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Delta-aminolevulinic acid dehydratase activity (ALA-D) in red mullet (*Mullus barbatus*) from Mediterranean waters as biomarker of lead exposure

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ABSTRACT

The enzyme delta-aminolevulinic acid dehydratase (ALA-D) has been investigated as biomarker of lead (Pb) exposure in red mullet (*Mullus barbatus*) from the Spanish continental shelf. Concentrations of Pb and Zn in muscle and organosomatic indices were also measured to explore causality. Blood ALA-D assay conditions were optimized; the optimum pH for this species has been set to 6.5. Results showed that ALA-D activity ranged from 3.2 to 16.9 nmol PBG min^{-1} mg^{-1}. No significant differences on ALA-D levels between genders have been detected. ALA-D Baseline level and Background Assessment Criteria (BAC) for this species have been set to 9.1 and 6.6 nmol PBG min^{-1} mg^{-1}, respectively. There have been detected significant differences on ALA-D activity levels among areas, though the markedly low levels of Pb measured in fish muscle seemed not to be able to produce a relevant suppression on ALA-D. In spite of this, a weak inverse relationship detected between ALA-D and Pb concentrations pointed out the potential of this biomarker in red mullet to reflect Pb bioavailability in marine environment. Nevertheless, subsequent research on ALA-D in marine fish species is recommended to be limited to areas where environmental Pb is effectively accumulated by fish.

1. Introduction

Lead (Pb) is a major environmental pollutant whose presence in aquatic ecosystems is largely due to the burning of leaded fuels, metal smelters, mining activities, paints, cosmetics, human medicines or food supplements. Some modern uses of Pb include manufacturing ammunition, batteries, chemical compounds, explosives, glassware, metal products or pesticides (UNEP, 2010). Pb can be accumulated in fish tissues; its concentration is the result of the equilibrium between absorption and depuration rates and reflects Pb abundance and bioavailability in the aquatic environment (Hadson, 1988; Mansour and Sidky, 2002). Pb has been probed to produce hematological, renal, reproductive, behavioral and neurological deleterious effects in fish, such as reduction in bone strength, black tail, stippled erythrocytes, spinal deformities, paralysis or muscular atrophy (see review in Hoffman et al. (2002)). Pb toxicity mainly arises from its ability to mimic biologically essential metals such as Ca, Fe and Zn, provoking them to fail to carry out their primary tasks (Ballatori, 2002). Furthermore, Pb is able to inhibit several enzymes in the heme biosynthesis pathway, such as coporphyrinogen oxidase, ferrochelatase and delta-aminolevulinic acid dehydratase (ALA-D) (Kelada et al., 2001). The ALA-D enzyme, also called porphobilinogen (PBG) synthase, catalyzes the formation of one molecule of PGB, a hemoglobin (Hb) precursor, from two molecules of aminolevulinic acid (ALA). ALA-D requires Zn^{2+} cofactors in order to function and contains highly active thiol groups, therefore other metals may bind ALA-D, inhibiting its enzymatic activity and decreasing PBG levels (Finelli, 1977). The affinity of ALA-D is almost 25 times greater for Pb than for Zn, thereby Pb easily displaces Zn from the active site, giving an identical molecular structure but an improper functioning (Rodrigues et al., 1989; Bergdahl et al., 1997; Godwin, 2001). As a rate-limiting enzyme in Hb synthesis, ALA-D inhibition may lead to anemia in some vertebrates, such as mammals and birds, though this does not appear to be the case for fish (Hoffman et al., 2002). Indeed, in fish ALA-D inhibition has not been directly related with deleterious effects in health, and the relationship between reduced ALA-D levels and hematological and physiological changes is poorly defined. In spite of this, ALA-D in fish has an outstanding eco-toxicological interest, as it is inhibited at low levels of Pb exposure long before any other deleterious effects becomes perceptible (see for instance Schmitt et al. (1984), Haux et al. (1985), Dwyer et al. (1988), Addison et al. (1990), Nakagawa et al. (1997) and Burden et al. (1998)). Thus, ALA-D has been

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considered a selective and fast responding biomarker of Pb exposure and bioavailability in the aquatic environment (Hodson et al., 1984; Haux and Förlin, 1989; Mayer et al., 1992) and proposed as feasible tool to ascertain if Pb concentrations are at levels giving rise to pollution effects, especially in marine areas where Pb is significantly above background levels (ICES, 2006). In this context, we have investigated for the first time ALA-D in *Mullus barbatus* (red mullet) from several areas along the Spanish continental shelf (Western Mediterranean Sea), with particular reference to Cartagena (SE Spain). Cartagena is an area highly impacted by metals as a consequence of mine tailing dumping into the sea performed in the past decades from Cartagena-La Unión mining district (Oyarzun et al., 2013). In fact, concentrations of Pb and Zn in surface sediments in the vicinity of Cartagena has been reported to be one order of magnitude higher than in other Spanish Mediterranean coastal areas (Martínez-Gómez et al., 2012). Also concentrations of Pb in mussels (Fernández et al., 2010) and red mullet (Benedicto et al., 2008; Martínez-Gómez et al., 2012) from Cartagena area are the highest ones recorded along the Spanish Mediterranean coast. The objective of this study was to assess the potential of ALA-D as biomarker of Pb exposure in red mullet, a recommended target species for pollution monitoring in the Mediterranean Sea (FAO/UNEP, 1993). With this aim we have optimized blood ALA-D assay conditions (pH and sample enzyme extract volume), we have determined ALA-D levels in fish collected at several areas of the Spanish shelf, and we have investigated the relationship between ALA-D, sex, condition factor (CF), organosomatic parameters (gonadosomatic index-GSI and hepatosomatic index- HSI) and bioaccumulation of Pb and Zn in fish muscle.

2. Material and methods

2.1. Fish sampling

Fish specimens, *M. barbatus,* were caught by 15 min bottom trawls in October 2008 and 2010, a non-spawning period for red mullet in the Western Mediterranean Sea (Miramand et al., 1991). Sampling stations included 13 soft bottom locations from 9 areas of the Iberian Mediterranean shelf (Fig. 1). Detailed geographical and environmental information is provided in Supplementary Table S1. Briefly, Barcelona, Tarragona, Valencia and Cartagena areas are priority pollution hotspots in the Mediterranean Sea under the influence of relevant industrial and urban centers, ports and rivers; Ebro Delta is a priority pollution sensitive area with natural and socio-economic value located under the influence of the activities developed along the basin of the River Ebro (UNEP/MAP/MED POL, 2005); Málaga and Almería areas are close to urban and industrial nuclei, but they are subjected to a minor degree of anthropogenic activity than the previous ones; and Santa Pola and Palos Cape areas are the lesser human-impacted areas studied, especially Palos Cape, which is a marine reserve. Mature fish within the length 12–18 cm, representing age class I–III (Kinacigil et al., 2001; Kokokiris et al., 2014), were selected. Once collected, fish were maintained in aerated tanks aboard until blood sample extractions were performed. Sex, total length (L), eviscerated weights (W), and gonad and liver weights were recorded. Condition Factor (CF), gonadosomatic index (GSI) and hepatosomatic index (HSI) were calculated as follows: $CF = 100 \times W/L^3$; $GSI = gonad\ weight \times 100/W$; $HSI = liver\ weight \times 100/W$. Water bottom temperature, salinity and dissolved oxygen were registered at each area using a conductivity-temperature-depth instrument

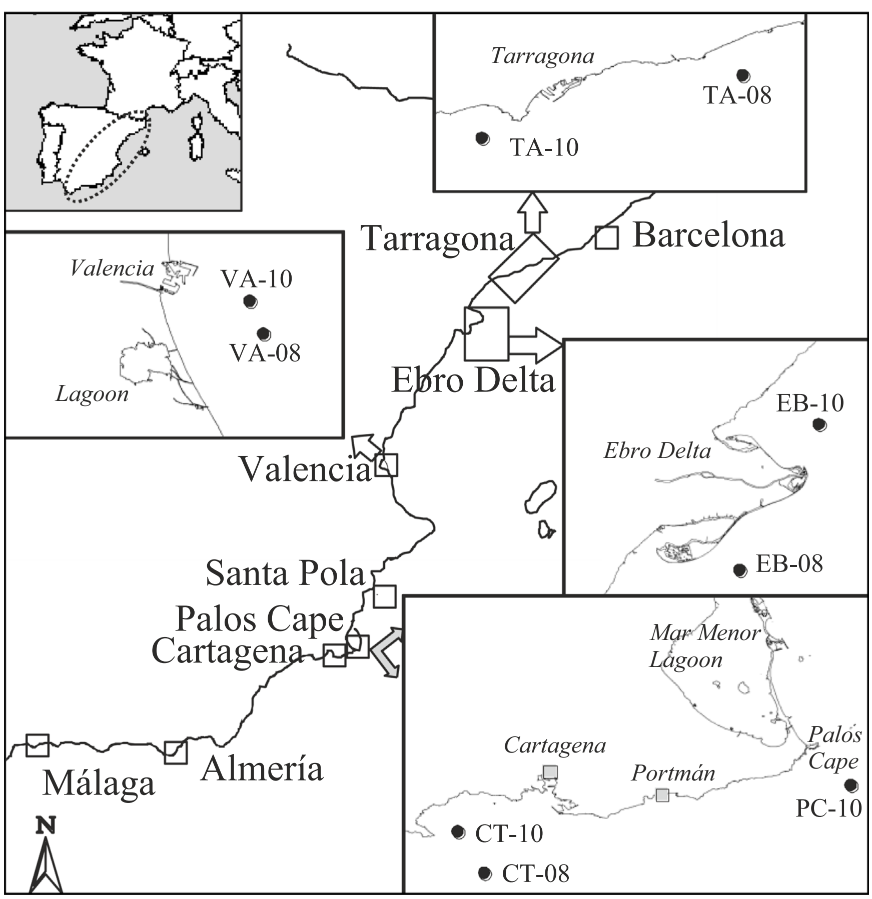

Fig. 1. Areas and stations of the Spanish Mediterranean coast studied for ALA-D in *M. barbatus*.