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Effect of diet quality on mussel biomarker responses to pollutants

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ABSTRACT

The effect of the quality of two microalgal species on select biological and biochemical responses used as indicators of pollution were assessed. Mussels were conditioned for 6 weeks with the diatom *Chaetoceros neogracile* and the dinoflagellate *Heterocapsa triquetra*, chosen for being two clearly different types of primary production quality that differ in both biometric and biochemical characteristics. After dietary conditioning, the mussels were exposed to a polycyclic aromatic hydrocarbon, fluoranthene (FLU), for 1 week followed by 1 week of depuration. Results showed higher FLU accumulation in mussels fed on *C. neogracile* compared to those fed on *H. triquetra*. Concomitantly, a greater impact of this toxicant was observed in the biomarker responses of mussels fed on *C. neogracile*. These mussels showed an increase in the percentage of dead hemocytes, an activation of phagocytosis and ROS production of hemocytes after exposure. Some enzymatic activities also increased upon FLU exposure (superoxide dismutase –SOD-, catalase –CAT-, and glutathione reductases –GR-) and after depuration (glutathione-s-transferase –GST-). Results suggest that FLU exposure as well as food quality influence biomarker responses, with higher values of SOD, CAT and GR in non-exposed mussels fed on *C. neogracile*. In addition, upon exposure to the same FLU concentration, GR response varied according to dietary conditioning, suggesting that diet could act as a confounding factor in biomarker responses to pollution. Consequently, trophic conditions should be considered in marine pollution monitoring programs for a better interpretation of biomarker responses.

1. Introduction

In recent decades, marine pollution monitoring programs have incorporated the analysis of biological effects caused by exposure to chemical pollution in sentinel species (biomarkers), to assess marine environmental quality (e.g. Davies and Vethaak, 2012). Mussels, such as *Mytilus galloprovincialis*, are among the most commonly-used sentinel organisms in integrated monitoring programs due to their sedentary nature, wide-spread geographical distribution, capacity for accumulating contaminants and ease of sampling (Kimbrough et al., 2008; Nakata et al., 2012; Sericano et al., 2014; Thébault et al., 2008; Widdows et al., 2002).

Biological responses, used as pollution biomarkers, also play a primary role in the normal homeostasis of the organism, and can therefore be affected by environmental conditions (Nahrgang et al., 2010). Previous studies highlighted the effect of the biological condition of mussels on antioxidant and physiological biomarker responses (Albentosa et al., 2012; Bellas et al., 2014; González-Fernández et al., 2015a). The high degree of geographical variability observed in the growth and reproductive conditions of the mussels was linked to the differences in food availability between mussel sampling areas. Different food rations (González-Fernández et al., 2015b) or reproductive stages (González-Fernández et al., 2015c), under laboratory conditions, showed the strong effect that mussel nutritive or reproductive conditions have on biomarker responses and their interpretation.

Coastal waters are characterized by large variations in food supply which are not only related to food availability, but also

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to the species composition of the phytoplankton community (Bayne, 1993), which varies both temporally and spatially in response to physical and biological factors. Among the different phytoplankton groups living in coastal ecosystems, diatoms and dinoflagellates are known to prevail under different oceanographic conditions (Oliveira et al., 2009). Natural (upwelling) and anthropogenic eutrophication processes modify phytoplankton patterns depending on their specific nutrient requirement. In general, diatom blooms prevail during the spring and summer upwelling fertilization events, whereas dinoflagellate blooms are more abundant in nutrient-poor waters or during stratified conditions in summer (Oliveira et al., 2009; Smayda and Trainer, 2010). In addition, anthropogenic eutrophication, characteristic of semi-enclosed coastal ecosystems, also modifies nutrient composition which in turn affects the species composition of phytoplankton communities. Excessive N-inputs in eutrophic systems cause a decrease in Si:N ratios which lead to a proliferation of non-siliceous species such as dinoflagellates to the detriment of diatom dominance (Fouillaron et al., 2007). Such a modification in plankton structure can impact higher trophic levels, especially primary consumers such as filter feeders, for example mussels (Chauvaud et al., 2001; Bougrier et al., 1997; Rouillon and Navarro, 2003).

Differences among phytoplankton species are related to cell size, toxicity, cell wall composition, proximal composition or essential nutrients such as fatty acids, which modify energy and nutrient uptake by filter feeders. Food properties such as availability (food quantity) and quality can affect biological responses used as pollution biomarkers. However, the way in which changes in primary production quality could affect biomarker responses upon exposure to pollutants in large-scale monitoring programs where a wide range of phytoplankton communities can be found at the same time remains unclear. To validate the use of biomarkers in marine monitoring programs it is critical to understand how they are influenced by primary production quality.

This study examined selected biomarkers at different biological organization levels (biochemical, cellular and organism) to obtain a better picture of the effects of dietary conditions on biomarker responses in mussels. Hemocyte parameters such as hemocyte counts, percentage of dead hemocytes, hemocyte phagocytosis rate and capacity, and hemocyte reactive oxygen species (ROS) production in mussels were measured. Some of the most useful biochemical biomarkers used in monitoring programs were also included: superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione-S-transferase (GST) activities, and lipid membrane peroxidation (LPO), which were previously proven to reveal exposure to a wide range of pollutants such as metals or organic compounds (Campillo et al., 2013; Fernández et al., 2010, 2012; Regoli, 1998). Furthermore, the fatty acid composition of mussel membrane and reserve lipids were measured to assess the extent of dietary modification after conditioning.

Two common phytoplankton species in temperate coastal ecosystems, the diatom *Chaetoceros neogracile* and the dinoflagellate *Heterocapsa triquetra*, were selected as well-differentiated types of primary production quality. These microalgae species differ in several aspects: cell size, mobility, cell wall and fatty acid composition, all of which can influence the growth, reproduction, defense/detoxification systems and physiology of mussels (Parrish et al., 2000). Regarding pollution simulation, we selected the toxicant fluoranthene (FLU) as a model polycyclic aromatic hydrocarbon (PAH) because it is included in the lists of priority substances in the field of water policy of both the European Commission (EC) and the United States Environmental Protection Agency (USEPA). This PAH is considered to be among the most toxic PAHs for marine biota in the short term (Othman et al., 2012).

The experimental design consisted in conditioning mussels for 6 weeks to two different diets so as to obtain a different mussel dietary status, after which the mussels were exposed to FLU for 1 week. They were then depurated for 1 week to assess their recovery capacity after exposure under the different dietary scenarios. This paper identifies (i) the effect of primary production quality on a battery of cellular and biochemical biomarkers and (ii) the combined effect of pollutant and mussel dietary condition on those biomarkers.

2. Material and methods

2.1. Mussel collection and conditioning

Mussels (*Mytilus spp.*) from *Pointe d'Armorique* in the Bay of Brest (France) were collected at low tide in September 2013 and equally distributed into two 100 L tanks to acclimatise them to laboratory conditions (1 μm-filtered seawater, 17 °C, and an aerated open flow system). Two different diets, *Chaetoceros neogracile* (*C*) and *Heterocapsa triquetra* (*H*), were supplied at 3% of microalgal organic matter per mussel tissue dry weight for 6 weeks to prepare 2 different dietary conditioned mussel groups: *C*-mussels (mussels fed on *C. neogracile*) and *H*-mussels (fed on *H. triquetra*). The concentration of algae was measured daily by flow cytometry prior to feeding the mussels.

2.2. Exposure

After the conditioning period, mussels from each dietary conditioned group (*C*-mussels and *H*-mussels) were distributed into 40 L tanks (N = 3 per treatment). A total of 6 tanks per dietary conditioning (3 non-exposed, control tanks and 3 FLU-exposed tanks) were prepared with 25–28 mussels in each tank. FLU exposure was carried out for 1 week with the food, at the amount stemming from concentrations of 30 $\mu g\,L^{-1}$ FLU, per day. FLU was dissolved in acetone at 1 $g\,L^{-1}$ and mixed with the algal culture 0.04% (v/v), for each algal species separately, for 45 min in the dark, to final concentration of 0.003% (v/v) in the tank, before being fed to mussels. Contaminated algae were supplied with a peristaltic pump giving 1.5% of microalgal organic matter per soft tissue dry weight (*C* or *H*) in an aerated closed system. Control treatments (solvent only) were performed with the same ration of algae. The water was renewed every day.

2.3. Depuration

Each mussel dietary conditioning was maintained for one more week, the mussels being fed with 1.5% of microalgal organic matter per mussel tissue dry weight with a peristaltic pump, with no toxicant. The water was renewed every day.

2.4. Sample preparation

Mussels were sampled twice, seven days after the start of FLU exposure and after seven days of depuration. At each sampling time, 22 mussels were collected per diet group and FLU treatment. Ten mussels were used to determine the biometric parameters and the rest were used for biomarker analyses. Hemolymph from 12 mussels was extracted and the digestive glands were subsequently dissected in RNase-free conditions, quickly flash-frozen in liquid nitrogen and stored individually at −80 °C. Organs were homogenized using a mixer mill MM400 (Retsch) under liquid nitrogen and divided for further chemical, biomarker and lipid analyses.

2.5. Chemical analyses

Chemical analyses were conducted in two different ways to quantify i) FLU accumulation in whole mussels using 3 pools of 5