: low food condition (N1), medium food condition (N2) and high food condition (N3), which respectively promote negative, maintenance and positive mussel energy balances.. In all cases, mussels were exposed to two nominal fluoranthene (FLU) concentrations (3 and 60 $\mu\text{g L}^{-1}$), for 3 weeks.

2. Materials and methods

Blue mussels, *Mytilus galloprovincialis*, were collected from an unpolluted site of the North-Atlantic Spanish coast and were transported to the IEO's laboratories (Murcia). Mussels were conditioned at three food rations: low (N1, 0.05% of the mussel weight), medium (N2, 0.15%), and high (N3, 0.3%). After conditioning, individuals were distributed equally in 30L-tanks where mussels were exposed to two nominal FLU concentrations (3 and 60 $\mu\text{g L}^{-1}$) for 3 weeks which is equivalent to exposition rations of 100 and 2000 mg FLU (kg mussel dw)⁻¹, respectively. Acetone was used as carrier and FLU was added to microalgae suspension prior to supply.

Mussel biochemical components (lipids -LIP-, carbohydrates -CH- and proteins -PROT-) were quantified in three pooled samples of five mussels each one. Protein content was determined using bovine serum albumin as a standard^[3]. Carbohydrates were extracted by boiling the samples with 5% TCA and determined using oyster glycogen as a standard^[4]. Extraction of total lipids was carried out with mixtures of chloroform-methanol-water^[5], using tripalmitin as a standard.

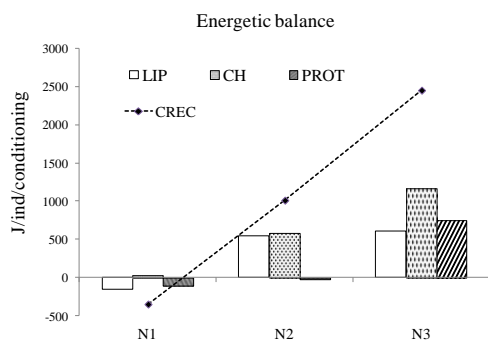
Digestive glands from 10 mussels of each condition were pooled into groups of two. Digestive glands were homogenized (1:4, w/v) in K-phosphate buffer 100mM, pH 7.6, containing 0.15 M KCl, 1mM DTT and 1 mM EDTA. After sequential centrifugations at 10.000xg for 20 min and 36.000xg for 60 min, the resulting microsomal pellet (microsomal fraction) was separated from the supernatant (cytosolic fraction) and resuspended in approximately 0.5ml of microsomal buffer (50mM Tris-HCl pH 7.6, containing 20% glycerol, 1mM DTT and 1mM EDTA). Measured enzymatic activities included: CAT^[6], GRx^[7], GPx^[8], GST^[8], SOD^[9] in the cytosolic fraction and LPO^[10] in the microsomal fraction. Enzymatic activities were expressed as nmol min⁻¹ mg protein⁻¹, apart from CAT, which was expressed as $\mu\text{mol min}^{-1}$ mg protein⁻¹ and SOD, which was expressed as U min⁻¹ mg protein⁻¹, being U defined as units of SOD, that is the amount of enzyme which inhibits 50% of reaction. LPO was expressed as nmol MDA mg protein⁻¹. Protein concentrations in both fractions (microsomal and cytosolic) were measured using bovine serum albumin as a standard^[3].

3. Results and discussion

3.1. Mussel nutritional status

After the conditioning period, nutritive-stressed mussels (N1) showed lower values of all biochemical components (LIP, CH and PROT) and this resulted in a negative growth. Conversely, medium-fed (N2) and high-fed (N3) mussels showed a positive growth and higher values of biochemical components, CH being those exhibiting the highest values. Therefore, after conditioning and before exposition, we obtained mussels at 3 different metabolic statuses: N1, catabolic, N2, maintenance and N3, anabolic metabolism.

Figure 1: Energetic balance of mussel (*M. galloprovincialis*) at the end of conditioning



3.2. Biomarkers

CAT, GPx and GST were affected by mussel nutritional status and evidenced the same behavior: nutritional stressed mussel (N1) had the highest enzymatic activity, contrary to N3-mussels which showed the lowest enzymatic activity. Regarding the effect of FLU on biomarkers, well fed mussels (N2 and N3) showed an increase in some antioxidant responses, as GST, GR and GPx, only at low FLU concentration. Considering these results it could be

suggested that the nutritional status responses seem to be higher than the pollutant response (Figure 2). Two important metabolic processes could be responsible for this behaviour. On the one hand, nutritive-stressed mussels seem to be more resistant to pollution than well-fed mussels, and on the other hand, it seems that some biomarkers have a "hormetic response" to FLU due to the low-concentration biomarker stimulation observed which was not detected at higher

FLU concentration.

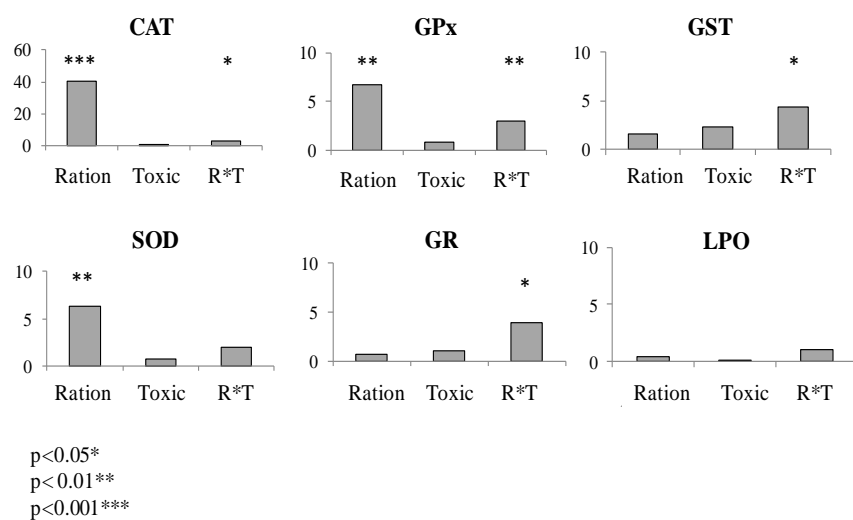


Figure 2: Multifactorial ANOVA results of biomarkers. F-ANOVA for the effect of Ration, Toxic and the interaction between them (R*T)

4. Conclusions

The results obtained in this study highlight the fact that the nutritional status could be more relevant than the effect of pollution in some

biomarkers commonly used in monitoring programs. Therefore, it needs to be considered in large scale monitoring programs where both variables, pollution and food conditions, can be interacting.

5. References

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Acknowledgement – We acknowledge F.Gómez and E.Vergara for their assistance. This study has been founded by the Spanish National R& D Program: BIOCUM project (CTM2012-30737).