



## Is there a direct relationship between stress biomarkers in oysters and the amount of metals in the sediments where they inhabit?



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### ABSTRACT

The effects exerted by metals in oysters are still a matter of debate and require more detailed studies. In this work we have investigated whether the health status of oysters are affected by the amount of metals present in the sediments of their habitat. Sediments and oysters were collected in the tidal part of the estuary of the Oka River (Basque Country), representative of other mesotidal, well mixed and short estuaries of the European Atlantic coast. The concentrations of 14 elements were determined in all the samples. Several biomarkers were also measured in the soft tissues of oysters. According to the concentrations found, the sediments were classified as non-toxic or slightly toxic. In good agreement, the histological alterations observed in oysters were not severe. Interestingly, in those sampling sites where the sediments showed relatively high metal concentrations, the metallic content in oysters was lower, and *vice versa*.

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### 1. Introduction

Estuaries are areas of high concern due to their high biological productivity. At the same time, they are close to densely populated areas and subjected to a great amount of potentially polluting activities like aquaculture, shipyards, industry and intensive agriculture and mining. Many pollutants of different origin and nature may consequently enter the estuary as a result of anthropogenic activity (Abraham et al., 2007; Reboreda et al., 2008; Szava-Kovats, 2008). Metals and metalloids have been largely recognised as highly toxic contaminants and their fate in estuaries has been investigated for decades (Pereira et al., 2015; Venkatraman et al., 2014). The fate and reactivity of metals in estuaries highly depends on the physico-chemical conditions of the system. Metals can be stored in sediments, remobilised to the water column, adsorbed onto suspended particulates, associated to fulvic and humic acids or accumulated by living organisms in their tissues (Dekov et al., 1998; Franco et al., 2002). The extent of these processes is governed by a wide number of environmental variables. As estuaries are highly dynamic systems with a remarkable variability in time and space of parameters such as; salinity, pH, dissolved oxygen, redox potential, amount of suspended particulate matter and organic carbon, the partition of metals within different compartments is also complex and difficult to predict (van Ryssen et al., 1999).

Sediments, water and molluscs like oysters are highly connected factors in estuaries. As oysters live in close contact with sediments and obtain food by filtering water, it becomes evident that the metal content in these three compartments must be highly interconnected. In fact, sediments and oysters have been frequently used to monitor metal pollution in estuarine waters (Fdez-Ortiz de Vallejuelo et al., 2014; Gredilla et al., 2015; Raposo et al., 2009). Both sediments and oysters show a high capacity to accumulate the low concentrations of trace elements usually found in water. Sediment is more conservative than water, as it accumulates historical data on processes within water bodies and the effect of anthropogenic factors on these processes. On the other hand, bioaccumulation and biomagnification processes may lead to extremely high concentrations of metals in oyster tissues.

The presence of metals in oyster tissues may result in histopathological alterations, metallothionein induction and accumulation in specific parts of the cell. The digestive gland of molluscs is a key organ in relation with accumulation, detoxification and elimination of xenobiotics including metals (Moore and Icarus Allen, 2002), and is the most studied organ in pollution monitoring programmes. Histopathological alterations are effect biomarkers that allow the identification of pathological lesions in tissues induced by environmental contamination and disease (Arab et al., 2011). The use of histopathological examination techniques in wild molluscs provides sensitive and useful indications of the overall health status of bivalves and can be used for the assessment of historical exposure or effect, of a contaminant (Izagirre et al., 2014). Metallothioneins (MT) are cysteine-rich metal binding proteins and

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have been widely used as markers of metal contamination (Viarengo et al., 1999) since they are involved in the detoxification of heavy metals, free radical scavenging, and in inflammatory responses (Gueguen et al., 2003). Changes in MT levels have been described after the study of cadmium, copper, zinc, mercury or lead exposure in a great variety of aquatic organisms (Sheehan and Power, 1999). The presence of metals in selected cell-types can be visualized with the aid of histochemical techniques such as autometallography (AMG). This technique allows the localization of metal ions (as black silver deposits, BSD) in biological tissues and has been used to determine metal levels in aquatic molluscs (Soto et al., 2002). The use of cell and tissue level biomarkers can be influenced by the season and related changes such as gamete development, food availability, or general metabolic activity (Bocchetti and Regoli, 2006). Therefore, the understanding of the natural variability of biomarkers is necessary in order to make correct interpretations of the obtained results.

In this work we have measured the concentration of selected metals and metalloids in sediments and oysters collected at different sites of the estuary of the Oka River (Bay of Biscay, Basque Country) with the aim i) to investigate the occurrence and geographical distribution of metal pollution within the estuary, ii) to determine the overall health status of the estuary based on cell and tissue level biomarkers measured in oysters, iii) to check if the concentration of metals in sediments and oysters is correlated or not, and iv) to study if the existence of alterations in oysters depends or not on the presence of specific metals in their tissues or in sediments.

## 2. Materials and methods

### 2.1. Study area, sampling and sample treatment

The area of Urdaibai (Bizkaia, Basque Country), including the estuary of the Oka River, was declared Reserve of the Biosphere by the UNESCO in 1984 (Fig. 1). The estuary is generally accepted as a clean area and major pollution inputs have been related with urban discharges and dredging due to the presence of a shipyard since 1943 in Murueta (Bartolome et al., 2006). A large amount of industrial activity remains nowadays, however, in the surroundings of Gernika-Lumo (~17,000 inhabitants), the most important town in the area. Leisure activities, including recreational boats and sport fishing, are also important. During summer time, traffic exhaust increases considerably due to the massive affluence of visitants to the beaches nearby the estuary. The presence of two marinas (in Sukarrieta and Laida) and a little port with a few medium-size fishing boats in Mundaka (~2000 inhabitants, located in the left bank close to the mouth of the estuary) also contribute as potential sources of contaminants. The concentrations of chemicals in sediments, fishes and molluscs from the estuary have been previously measured (Bustamante et al., 2012; Puy-Azurmendi et al., 2010; Zabaljauregui et al., 2007) and the existence of harmful effects on organisms derived from the presence of these chemicals have also been investigated previously (Orbea et al., 2002; Puy-Azurmendi et al., 2013; Uriarte and Villate, 2004). In general low metal pollution



Fig. 1. Sampling sites in the estuary of the Oka River (Urdaibai Reserve of the Biosphere): Mundaka MK, Laida LA, Sukarrieta SU, Axpe AX, Kanala KA, Murueta MU and Arteaga AR.

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