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Biomarkers of physiological responses of *Octopus vulgaris* to different coastal environments in the western Mediterranean Sea

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ABSTRACT

The increase of pollutants in coastal seawater could produce several harmful biological effects on marine organisms related to the production of reactive oxygen species (ROS) causing cellular and tissue damages through oxidative stress mechanisms. Common octopuses (*Octopus vulgaris*) inhabiting coastal areas under high anthropogenic activity of Mallorca (W-Mediterranean Sea) have the ability to control oxidative damage by triggering antioxidant enzyme responses. Analyzing the digestive glands, octopuses from human-altered coastal areas showed higher activity of superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST) compared to octopuses from non-influenced coastal waters (i.e. marine reserve area). Higher metallothionein (MT) concentrations and lack of malondialdehyde (MDA) variations also reflect adaptations of *O. vulgaris* to polluted areas. This is the first study assessing the levels of the oxidative stress biomarkers on *O. vulgaris* in the Mediterranean Sea, revealing their usefulness to assess diverse environmental pollution effects on this relevant ecological and commercial species.

1. Introduction

In the last decades, coastal areas have suffered several changes and strong anthropogenic pressures (harbours, urban/touristic pressure, village residues, urban wastes, etc.). These impacts can have direct and indirect effects on coastal marine ecosystems, affecting resources, modifying species diversity, habitats and population dynamics (Claudet and Fraschetti, 2010; Micheli et al., 2013). In consequence, coastal species inhabiting human-altered ecosystems could be affected by these anthropogenic activities and their survival will depend on their ability to adapt to different environmental conditions. Common octopus, *Octopus vulgaris* (Cuvier 1797), is a cosmopolitan species that mainly lives on littoral waters of the Mediterranean Sea and Eastern Atlantic (Mangold, 1983). This species is an opportunistic predator with a short life span, between 1 and 2 years (Otero et al., 2007). It is essentially a solitary and sedentary species (Otero et al., 2005) capable to quickly adapt to different environments (Mangold, 1983). Moreover, it is one of the most important cephalopod worldwide due to its economic and fishing interest, being one of the most appreciable species in the worldwide gastronomy (43,334 t of global capture production in 2014; FAO, 2014). There are many studies on this species, though many aspects of the dynamic and adaptations of octopus populations living on coastal ecosystems are still unknown (Guerra, 1982; Arechavala-Lopez et al., 2018). Due to its territorial nature and small-scale activity area (Arechavala-Lopez et al., 2018), octopus can provide useful information about adaptations to coastal habitats, but also they can act as bio-indicators about the quality of the coastal environments (Boyle and Knobloch, 1982).

The increase of organic pollutants and metals in sea water results in a serious concern to marine organisms causing several biological effects from molecular to ecological order depending on the time of exposure and concentration levels (Fasulo et al., 2010; Sureda et al., 2011, 2013; De Domenico et al., 2013; Jebali et al., 2014). The oxidative metabolism of cells is a continuous source of reactive oxygen species (ROS), resulting from univalent reduction of O_2, that can damage most cellular components (Livingstone, 2001; Regoli et al., 2002a). Cells contain a complex network of antioxidant defense that avoids damages related to ROS production (Halliwell and Gutteridge, 1989; Lesser, 2006; Box et al., 2007; De Domenico et al., 2013; Giannetto et al., 2014; Brandão et al., 2015; Cappello et al., 2016a, 2016b). If the balance between pro-oxidants and antioxidants is broken, oxidative stress can occur and ROS can cause tissue damage, impair cellular functions, alter the

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physicochemical properties of cell membranes and, finally, disrupt vital functions (Manduzio et al., 2005). The antioxidant system involves enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) that act by detoxifying the generated ROS and glutathione reductase (GR) that reduces glutathione disulfide (GSSG) to glutathione (GSH), contributing to the maintenance of the cellular redox status. Glutathione S-transferases (GST) contribute to remove xenobiotic substances which can be either direct contaminants or oxidation products. In polluted coastal waters, living organisms are exposed to chemical contaminants and, consequently, these organisms developed adaptations to the presence of ROS by enhancing the activity of antioxidant enzymes (Livingstone, 2001; Semedo et al., 2012). A well-known detoxification strategy in marine invertebrates consists of inducing metallothioneins (MTs), proteins that bind to metals, preventing oxidative stress to the organism (Bebianno and Langston, 1991; Roesijadi, 1992; Raimundo et al., 2010). Metallothionein induction as a response to metal exposure is well documented in many species and is known to play a role in the detoxification of toxic metals (Amiard et al., 2006; Ciacci et al., 2012). The production of MTs has also been recorded in organisms exposed to complex mixtures of contaminants under environmental conditions (Geffard et al., 2002; Bebianno and Serafim, 2003).

The activity of antioxidants enzymes and MT concentrations have been extensively used as biomarkers of oxidative stress (Langston et al., 1998; Ferreira et al., 2005, 2007; Sureda et al., 2006, 2013; Box et al., 2007; Fernández et al., 2010; Raimundo et al., 2010; Semedo et al., 2012; Oaten et al., 2015). However, contaminant associated production of ROS can overwhelm antioxidant defenses and oxidative damage in lipids, lipid peroxidation, will occur in tissues of exposed organisms (Ahmad et al., 2008). Malondialdehyde (MDA) is used as marker of oxidation of membrane phospholipids through lipid peroxidation. An increase in MDA levels in organisms can be related to degradation of an environmental site by decreasing the water quality (Charissou et al., 2004; Box et al., 2007). As for antioxidant enzyme activities, oxidative damages have been used in aquatic organisms as biomarkers of oxidative stress (Valavanidis et al., 2006; Box et al., 2007; Ferreira et al., 2008).

The use of biomarkers to analyze the effects of exposure to chemical contaminants in the aquatic environment is more extended in the actuality (Cossu et al., 2000; Regoli et al., 2002b; De Luca-Abbott et al., 2005; Ferreira et al., 2005, 2007; Sureda et al., 2011; Semedo et al., 2012; Natalotto et al., 2015). Moreover, measuring the same biomarkers in different localities simultaneously gives us information about the pollution status and provides a better comprehension of the mechanistic mode of action of environmental pollutants on the organisms (Frenzilli et al., 2004). Regarding biomarkers of oxidative stress in *Octopus vulgaris*, there is only one study that applies biomarkers in order to evaluate the physiological response of this species associated to contaminant related oxidative stress in the Atlantic coast of Portugal (Semedo et al., 2012). The Mediterranean Sea is mostly an enclosed sea that has limited exchange of water with outer oceans and, therefore, the pollution due to anthropogenic effects is higher (Duarte et al., 1999). For this reason, this study might be of particular interest given that the characteristics of the water masses of the Atlantic differ from those of the Mediterranean. The aim of this work was to use the response of the antioxidant enzyme activities, MT contents and changes in lipid peroxidation (MDA levels) in the digestive gland of the common octopus, *O. vulgaris*, as biomarkers of the physiological status of three octopus populations inhabiting coastal areas of Mallorca with different degree of human activities.

2. Material and methods

2.1. Study area and octopus sampling

For this study, *O. vulgaris* were captured at three locations selected along the southwest coast of Mallorca (Fig. 1), approximately 20 km distant each other and attending to different human activities (Table 1). The easternmost area was the Palma Bay Marine Reserve, characterized by a low pressure and anthropogenic impacts given that human activities are regulated by zonation and restrictive use of different zones (RD33/2007) (Boletín Oficial de las Islas Baleares, 2007). The westernmost area was Andratx Harbour, a small and narrow bay that presents a wide range of anthropogenic activities and low water quality due to the presence of commercial and recreational vessels, building-up pressure, coastal modification, urban wastes and low water exchange. In between, the area of Magaluf is also considered as high polluted given the high presence of urbanizations, urban wastes, high touristic impact and coastal modification (Natalotto et al., 2015). A total of 25 *O. vulgaris* individuals were captured during the period of March–May 2017 using spear-guns and traps (this latter only in Andratx Harbour given the low visibility) in shallow coastal waters ranging from 2 to 10 m depth at the three study areas. Captured individuals were immediately sacrificed and digestive glands were collected from each individual, frozen and taken to the laboratory where they were stored at −80 °C until further use. Digestive gland was selected as the best tissue due to its role in the storage/accumulation and detoxification of organic and inorganic contaminants (Semedo et al., 2012; Rodrigo and Costa, 2017). Fishing procedures were carried out under previous permission by the local government (Direcció General de Pesca i Medi Marí, Conselleria de Medi Ambient, Agricultura i Pesca del Govern de les Illes

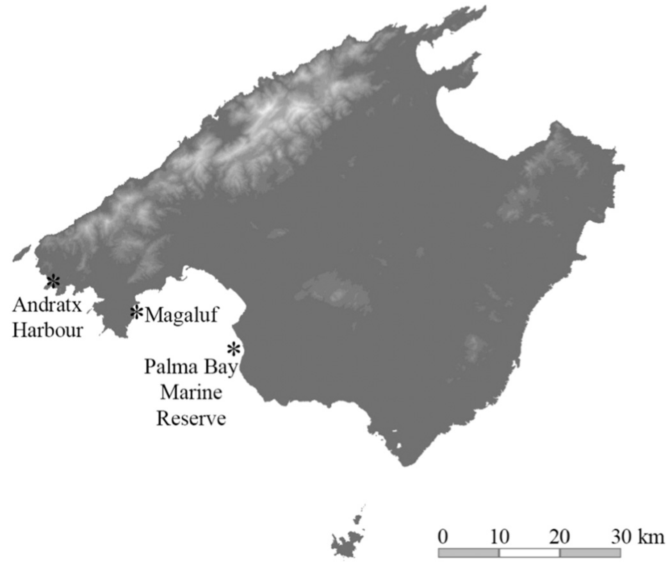

Fig. 1. Geographical location of the three sampling areas (asterisks) in the southwest of Mallorca Island, Spain (W-Mediterranean Sea).

Table 1
Human activities and degree of potential impact on coastal ecosystems at the three sampling areas.

Site	Human activities/impacts	Degree of impact
Palma Bay Marine Reserve	• Regulated and restricted coastal waters use	Low
Andratx Harbour	• Marina • Commercial harbour • Urban wastes, urbanizations • Tourist use • Experimental Fish farms	High
Magaluf	• Tourist use (high number of hotels) • Population • Urban wastes, urbanizations	High