



# Muscular cholinesterase and lactate dehydrogenase activities in deep-sea fish from the NW Mediterranean



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## ABSTRACT

Organisms inhabiting submarine canyons can be potentially exposed to higher inputs of anthropogenic chemicals than their counterparts from the adjacent areas. To find out to what extent this observation applies to a NW Mediterranean canyon (*i.e.* Blanes canyon) off the Catalan coast, four deep-sea fish species were collected from inside the canyon (BC) and the adjacent open slope (OS). The selected species were: *Alepocephalus rostratus*, *Lepidion lepidion*, *Coelorrhinus mediterraneus* and *Bathypterois mediterraneus*. Prior to the choice of an adequate sentinel species, the natural variation of the selected parameters (biomarkers) in relation to factors such as size, sex, sampling depth and seasonality need to be characterised. In this study, the activities of cholinesterases (ChEs) and lactate dehydrogenase (LDH) enzymes were determined in the muscle of the four deep-sea fish. Of all ChEs, acetylcholinesterase (AChE) activity was dominant and selected for further monitoring. Overall, AChE activity exhibited a significant relationship with fish size whereas LDH activity was mostly dependent on the sex and gonadal development status, although in a species-dependent manner. The seasonal variability of LDH activity was more marked than for AChE activity, and inside-outside canyon (BC–OS) differences were not consistent in all contrasted fish species, and in fact they were more dependent on biological traits. Thus, they did not suggest a differential stress condition between sites inside and outside the canyon.

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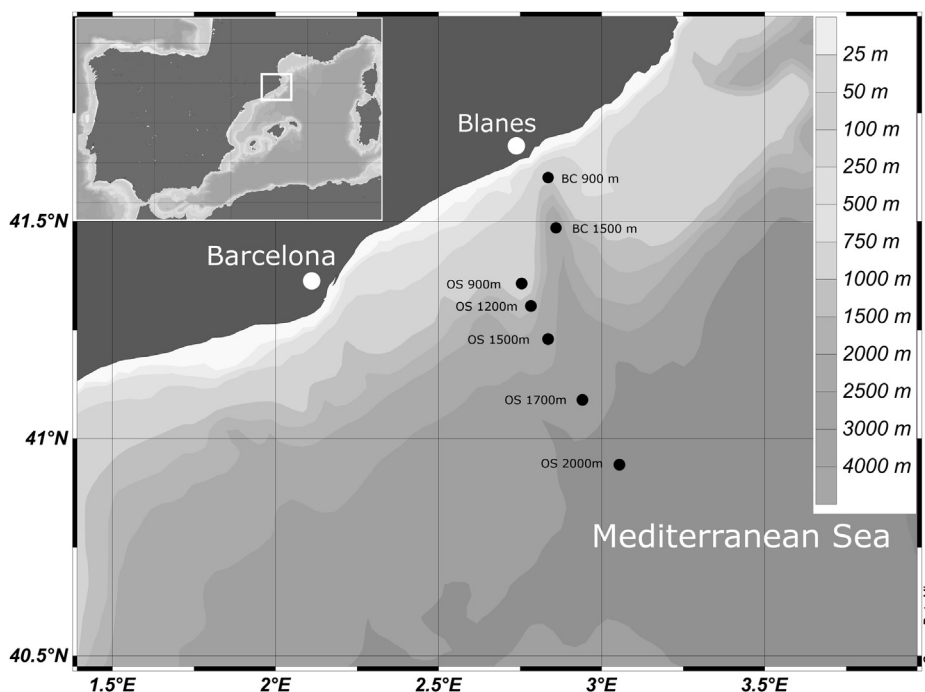
## 1. Introduction

It is well known that submarine canyons act as conduits from coastal anthropogenic xenobiotics to deeper waters and therefore these waters could be subject to higher contaminant input (Jesus et al., 2010). In the particular case of the Blanes canyon in the NW Mediterranean, due to its shape and proximity to the coastline, it constitutes an important transport route for sediments and particulate matter from the adjacent coastal areas into the deep-sea (Palanques et al., 2006). Furthermore, episodic dense-shelf water cascading events, which have been shown to take place every 6–10 years in the NW Mediterranean and affect their population dynamics (Company et al., 2008), could contribute to an increased pollution input into submarine canyons. In addition, the role of these canyon environments as essential habitats for different deep-sea organisms is currently under investigation, which reinforces the importance of assessing the anthropogenic impact on submarine canyon ecosystems.

Even though a broad range of anthropogenic contaminants have been shown to accumulate in Mediterranean deep-sea biota and biomagnify along the food chain (Borghgi and Porte, 2002; Koenig et al., 2013a, 2013b; Siscar et al., 2013; Solé et al., 2001; Storelli et al., 2007), thus far only a limited number of studies have investigated the potential adverse effects of these pollutants on Mediterranean deep-sea organisms (Escartin and Porte, 1999; Koenig et al., 2013c; Koenig and Solé, 2012; Porte et al., 2000) including the effects of metal exposures on the biomarkers selected in the present study but from a broader interspecific perspective (Siscar et al., 2013). In this context, the use of biomarkers (functional measures of exposure to stressors expressed at the sub-organismal, physiological or behavioural level) has been advocated as a means to determine adverse effects resulting from contaminant exposure in marine organisms (van der Oost et al., 2003).

In the present study we analysed two biomarkers, namely cholinesterases (ChEs) and lactate dehydrogenase (LDH) in muscle tissue of four deep-sea fish species belonging to four different phylogenetic families caught within the Blanes submarine canyon, NW Mediterranean, and on the adjacent continental slope. Cholinesterases, which include the neurotransmission enzyme acetylcholinesterase (AChE) and the pseudocholinesterases butyrylcholinesterase (BuChE) and

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**Fig. 1.** Map of the sampling sites and depths within the open slope (OS) and inside the Blanes canyon (BC). The map was created using the Ocean Data View (ODV) software package by Schlitzer, R., Ocean Data View, <http://odv.awi.de>, 2010.

propionilcholinesterase (PrChE), are widely used in toxicological as well as pollution monitoring studies (Kopecka and Pempkowiak, 2008) mostly as specific indicators of organophosphorus and carbamate pesticide exposure (Fulton and Key, 2001). However, there is growing evidence of their responsiveness to a broader range of chemicals including tributyltin (TBT), polycyclic aromatic hydrocarbons (PAHs), surfactants and metals (Greco et al., 2007; López-Galindo et al., 2010; Vieira et al., 2008, 2009), highlighting their potential use as biomarkers of exposure to a broad range of chemicals that have been found in deep-sea biota. LDH is an important glycolytic enzyme involved in the supply of energy demand under oxygen-limiting conditions and LDH activity in white muscle reflects the anaerobic power generating capacity during burst swimming (Childress and Somero, 1979). LDH activity has been associated with the species locomotory performance as a quick response for energy demands under low oxygen conditions, also in benthic species from deep-sea environments (Drazen and Seibel, 2007). In the context of ecotoxicology, some studies have considered muscular LDH activity as indicator of contaminant-induced metabolic disturbances (Greco et al., 2007; Moreira et al., 2010; Vieira et al., 2009).

However, due to the intrinsic natural variability of enzyme activities as a result of environmental as well as biological traits, the characterisation of baseline activities are prerequisite before using these biomarkers as proxies for environmental stress conditions. In the present study, the potential effect of variations in fish size, sex, sampling depth and season on the enzyme activities in the four species was therefore evaluated. The Mediterranean deep-sea environments below 400 m depth are characterised by relatively constant physical water parameters and variations of hepatic enzyme activities of some shared deep-sea organisms have been shown to vary according to seasonal fluctuations of food availability and biological traits (Koenig and Solé, 2012).

The overall aim of the study was to evaluate the applicability of two selected muscular parameters (AChE and LDH) as biomarkers of pollution in four deep-sea fish from the NW

Mediterranean Sea and subsequently identify potential sentinel species for future monitoring studies. To achieve this goal: (1) muscular cholinesterases in four deep-sea fish species (*i.e.* *Alepocephalus rostratus*, *Lepidion lepidion*, *Coelorrhinus mediterraneus* and *Bathypterois mediterraneus*) were characterised; (2) intra-species variation of enzymatic activities in relation to body size, gender, sampling depth and seasonality were evaluated and (3) potential contaminant exposure differences between fish living inside the Blanes submarine canyon (BC) and those from the adjacent slope (OS) were contrasted using the two selected enzymatic markers.

## 2. Materials and methods

### 2.1. Study area and sampling sites

The Blanes canyon is located on the Catalan coast and is one of the largest submarine canyons in the NW Mediterranean Sea. The situation of the sampling depths and sites from the adjacent open slope (OS) and within the Blanes canyon (BC) where fish were collected are detailed in the map (Fig. 1). This area receives the influence of the Tordera river and the predominant local currents flow from North to South. More information on the characteristics of the canyon will be published in a monographic issue of which some of the referenced works are part (Fernandez-Arcaya et al., 2013; Koenig et al., 2013d).

### 2.2. Sampling procedure

Seasonal sampling cruises were conducted onboard the R/V *García del Cid* in Winter (January 2009), Spring (May 2009), Summer (September 2009) and Autumn (November 2009). Fish were caught using an OTMS otter trawl at various water depths ranging from 900 m to 2000 m, selecting the most abundant species for further analyses. Fish size, weight and sex were recorded and a

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