The effect of food quality on immune and oxidative stress biomarkers of wild mussels, *Mytilus spp*.

C González-Fernández[1], C Lacroix[2], I Paul-Pont[2], M Albentosa[1], JA Campillo[1], J Bellas[3], L Viñas[3], F Le Grand[2], H Hegaret[2], P Soudant[2].

1Instituto Español de Oceanografía, Centro Oceanográfico de Murcia, Varadero, 1, 30740 San Pedro del Pinatar, Murcia, Spain.
2 LEMAR UMR 6539 UBO-CNRS-IRD-IFREMER, IUEM, rue Dumont d'Urville, Plouzané, France
3 Instituto Español de Oceanografía, Centro Oceanográfico de Vigo, Subida a Radio Faro 50, 36390 Vigo, Spain
carmen.gonzalez@mu.ieo.es

1. Introduction

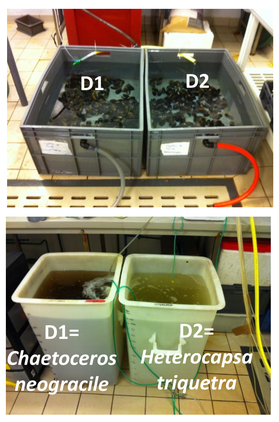
Blue mussel (*Mytilus spp*.) is commonly used in **pollution monitoring programs** due to its sessile character, wide distribution and ability to accumulate contaminants in tissues. Natural and anthropogenic discharges encourage the increase of eutrophication process, which promotes the proliferation of different algae types. This modification may influence the **nutritive condition** of marine organisms such as mussels, and thus affect their biological responses to pollutant exposures.

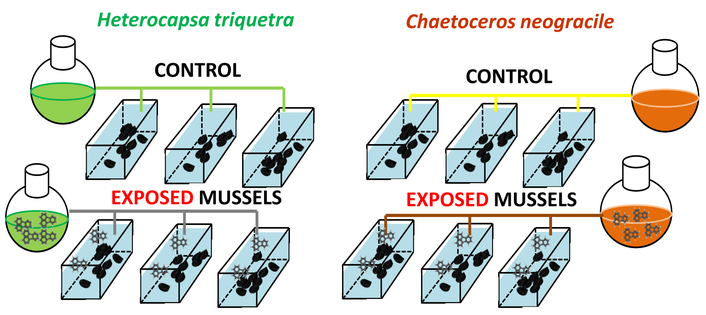
OBJECTIVE

The aim of this study was to evaluate the effect of food quality on pollutant bioaccumulation and mussel biomarker responses upon exposure to the polycyclic aromatic hydrocarbon (PAH) fluoranthene (FLU).

2. Materials and Methods

Mussels were conditioned to two algae species (D1, D2) that differed in biochemical composition for 2 months before being exposed to 30µg/L of Fluoranthene for 1 week.

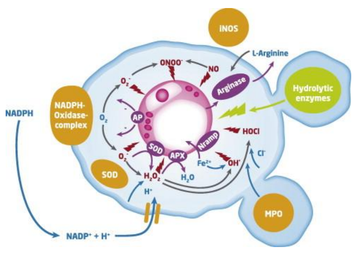




IMMUNOLOGICAL ANALYSES

Immune parameters were measured in mussel´s haemolymph through flow cytometry:

- Hemocyte mortality **(Delaporte et al., 2006)**
- Phagocytosis activities: phagocytosis capacity and phagocytosis rate **(Hégaret et al., 2003)**
- Hemocyte´ROS production **(Lambert et al. 2003)**



Production of Reactive Oxygen Species (ROS) by hemocytes (Soudant et al., 2013).

BIOCHEMICAL ANALYSES

Determination proteins **(Lowry *et al.1951)***
Determination enzymatic activities:

- **CAT: Claiborne (1985)**
- **SOD: Sigma-Aldrich SOD determination kit**
- **GR: Ramos-Martínez *et al.* (1983)**
- **GST: Habig *et al.* (1974)**
- **LPO: Buege and Aust (1978)**

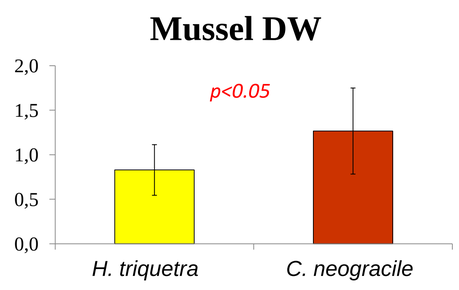
ANALYSIS	FUNCTION
CAT, SOD, GRx	Antioxidant enzymes
GST	Enzyme phase II biotransformation
LPO	Oxidative damage indicator

STATISTICAL ANALYSES

Multifactorial ANOVA analyses bewteen treatments and diets. T-Student tests to compare treatments (Toxic) and mussel nutritional conditions (diets) individually.

3. Results

MUSSEL DIETARY CONDITIONING



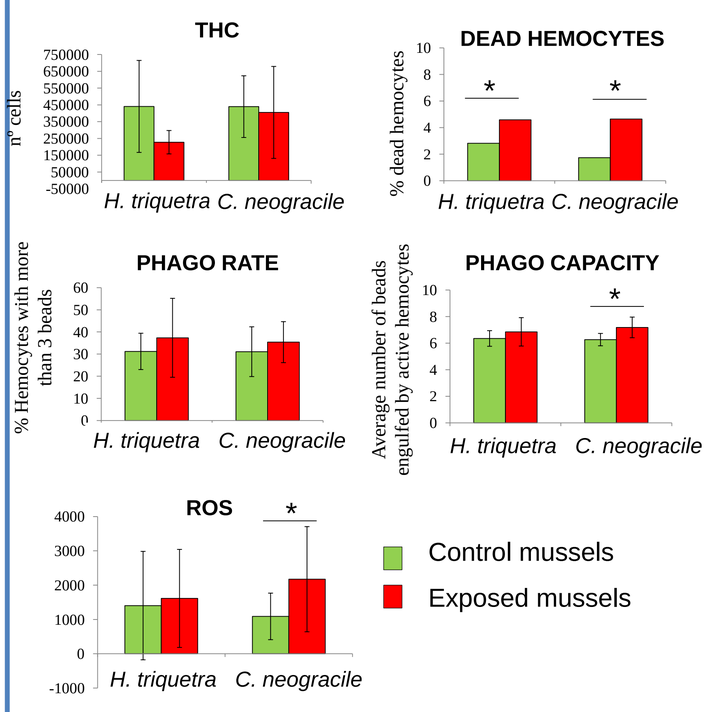
Mussel dry weights after dietary conditioning. Higher mussel dry weights were found in mussels fed with C.neogracile after dietary conditioning.

MUSSEL BIOACCUMULATION

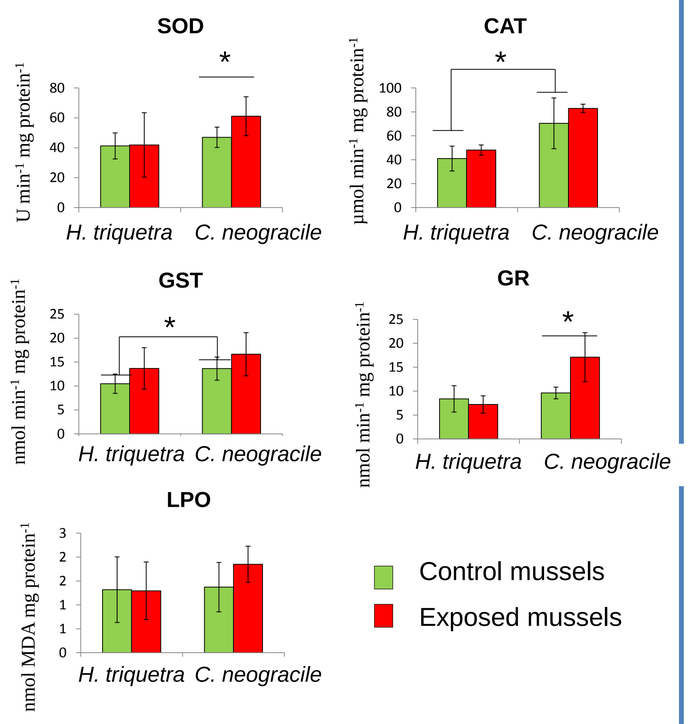
Diet	Treatment	time: Initial	time: After exposure
Heterocapsa spp.	Control		1.32±0.37^{aA}
	Fluoranthene	0.61±0.13^{a}	44.16±9.18^{bA}
Chaetoceros spp.	Control		0.94±0.13^{aA}
	Fluoranthene	0.61±0.13^{a}	106.74±7.87^{bB}

Fluoranthene accumulation in whole mussel tissues (µg/g mussel WW) at initial time and after exposure. T-Student test between treatments (Lowercases) and diets (capitals). Significant differences were observed in mussel accumulation. Higher Fluoranthene concentration was registered in mussels fed with *C.neogracile.*

IMMUNOLOGICAL BIOMARKERS



BIOCHEMICAL BIOMARKERS



Mean and standard deviations of mussel immunological parameters. After exposure, both diets showed an increase of dead hemocytes. Phagocytosis capacity ($p<0.01$) and ROS production ($p<0.05$) were increased upon FLU exposure in hemocytes of mussels fed with *C. neogracile.*

Mean and standard deviations of mussel biochemical biomarkers. T-student test showed that the antioxidant biomarker (CAT) and the phase II detoxification enzyme (GST) were affected by diet. Higher value of these activities was found in *C.neogracile* fed mussels. Furthermore, a significant effect of Fluoranthene was observed on SOD and GR acitivities in mussels fed with *C.neogracile*.

➡ Mussels fed with *C.neogracile* showed higher dry weights probably due to a better digestibility of diatoms. The better digestibility of diatoms could encouraged higher FLU accumulation in mussel´s tissues. As a result of higher FLU concentration in mussel tissues, mussels fed with *C.neogracile* displayed an increase of some immunological (phagocytosis capacity and ROS production) and biochemical (SOD and GR) activities.

➡ In addition, control mussels fed with *C. neogracile* showed higher CAT and GST activities. The highest fraction of absorbed food by these mussels, could promote the increase of ROS production, which was compensated by the increase of these enzymatic activities.

4. Conclusions

- Different mussel´s responses to pollutant were observed depend on dietary conditioning.
- Mussel fed with diatoms showed several immune **(%viability, phagocytosis rate and phagocytosis capacity**) and biochemical biomarkers (**SOD and GR**) **affected by toxic exposure**.
- **Mussel´s nutritive condition** need to be considered in pollution monitoring programs as a confounding factor on biomarker responses.

This study has been funded by the BIOCOM project (CTM2012-30737) funded through the Spanish National Plan R&D and the MICRO EU Interreg-funded project (MICRO 09-002-BE). C. González-Fernández was supported by a pre-doctoral IEO grant.

REFERENCES

Buege and Aust (1978). Methods in Enzymology Vol.52. Academic Press, New York, pp. 302-310.
Claiborne (1985). Handbook of Methods for Oxygen. Radical Research. CRC Press, Boca Raton pp.283-284
Ramos-Martínez *et al*. (1983). Comp. Biochem.Physiol.75B: 689-692.
Habig et al. (1974). J. Biol. Chem. 249: 7130-7139.
Lowry *et al.* (1951). J.Biol.Chem.193: 266–275.
Hégaret et al. (2003). J Exp Mar Bio Ecol 293: 249–265
Lambert et al. (2003). Fish Shellfish Immunol 15: 225–240
Delaporte et al. (2006). Aquaculture 254, 571–582.