ESTERASES AND LIPID PEROXIDATION LEVELS IN TWO COMMERCIAL FISH, *PHYCIS BLENNOIDES* AND *MICROMESISTIUS POUTASSOU* AND THE DEEP-SEA DECAPOD CRUSTACEAN *ARISTEUS ANTENNATUS* FROM THE NW MEDITERRANEAN

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Abstract

Few studies have monitored neurotoxicity biomarkers in offshore marine areas. To partly fill this gap in information we sampled the gadiform fish *Phycis blennoides* and *Micromesistius poutassou* and the crustacean decapod *Aristeus antennatus*, all species of great commercial interest in the Mediterranean, offshore the coast of Blanes (41.0N 3.0E; NE Spain) in two different grounds located in a submarine canyon. The trawling took place in July 2006, at depths of 585 m, canyon head close to the coast, and at 841 m, in the canyon axis and further from the coast. Esterase activities and lipid peroxidation levels were measured individually in muscle tissue and activities/levels recorded are discussed in relation to sampling depths, animal sex and size.

Keywords : Crustacea, Deep Waters, Ecotoxicology, Fishes.

Cholinesterases (ChEs), namely acetylcholinesterase-AChE- (EC 3.1.1.7), regulate the nerve impulse transmission in vertebrates and invertebrates, its inhibition causes a build up of acetylcholine that derivates in tetany, paralysis and eventual death. The physiological role of other ChEs such as butyrylcholinesterase-BChE- (EC 1.1.1.8) and propyonylcholinesterase-PrChE- is not so well established, although in some marine fish, BChE has shown to be a better marker than AChE [1]. Inhibition of AChE in marine pollution monitoring of the Mediterranean has been extensively applied using either fish or invertebrates in coastal areas [2]. However, to the best of our knowledge no studies are available on these markers in deep water species, this despite levels of pollutants in some regions being considerably high [3]. Carboxylesterases-CbE- (EC 3.1.1.1) catalyze the hydrolysis of a wide range of xenobiotic esters, amides and thioesters. Although more dominant in the liver, we included these enzymes for their role in the metabolism and subsequent detoxification of many xenobiotics, and endogenous compounds. Lipid peroxidation (LP) is considered a biomarker of effect. It is also included in many monitoring programs as it integrates the negative effects caused on the lipid membranes by reactive oxygen species (ROS). LP occurs when the natural antioxidant defences are overcome and membrane and membrane-bound enzyme destabilisation takes place [4]. A biomarker approach, using esterases responses in pollution monitoring seems adequate as it will integrate the effects on the targeted species, regardless of the chemical(s) involved. Even sublethal levels of neurotoxic compounds may have a negative result on the marine species, compromising their behaviour and altering predator-prey interactions, thus having a negative consequence at the ecological level [5].

The gadiform fish, *P. blennoides* (Phycidae) and *M. poutassou* (Gadidae) and the red shrimp, *A. antennatus*, (Crustacea, Aristeidae) are important species in Mediterranean fisheries. Nevertheless, they have not been considered in monitoring studies due to their habitat being situated far from pollution sources. To the best of our knowledge, there are no many studies reporting on esterase activities and LP levels for any of the species here described. For that, the aim of our study was to provide baseline data that could support their future inclusion in monitoring neurotoxicity in offshore fishing grounds of the NW Mediterranean.

During the sampling, on board of the fishing vessel, all specimens were immediately sexed and measured, the fish as total length (TL $\pm$ 1 cm) and the crustacea as cephalotorax length (CL $\pm$ 1 mm). A portion of the muscle and liver/hepatopancreas was also dissected and frozen in liquid nitrogen for further biochemical determinations. In muscle, ChEs and CbEs activities and LP levels were determined as recently described (6). ChEs and CbEs activities are reported in nmol/min/mg protein (Figure 1) and LP is expressed as nmol malondialdehide (MDA)/g wet tissue. Statistical analysis of the data was done using the one-way ANOVA test to determine differences due to either sex, size or sampling depth. The post-hoc Newman-Keuls test was used to show were statistical significance lay. Level of significance was set at $p<0.05$. Values are presented as mean $\pm$ SEM (n =7-11).

In fish, AChE was the predominant form (61-66%) followed by PrChE=CbE (14-15%) and BChE. (6-8%). In the crustacea, AChE was also dominant (46-49%) although the contribution of other esterases (e.g. CbE) was quite significant (26-30%). In fish a relationship between esterases and fishing depth was seen but not in the animal sex or size. In crustacea, however, esterase activities were seen as sex and size dependent but not related to sampling depth. LP levels varied between 0.86-3.35 nmol MDA/g w.w. In all cases esterases correlated well between themselves but not with LP levels. In view of our results, in these deep sea water species, the choice of AChE for measuring neurotoxicity seems adequate, however, in the crustacea, CbE in hepatopancreas needs to be further investigated as a potentially more adequate marker of neurotoxicity.

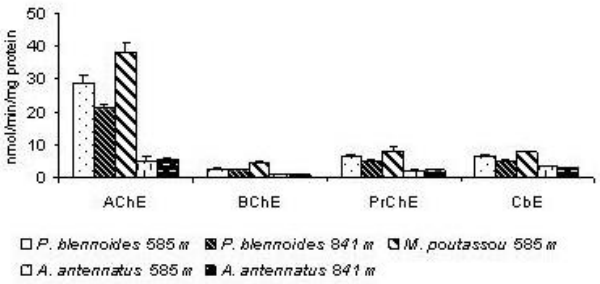

Fig. 1. Activity levels of esterases (AChE, BChE, PrChE and CbE) in the gadiform fish *Phycis blennoides* and *Micromesistius poutassou* and the decapod crustacean *Aristeus antennatus* at two sampling depths from the Blanes canyon (NW Mediterranean). (n.b. *M. poutassou* was only found at one fishing depth).

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