



Effect of mussel reproductive status on biomarker responses to PAHs: Implications for large-scale monitoring programs



Carmen González-Fernández^a, Marina Albentosa^{a,*}, Juan A. Campillo^a, Lucía Viñas^b, Angeles Franco^b, Juan Bellas^b

^a Instituto Español de Oceanografía, IEO, Centro Oceanográfico de Murcia, Varadero 1, E-30740 San Pedro del Pinatar, Murcia, Spain

^b Instituto Español de Oceanografía, IEO, Centro Oceanográfico de Vigo, Subida a Radio Faro 50, E-36390 Vigo, Spain

ARTICLE INFO

Article history:

Received 10 January 2016

Received in revised form 6 June 2016

Accepted 16 June 2016

Available online 16 June 2016

Keywords:

Biomarkers

Mussels

Reproductive status

Fluoranthene

Confounding factors

Monitoring programs

ABSTRACT

Biomarkers are useful tools to assess biological effects of pollutants and have been extensively used in monitoring programs to determine ecosystem health. In these programs, a wide range of environmental conditions are covered and sometimes, obtained data are difficult to interpret because of natural variables are affecting biomarker responses. Among these variables, mussel's reproductive status has been considered one of the most changing variables between sites in a monitoring survey. Thus, the main aim of this work was to identify the effect that mussel reproductive status has on biomarker responses. For that purpose, mussels sampled at two periods in the reproductive cycle (reproductive and resting stages) were conditioned to the same laboratory conditions and exposed to fluoranthene (FLU) for three weeks. Studied biomarkers covering a wide range of organism responses were included: bioaccumulation, physiological rates (clearance rate –CR–, absorption efficiency –AE–, respiration rate –RR– and their integration in the scope for growth –SFG–), antioxidant enzyme activities (superoxide-dismutase –SOD–, catalase –CAT–, glutathione reductase –GR–, glutathione peroxidase –GPx–, glutathione-S-transferase –GST–) and biochemical damage responses (lipid membrane peroxidation –LPO–). The results obtained evidenced that the levels of the biomarkers studied (RR, SOD, CAT and GPx) were higher at reproductive than at resting stage. On the other hand, the effect of toxicant was observed in SFG, CAT and GPx but this effect was only detected during the resting period. Moreover, there was a deterioration of mussel gonadal tissue with FLU exposure during reproductive stage. FLU accumulation in mussel tissues was also dependent of the reproductive status with higher internal concentrations during resting than reproductive period. In conclusion, there was a strong effect of reproductive status on studied biomarkers which seems to mask the effect of FLU at reproductive stage. The present study evidences the need to include the measurement of mussel biological status in marine pollution monitoring programs for a correct interpretation of biomarker data.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Mussels (*Mytilus* spp.) have been extensively used as sentinel species in marine monitoring programs to determine pollution level, mainly due to their wide distribution, and also because they tend to accumulate the pollutants contained in the water column, reaching very high concentrations (Kimbrough et al., 2008; Sericano et al., 2014; Widdows et al., 2002). The implementation of these programs in Europe is now determined by the Marine Strategy Framework Directive (MSFD, European Commission Directive 2008/56/EC), which demands an approach focused not only on

the analytical chemistry of pollutants, but also on evaluating their effects on the ecosystems. Thus, for water quality assessment, it is essential to establish a link between pollutant levels and their harmful effects on marine ecosystems (Bellas, 2014; Davies and Vethaak, 2012).

Biomarkers are common tools used in marine pollution monitoring programs as they seem to be indicative of xenobiotic exposure and/or effect. Among them, the measurements of the activity of antioxidant enzymes in mussels have been widely used as biomarkers of exposure to environmental pollutants (Lam, 2009). In this study, some of the most frequently used biomarkers of oxidative stress have been considered: superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) activities. Furthermore, an oxidative damage indicator was also considered,

* Corresponding author.

E-mail address: marina.albentosa@mu.ieo.es (M. Albentosa).

the lipid membrane peroxidation (LPO), which indicates the damage to cellular membrane lipids caused by ROS and is useful for assessing exposure to, and the effects of pollutants in mussels (Campillo et al., 2013; Fernández et al., 2010, 2012). In addition, mussel physiological biomarkers (the scope for growth, SFG) have been included in this study, as a measure of stress effects at individual level caused by pollutants (Albentosa et al., 2012; Bellas et al., 2014; Widdows et al., 2002). The SFG gives the energy available for growth under standardized laboratory conditions (Widdows and Staff, 2006).

The establishment of links between chemicals in the environment and measured biomarker responses in organisms is sometimes difficult in field conditions, where several biotic and abiotic factors interact, affecting biological responses to pollution. Studies carried out by our group (Albentosa et al., 2012; Bellas et al., 2014; González-Fernández et al., 2015a), suggested that some biological variables, for instance mussel condition, act as confounding factors hindering the use of biomarkers in monitoring programs. This challenge is especially critical in large-scale monitoring programs where large geographical variability in environmental characteristics is observed. Mussel condition is dependent on both nutrition and reproduction, which are closely associated. Gonadal development is a process demanding much energy, and thus requires high food availability conditions or body reserves (Gabbott, 1975). In large-scale programs, variability of natural trophic conditions between sampling sites leads to differences in mussel gonadal development in a way that the whole range of gametogenic stages coexist at the same sampling period, even when sampling is carried out during the theoretically resting stage for the species considered. This is especially true for opportunistic species as mussels, which, if environmental conditions (food availability and temperature) are favorable, builds up an important gonadal development (Bayne, 1976). For that reason, in low latitudes, as is the case of the above-mentioned studies (N-NW Spanish Atlantic-Cantabrian coast), the cyclical nature of gametogenesis in *Mytilus* is less evident (González-Fernández et al., 2015a).

In field studies, it is thus impossible to discern which process, gametogenesis or nutrition, is responsible of animal condition, which, in turn, influences biomarker responses to pollution. In order to establish the specific contribution of each factor, two experimental studies were done under controlled laboratory conditions. The first one (González-Fernández et al., 2015b) aimed to assess the biological responses to pollution under different nutritive regimes. Mussels with inactive gonads were conditioned to 3 food concentrations; consequently, high body condition of mussels was achieved only by nutrition without the influence of gametogenesis. This study evidenced that most of the studied biomarkers were strongly affected by mussel nutritive status, showing higher biomarker values in the mussels with lower nutritive status and masking the effect of contaminants. The referred study demonstrated that toxicants were not the only source of variability modulating pollution biomarkers, and confirmed nutritive status as a major factor altering biomarker responses.

The second experimental study, designed to ascertain the effect of reproduction on the pollution responses of biomarkers, is presented here. Similarly to the nutrition experimental study, the variable reproduction was isolated from the variable nutrition, and two gonadal stages were used under the same nutritive conditions. It has been difficult to find, in the literature, studies on mussels which consider the reproductive cycle as key factor on pollution biomarker responses under controlled laboratory conditions. The closest literature on this subject is on field studies about the seasonal variability on biomarker responses in native (Bocchetti and Regoli, 2006; Borković et al., 2005; Nahrgang et al., 2013; Power and Sheehan, 1996; Schmidt et al., 2013; Sheehan and Power, 1999; Vidal-Liñán et al., 2010) or transplanted mussels (Bodin et al., 2004;

Palais et al., 2012). However, as both food availability and gonadal development are cyclical processes that follow an annual pattern (Bayne, 1976; Gabbott, 1976), it is difficult to identify the effect of gametogenesis on biomarker responses in these seasonal studies.

Thus, the aim of the present work was to identify the effect of the reproductive status on biomarker responses in mussels (*Mytilus galloprovincialis*) maintained under the same environmental conditions of food availability and temperature, although mussels differed on the developmental stage (mature and immature). After acclimation to laboratory conditions, mussels from each reproductive status were exposed to the toxicant fluoranthene (FLU), as a model polycyclic aromatic hydrocarbon (PAH). Thus, this study intends to identify (i) the effect of mussel reproductive status on a battery of biochemical and physiological biomarkers and (ii) the combined effects of a pollutant and the mussel reproductive status on those biomarker responses.

2. Materials and methods

2.1. Mussel collection and acclimation

Wild mussels (length 4.5 cm), *M. galloprovincialis*, were collected from an unpolluted site at Galicia (NW Spain) at two different occasions with the aim of covering two different events of the annual reproductive cycle: reproductive and resting stages. Collection site was selected due to its localization far from polluting industries or cities, showing background levels of pollutants (González-Fernández et al., 2015a). Mussel sampling was distributed throughout the year to meet these experimental premises. Mussels collected in February (reproductive stage) and September (resting stage) of 2013 were transported in cold and air-exposed via overnight express delivery services (less than 24 h) to the laboratory of the Spanish Institute of Oceanography (IEO) in Murcia.

At the two seasons considered, mussels were acclimated to standardized laboratory conditions (filtered seawater 0.5 μm , 15 °C, fed with a ration of 0.17% of microalgal organic matter (OM) per mussel live weight of the microalgae *Isochrysis galbana*, clone t-ISO) in an aerated closed system for ten days, in order to harmonize the condition of mussels under the same environmental conditions.

Moreover, after acclimation 15 mussels were collected (initial time) at each sampling period in order to determine biological and histological characterization prior exposure with the objective of (i) confirming that mussels were at the reproductive stage desired and (ii) determining mussel weight in order to expose mussels to the same toxicant dose in the two reproductive stages studied.

2.2. Mussel gametogenic development

The gametogenic development of mussels was checked in 15 mussels which were dissected and their mantle tissues preserved in a 10% v/v formaldehyde solution for fixation during at least 24 h, and were subsequently processed for the histological study. Dehydration of tissues was carried out in successive and increasing alcohol baths, followed by the replacement of alcohol for Ultra-clear as clearing agent. Then, tissues were embedded in paraffin, cut at 3 μm , and stained with haematoxylin and eosin. Histological sections were observed under an optical microscope to identify sex and stage of gonadal development. Five stages were considered (Kim et al., 2006): Stage 0, inactive gonad; Stage 1, gametogenesis has begun although no ripe gametes are visible yet; Stage 2, ripe gametes are present and gonias occupy about one-third of the section; Stage 3, gonias increased in area to about half the fully ripe condition; Stage 4, gonad fully ripe.

The proportion of gonadal tissue that is comprised of follicles containing developing or ripe gametes is reported as the gamete

Download English Version:

<https://daneshyari.com/en/article/6382040>

Download Persian Version:

<https://daneshyari.com/article/6382040>

[Daneshyari.com](https://daneshyari.com)