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Influence of mussel biological variability on pollution biomarkers

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ABSTRACT

This study deals with the identification and characterization of biological variables that may affect some of the biological responses used as pollution biomarkers. With this aim, during the 2012 mussel survey of the Spanish Marine Pollution monitoring program (SMP), at the North-Atlantic coast, several quantitative and qualitative biological variables were measured (corporal and shell indices, gonadal development and reserves composition). Studied biomarkers were antioxidant enzymatic activities (CAT, GST, GR), lipid peroxidation (LPO) and the physiological rates integrated in the SFG biomarker (CR, AE, RR). Site pollution was considered as the chemical concentration in the whole tissues of mussels.

A great geographical variability was observed for the biological variables, which was mainly linked to the differences in food availability along the studied region. An inverse relationship between antioxidant enzymes and the nutritional status of the organism was evidenced, whereas LPO was positively related to nutritional status and, therefore, with higher metabolic costs, with their associated ROS generation. Mussel condition was also inversely related to CR, and therefore to SFG, suggesting that mussels keep an "ecological memory" from the habitat where they have been collected. No overall relationship was observed between pollution and biomarkers, but a significant overall effect of biological variables on both biochemical and physiological biomarkers was evidenced. It was concluded that when a wide range of certain environmental factors, as food availability, coexist in the same monitoring program, it determines a great variability in mussel populations which mask the effect of contaminants on biomarkers.

1. Introduction

Environmental monitoring programs have been traditionally designed for quantifying the concentration of chemical pollutants in different marine matrices: water, sediment and biota. These coastal pollution monitoring programs have included the analysis of biological effects caused in sentinel species (bioindicators) by the exposure to chemical pollution, in order to obtain combined chemical and biological data, to assess marine environmental quality (Bellas et al., 2014; Thain et al., 2008). Mussels are among the most commonly-used sentinel organisms in pollution studies due to its sedentary nature, its wide-spread geographical distribution, its great capacity for accumulating contaminants and its easy sampling (Kimbrough et al., 2008; Nakata et al., 2012; Sericano et al., 2014; Thébault et al., 2008; Widdows et al., 2002). Mussel biological responses (biomarkers) have been proposed as sensitive "early warning" tools to assess the environmental quality of coastal areas (Cajaraville et al., 2000; Lam, 2009) and some of them have been incorporated by different international pollution monitoring programs (OSPAR, 2012a; ICES, 2013). Thus, biomarkers are defined as quantitative measurements of changes occurring at cellular, biochemical, molecular, or physiological levels that can be measured in cells, body fluids, tissues or organs within an organism and that may be indicative of xenobiotic exposure and/or effect (Lam and Gray, 2003).

Pollutant-mediated generation of reactive oxygen species (ROS) is likely to induce antioxidant defense mechanisms in exposed organisms to prevent oxidative damage to cellular macromolecules (Livingstone, 2001). The measurement of the activity of antioxidant enzymes in mussels has been widely used as biomarkers of exposure to environmental pollutants (Lam, 2009). For the present study, we selected some exposure biomarkers (Catalase – CAT, Glutathione Reductase – GR and Glutathione S-transferase – GST activities) and an effect biomarker (Lipid peroxidation – LPO), which have been used to reveal the exposure to metals and a wide range of organic compounds in the environment (Campillo et al., 2013; Fernández et al., 2010a, 2010b, 2012; Fitzpatrick et al., 1997; Funes et al., 2006; Lee et al., 1988; Regoli, 1998; Vidal-Liñán et al., 2010). CAT is an extremely active catalyst for reduction of H_2O_2 to H_2O at high levels of H_2O_2, but at low levels it modulates

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the detoxification of other substances as phenols and alcohols through reactions coupled to the H_2O_2 reduction. Although the GR does not play a direct role in the elimination of oxygen radicals, it can be regarded as an essential antioxidant enzyme since it reduces oxidized glutathione (GSSG) and maintains the GSSG/GSH balance, which is essential for cellular homeostasis and the operation of other enzymes (Regoli and Giuliani, 2013). GST is a Phase II detoxification enzyme involved in the conjugation and detoxification of organic compounds, and also plays a protective role against oxidative stress by catalyzing a selenium-dependent glutathione peroxidase (Prohaska, 1980; reviewed by Sheehan et al., 2001). Finally, LPO 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; Cheung et al., 2001; ICES, 2013; Regoli and Principato, 1995; Regoli, 1998).

Regarding physiological biomarkers, the measurement of the scope for growth (SFG) has also been used as a measure of stress effects caused by pollutants in marine organisms (Albentosa et al., 2012a; Bellas et al., 2014; Halldórsson et al., 2005; Tsangaris et al., 2010; Widdows et al., 2002). SFG is a technique involving the calculation of the energy available for growth under standardized laboratory conditions. In short, it consists of evaluating the energy acquired by an organism after absorbing the food it has ingested, and that lost in the respiratory and excretory processes, being the difference the energy the organism has available for production (growth and reproduction) (Widdows and Johnson, 1988; Widdows and Donkin, 1989, 1991).

In general, biological responses used as biomarkers play a primary role in the normal homeostasis of the organism, and therefore, can also be influenced by natural biological and environmental cycles (Nahrgang et al., 2010). In fact, it has been previously highlighted that some confounding factors can alter biomarker responses to pollution (Coulaud et al., 2011; Lam, 2009). For instance, it has been reported that some enzymatic activities associated to biomarker responses change due to the natural physiological or reproductive cycles (Borkovic et al., 2005; Sheehan and Power, 1999), differences in food availability (Martínez-Álvarez et al., 2005) or water temperature (Viarengo et al., 1998), which may be misinterpreted as an exposure effect. These problems are magnified in large scale monitoring programs, as the present study, where organisms are subjected to a wide range of environmental conditions. Previous studies carried out within our group (Albentosa et al., 2012a; Bellas et al., 2014) have revealed that physiological and biochemical biomarkers seems to be more affected by mussel biological parameters than by chemical pollution. To validate the use of biomarkers in marine pollution studies it is indispensable to understand the importance of environmental variables on the studied biological responses (Tankoua et al., 2011).

The present study was carried out during the 2012 mussel Atlantic survey of the Spanish Marine Pollution monitoring programme, SMP, which is included within the framework of the Joint Assessment and Monitoring Programme (JAMP, OSPAR Commission). This survey is usually carried out in autumn (November), when mussels are in a more stable physiological state, according to the JAMP Guidelines for Monitoring Contaminants in Biota (OSPAR Commission, 2010a) and covers more than 2500 km of the N-NW Spanish coastline, located in the OSPAR Region IV. The SMP in this area consists of 40 sampling sites, from which 23 sites are monitored yearly. The sampling area includes two different oceanographic regions: the Atlantic and the Cantabrian coast in the Bay of Biscay, which differ in their trophic and hydrodynamic characteristics. The wind intensity and strength on the Galician platform provides upwelling processes mainly at the end of the spring, with maximum values in summer. The intensity of the upwellings decrease from Cabo Finisterre to Santander, which is considered the limit of the Iberian upwelling (Lavín et al., 2012; Molina, 1972). The Atlantic coast has also shown several periods of upwelling in late summer or autumn (Bode et al., 1996, 2011; Botas et al., 1990). The Cantabrian coast also shows the upwelling phenomenon between the late spring and summer but with less intensity and duration than in the Atlantic coast (Gil, 2008; Lavín et al., 1998; Llope et al., 2003). In summary, Atlantic waters are more productive than those of the Cantabrian zone, due to the greater abundance and intensity of upwellings processes from which nutrient-rich water rises from bottom fertilizing surface waters (Cermeño et al., 2006; Fraga, 1981).

The main objectives of the present study were to identify and characterize quantitative and qualitative biological variables (corporal and shell indices, gonadal development and reserves composition) that may affect the mussels' response to pollutant exposure in the populations of the SMP, and to establish the relationships between those biological variables and biomarkers responses, on the one hand, and between pollutants bioaccumulation and biomarkers on the other. The final objective of the study was to describe to what extent the variation in biomarkers responses can be explained by biological variables and by pollution.

2. Material and methods

2.1. Specimen sampling

Mussels (41.63 ± 1.06 mm in length), *Mytilus galloprovincialis*, were collected from 23 sites along the N-NW Spanish Atlantic coast, in November 2012, at low tide and transported in cold and air exposed, to the IEO's laboratories. These sampling sites were considered for the spatial distribution studies of the SMP, since they cover a wide range of environmental scenarios (pollution sources, industrial areas, cities, upwelling, etc.). Table 1 shows the

Table 1
Location and environmental characteristics of the 23 sampling sites along the N-NW Spanish coast. Numbers under Environmental Conditions relate to the degree of waves exposure (1: protected; 2: less protected; 3: Moderately exposed; 4: exposed and 5: heavily exposed) and letters are related to trophic conditions (H: hypertrophic and O: oligotrophic conditions) according to Ospina-Alvarez et al. (2014).

Sampling site	Code	Latitude	Longitude	Environmental conditions
		Atlantic coast		
Oia	2	41°58.212	08°53.199	5 H
Samil	3	42°13.177	08°46.604	2 H
Cabo Home	5	42°15.007	08°52.333	5 H
Pontevedra Raxó	7	42°24.172	08°44.968	2 H
Chazo	8	42°36.374	08°51.761	2 H
Punta Insua	12	42°46.423	09°07.550	5 H
Corme	14	43°14.569	08°56.642	3 H
A Coruña TC	16	43°22.178	08°23.160	5 H
Ferrol Palma	19	43°27.770	08°16.191	1 H
Cedeira	21	43°38.638	08°05.024	2 H
Espasante	22	43°43.346	07°48.302	4 H
		Cantabrian coast		
Ribadeo	24	43°31.570	07°01.032	1 O
Luarca	26	43°32.872	06°32.409	3 O
Avilés	28	43°34.759	05°58.180	4 O
Gijón	29	43°34.174	05°43.329	5 O
Ribadesella	30	43°28.051	05°03.742	2 O
SV Barquera	31	43°22.885	04°23.803	1 O
Suances	32	43°26.338	04°02.621	3 O
Santander Pantalán	33	43°25.937	03°47.476	1 O
Santander Pedreña	34	43°26.929	03°45.173	1 O
Castro Urdiales	36	43°21.868	03°11.663	3 O
Bilbao Azcorri	37	43°22.917	03°00.885	5 O
Hondarribia	41	43°22.119	01°47.474	1 O