Environment International 36 (2010) 202–211

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Muscular cholinesterase activities and lipid peroxidation levels as biomarkers in several Mediterranean marine fish species and their relationship with ecological variables

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ARTICLE INFO

Article history:
Received 20 July 2009
Accepted 20 November 2009
Available online 22 December 2009

Keywords:
Cholinesterases
Lipid peroxidation
Ecological parameters
Marine fish
NW Mediterranean

ABSTRACT

Muscular cholinesterase activities, as potential markers of neurotoxic exposure, and lipid peroxidation levels, indicative of oxidative stress damage, both currently used in early-warning pollution monitoring, were characterised in eighteen fish species of ecologic and/or economic importance. These species comprise five orders and eleven families of teleosts and two species of elasmobranchs, feed using different strategies (benthic, epibenthic, endobenthic and pelagic), belong to different trophic levels and express different swimming behaviour. Their habitat ranges from 50 to 60 m (shallow or continental shelf) and 600 to 850 m (middle continental slope). Sampling took place in front of the Barcelona coast (NW Mediterranean) during four seasonal cruises in 2007. In the summer sampling, another site potentially exposed to a different pollution load (Vilanova) was included for comparison.

Species, seasonal and site differences were tested and discussed in relation to chemical analysis of the local sediment, systematic position, habitat depth, feeding strategy, trophic level and swimming activity. Greater inter species differences rather than seasonal or site trends were seen in accordance to little pollution fluctuations. Higher cholinesterase activities were recorded in suprabenthos feeders, regardless of depth habitat, whereas LP levels were similar in all species except for the shark *Scyliorhinus canicula* in which they were consistently elevated. This study confirms and broadens former observations carried out with a more reduced number of fish species (Solé et al., 2008a).

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1. Introduction

In ecotoxicology the use of a battery of cellular and biochemical parameters to evaluate exposure/effects to chemicals, is strongly recommended (Cajaraville et al., 2000; van der Oost et al., 2003). Among the proposed biomarkers, cholinesterase (ChE) activities are considered reliable markers of exposure to anticholinergic chemicals such as organophospohorus (OP) pesticides and carbamates (Galloway et al., 2004). However, there is growing evidence that other chemical classes can also affect these enzymatic activities in fish: petrogenic chemicals including polycyclic aromatic hydrocarbons (PAHs; Vieira et al., 2008), polychlorinated biphenyls (PCBs; Buet et al., 2006), detergents (Jifa et al., 2005; Feng et al., 2008), metals (Gill et al., 1990; Zinkl et al., 1991), antifoulants (López-Galindo et al., 2009) as well as complex mixtures of pollutants (Payne et al., 1996; Moreira et al., 2004). The fact that the cholinergic synapses can be disrupted by a broad range of environmental contaminants has great ecological relevance, as acetylcholine is the main neurotransmitter involved not only in neuromuscular action but also in controlling respiration, feeding, swimming capacity, behaviour, prey–hunter relationships, hormonal function and reproduction. Thus, any alterations on this activity can potentially lead to ecological disturbances (Peakall, 1992; Labenia et al., 2007).

Acetylcholinesterase (AChE; EC 3.1.1.7) and pseudocholinesterases: butyrylcholinesterase (BChE) and propionylcholinesterase (PrChE) (EC 3.1.1.8), are all present in marine fish muscle, being AChE the predominant form. The physiological role of AChE is clear (breaking acetylcholine in neuromuscular junctions); however the role of BChE and PrChE is less understood due to the lack of a natural substrate. Nevertheless, pseudocholinesterases seem to be involved in detoxification processes, cell regeneration, lipid metabolism, neurogenesis and neural development (Mack and Robitzki, 2000). Moreover, the fact that in fish they respond to certain chemical classes, even more specifically than AChE, supports their consideration in pollution monitoring studies (Sturm et al., 1999, 2000; Kirby et al., 2000; Gold-Bouchot et al., 2006).

ChEs, and mostly AChE have been applied in fish from the Mediterranean (Stien et al., 1998; Bresler et al., 1999; Burgeot et al.,

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doi:10.1016/j.envint.2009.11.008

2001; Lionetto et al., 2004), but they are, for the most part, based on the use of *Mullus barbatus* as sentinel (Burgeot et al., 2001; Regoli et al., 2002; Porte et al., 2002; Lionetto et al., 2003, 2004). In more recent times, other fish species have also been considered: *Helicolenus dactylopterus* (Amato et al., 2006) and several fish species (Solé et al., 2006, 2008a) some of which are also considered in this study. ChE activities can also be modulated by biological parameters such as fish size/age and sex, although observations are often controversial and species dependent (Varó et al., 2003, 2007; Solé et al., 2006; Rodríguez-Fuentes et al., 2008). Thus, in order to avoid these potential confounding factors, the selection of balanced sex ratios and specimens of similar size/age is recommended.

Lipid peroxidation (LP) is a marker of effect, it reflects the action of reactive oxygen species (ROS) over the biological lipids and it can be measured by the presence of a lipid metabolite e.g. malondialdehyde (MDA). The occurrence of excess of lipid membrane peroxides may disrupt membrane functionality or react with proteins and DNA leading to protein inactivation and DNA adducts formation. ROS formation occurs in many biological processes but it can also be enhanced under exposure to xenobiotics (Livingstone, 2001; Valavanidis et al., 2006), which justifies its inclusion in monitoring studies using fish as sentinels (Avci et al., 2005; Solé et al., 2008b).

The selected study area is located at the mouth of the Besós river in the vicinity of Barcelona (NW Mediterranean). The presence of xenobiotics in the region was recently reported by levels in sediment (Castells et al., 2008) and in biota (Domingo and Bocio, 2007; Zorita et al., 2007). The presence of organic pollutants such as the persistent PCBs, DDTs and the PAHs in the local sediment was also carried out during our study. Moreover, as little is known about the natural variations and relationship between these biochemical markers and ecological parameters such as trophic habits (e.g. type of diet, trophic level), the habitat and/or the systematic position of the species, their swimming behaviour (Magnotti et al., 1994; Oliveira et al., 2007; Solé et al., 2008a), we explored the relationship between these sets of variables as relevant to the animal's physiology.

The aim of the study was to report, for a broad range of fish species, values on activities of muscle ChEs and LP levels. It complements and further explores the relationship formerly outlined between these two biomarkers and ecological variables (Solé et al., 2008a). The activities reported are discussed in relation to the fish systematic position, depth-range, trophic level, diet and swimming capacity. In addition, seasonal and site related differences were recorded for some selected fish species and related to sediment pollutant levels to further support the use of these markers in pollution monitoring.

2. Material and methods

2.1. Sampling area and species selection

Sampling took place in 2007 on board of a research vessel (R/V "Garcia del Cid") and a commercial trawling vessel ("Stella Maris III") in front of the Barcelona coast (NE Spain) at the mouth of the Besós river at the depths of 50–60 m (41° 24′N, 2° 16′E), 200 m (41° 20′N, 2° 15′E), 600–850 m (41° 12′N, 2° 28′E) and 1000 m (41° 10′N, 2° 30′E). Fish samples for the Besós transect were obtained at 4 different seasons along the year (winter: February; spring: April; summer: July; autumn: October 2007), while another site (Vilanova) was sampled only in July 2007 (summer) to assess site differences (Fig. 1). Once on board, fish were anaesthetised on ice; a portion of the axial muscle was dissected and stored in dry ice. Samples were transported to the laboratory where they were kept at −80 °C until biochemical analysis.

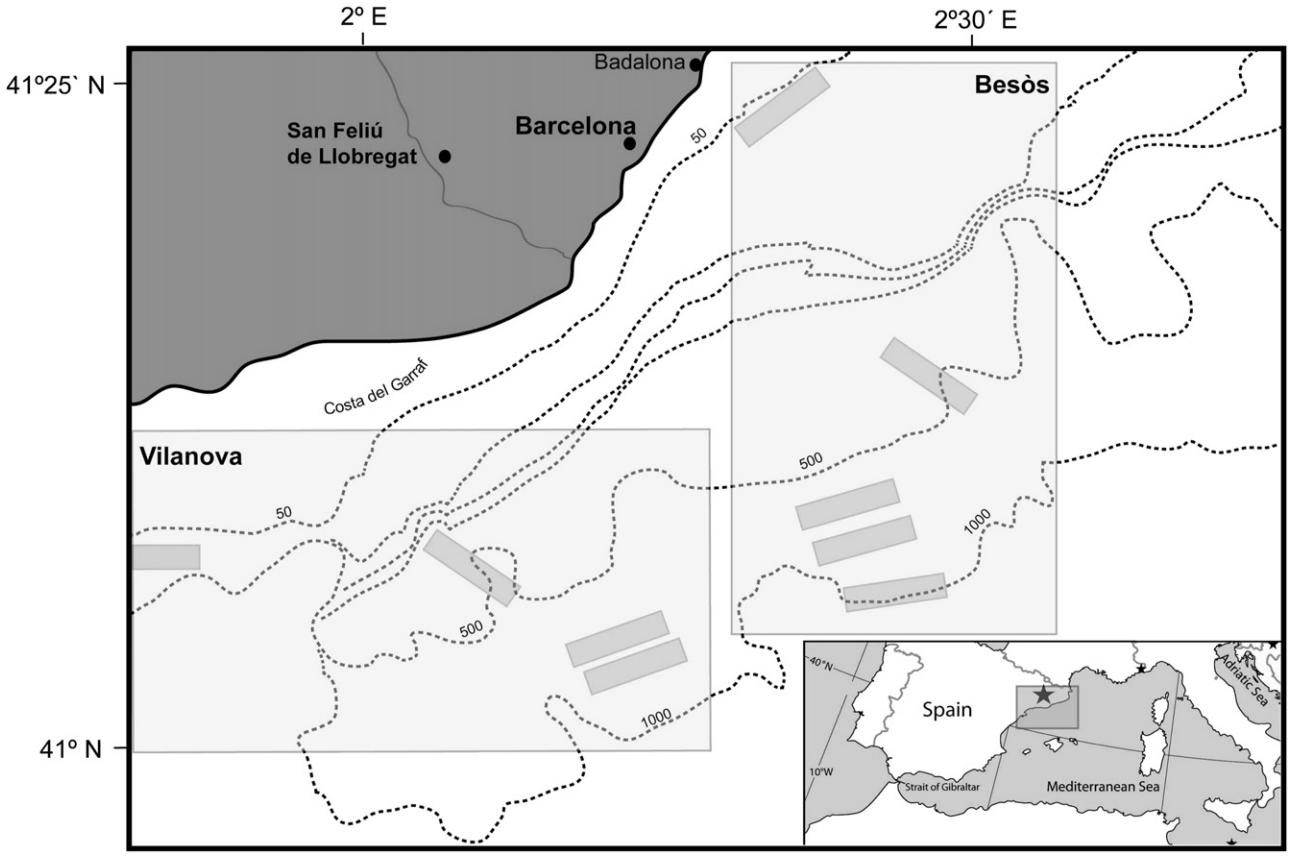

Fig. 1. Sampling sites (Besós and Vilanova) in the fishing grounds of the NW Mediterranean. Grey rectangles indicate the trawls done. Adapted by AE González.

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