Ecotoxicology and Environmental Safety 72 (2009) 1455–1462

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Characterisation of integrated stress biomarkers in two deep-sea crustaceans, *Aristeus antennatus* and *Nephrops norvegicus*, from the NW fishing grounds of the Mediterranean sea

Mertixell Antó, Susana Arnau, Elisenda Buti, Verónica Cortijo, Elena Gutiérrez, Montserrat Solé *

Institut de Ciències del Mar (ICM-CSIC), Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain

ARTICLE INFO

Article history:
Received 23 October 2008
Received in revised form
23 February 2009
Accepted 23 February 2009
Available online 24 March 2009

Keywords:
Deep-sea crustaceans
Aristeus antennatus
Nephrops norvegicus
Muscle and hepatic biomarkers

ABSTRACT

Several biomarkers indicative of stress were characterised in the crustaceans *Aristeus antennatus* and *Nephrops norvegicus* sampled off the Barcelona coast (NW Mediterranean). The biomarkers selected were cholinesterase (ChE) activities in muscle; and catalase, glutathione reductase (GR), total glutathione peroxidase (t-GPX), DT-diaphorase (DT-D), glutathione S-transferases (GSTs) and carboxylesterases (CbEs) in hepatopancreas tissue. Lipid peroxidation (LP) levels and total protein yield (PY) were also determined in muscle and hepatopancreas tissues. The activities and levels are discussed in relation to species and season, and differences in these two factors were observed for most biomarkers. AChEs and pseudocholinesterases were present in the muscles of both crustaceans. Catalase and GST activities were higher in *N. norvegicus*, whereas GR and t-GPX activities varied according to the season. Hepatic CbE activities were similar in the two crustaceans, whereas LP levels and PY were different between species. Seasonality and species particularities are factors to consider when these crustaceans are used as sentinels.

1. Introduction

Commercial fishing grounds in the NW Mediterranean are subjected to high anthropogenic pressure, not only due to intensive fisheries but also because of pollution inputs from industry, agriculture and aquaculture at nearby coastal sites. Little is known of the ability of deep-sea crustaceans to deal with environmental stressors (e.g. pollutants). In fact, we are not aware of any published data on the pollution biomarkers selected for this study in deep-sea crustaceans other than those recently published (Solé et al., 2006). Evidence of exposure to metals and organic pollutants in the deep-sea crustaceans *Aristeus antennatus* and *Nephrops norvegicus* from the Mediterranean has been reported (Drava et al., 2004; Rotllant et al., 2006; Perugini et al., 2006; Bocio et al., 2007); however, no associated biochemical responses have been considered. Studies measuring the capacity to respond to xenobiotocs have been conducted mainly with freshwater crustaceans (Ishizuka et al., 1998; Ashley et al., 1996; Escartín and Porte, 1998; McLoughlin et al., 2000; Li et al., 2008), estuarine and marine coastal crustaceans, such as *Carcinus aestuarii* (Fossi et al., 1998), *Carcinus maenas* (Orbea et al., 2002; Gowland et al., 2002; Martín-Díaz et al., 2008), *Palaemonetes pugio* (Oberdörster et al., 2000; Kuzmick et al., 2007), and amphipods (Correia et al., 2003).

The NW Mediterranean deep sea (>400 m) is considered to have very stable physical conditions but is not exempt from organic matter inputs with their associated chemicals from more polluted waters from the Gulf of Lions (Palanques et al., 2006). Marine species, and in particular crustaceans, are well adapted to their environment and their biological activities (e.g. reproduction and moulting) are generally regulated by food availability (Demestre, 1995; Sardà, 1995). The chemical inputs, xenobiotics, in their habitat may interfere with these naturally occurring biological events. Their ability to respond to chemical exposure and maintain cell homoestasis determines their individual performance and ultimately their ecological success.

The species selected for this study were the decapod red shrimp *Aristeus antennatus* (Penaeidea) and the Norway Lobster *Nephrops norvegicus* (Astacidea), two very important commercial species in the Mediterranean that feed on a large variety of preys within the bottom-dwelling community (Cristo and Cartes, 1998). They are both benthic species, although *A. antennatus* is more active (nektobenthic) while *N. norvegicus* burrows in sediments and leads a stricter benthic (endobenthic) lifestyle (Company and Sardà, 1998). Their reproduction follows a seasonal pattern. In *A. antennatus*, reproduction takes place in late spring–early summer, when most of the females are in the maturation stages V and VI; however, a high percentage of males are mature almost all year

* Corresponding author. Fax: +34 93 230 95 55.
E-mail address: msole@icm.csic.es (M. Solé).

0147-6513/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2009.02.007

round (Demestre, 1995). *N. norvegicus* females incubate their eggs for about 6 months and hatching occurs at the end of winter, which coincides with moulting. Males moult during the summer–autumn period (Sardà, 1995).

All organisms have the ability to transform xenobiotics (chemicals, food components) as well as endogenous molecules (hormones, fatty acids) in order to regulate their levels and make them more readily excretable. Some of these biochemical responses have been selected as markers of chemical exposure and/or effect (Cajaraville et al., 2000; Valavanidis et al., 2006). From among the possible biomarkers we selected the antioxidant enzymes catalase, glutathione peroxidase (t-GPX), glutathione reductase (GR) and DT-diaphorase (DT-D; NAD(P)H:quinone oxidoreductase). Catalase (EC 1.11.1.6) transforms H_2O_2 into H_2O and O_2, and t-GPX (EC 1.11.1.9) reduces H_2O_2 as well as other organic hydroperoxides to water and alcohols, respectively. GR (EC 1.6.4.2) maintains the balance GSH↔GSSG towards the reduced form and DT-D (EC 1.6.99.2) transforms quinones into hydroquinones via 2-electron reduction (Cadenas, 1995). We also considered other hepatic enzymes such as glutathione S-transferases (GSTs; EC 2.5.1.18) as a measure or conjugating metabolism with endogenous GSH (McLoughlin et al., 2000), and carboxylesterase (CbEs; EC 3.1.1.1). CbEs are responsible for the hydrolysis of a broad range of endogenous esters and play a significant role during moulting (Homola and Chang, 1997). Lipid peroxidation (LP) indicates lipid membrane damage as a consequence of exposure to reactive oxygen species (ROS) and insufficient antioxidant defences. Protein yield (PY) was adopted as an unspecific marker of enhanced protein synthesis. Acetylcholinesterase (AChE; EC 3.1.1.7) and the pseudocholinesterases (EC 3.1.1.8) butyrylcholinesterase (BChE) and propionilcholinesterae (PrChE) were characterised and measured as biomarkers of neurotoxicity in muscle.

The aim of our study was to provide baseline data on biochemical responses to environmental pollutants in two deep-sea decapod species from the NW Mediterranean. The comparative approach was used to show their differential capacity to deal with xenobiotics. Due to sample limitations, a study of the seasonal variation in the biomarkers selected could be carried out only in *A. antennatus*. Likewise, it was not always possible to test sex-related differences due to the unbalanced sex ratios in some samplings. However, to the best of our knowledge, this is the first study of this kind that deals with these hepatic markers in these two decapods. We also outline certain characteristics of the biochemical responses of the two crustaceans.

Table 1
Biological parameters of the crustacean *Aristeus antennatus* and *Nephrops norvegicus*.

	Season	Size (CL, mm)	(*n*)M:F	Development
Aristeus antennatus	Winter	36.9±8.3	(15)7:8	♀ Stages I–II
	Spring	42.6±8.9	(10)2:8	♀ Mature stages III–IV
	Summer	43.7±1.6	(8)0:8	♀ Mature stages V–VI
	Autumn	41.4±12.6	(6)2:4	♀ Stages I–II
Nephrops norvegicus	Spring	49.9±9.1	(10)10:0	All ♂
	Autumn	40.3±5.0	(7)3:4	♀ stages II–IV

CL = cephalothorax length, (*n* = number of individuals); M = male, F = female. Stages of maturity defined from I to IV (Demestre, 1995).

2. Materials and methods

2.1. Sampling area

Sampling took place offshore of the Barcelona coast (NW Mediterranean), in four consecutive campaigns: February 2007 (winter), April 2007 (spring), June 2007 (summer) and October 2007 (autumn). Sampling depths were either 600–650 m (2.4537°E; 41.2417°N) or 800 m (2.4330°E; 41.1654°N). The biometric characteristics of the species, the number of individuals analysed and their sex ratios are indicated in Table 1. In Table 2, the physico-chemical characteristics of their habitat are reported. Water values are considered to be more relevant for *A. antennatus*, whereas sediment values at 6 cm depth are more appropriate for *N. norvegicus*. Samples were taken by trawling with either a research vessel (Garcia del Cid, CSIC) or a commercial vessel (Stella Maris III). Size was measured as the cephalothorax length (CL±1 mm). Once the specimen had been measured and the sex identified, the hepatopancreas was dissected and frozen immediately in liquid nitrogen, while a portion of the muscle was stored in dry ice. Once in the lab, samples were stored at −80 °C until biochemical analysis.

2.2. Sample preparation of muscle

A portion of muscle, between 0.6 and 1 g, was homogenised in a 50 mM buffer phosphate pH 7.4 in a 1:5 (w:v) ratio using a polytron® blender. The homogenate was centrifuged at 10 000*g* for 20 min at 4 °C and the supernatant was used to characterise cholinesterase (ChE) activities and determine LP levels.

2.3. Sample preparation of hepatopancreas tissue

A portion of the hepatopancreas (0.2–0.5 g) was homogenised using a Teflon homogeniser in a 1:4 (w:v) of 100 mM buffer phosphate pH 7.4 containing 0.1 M KCl and 1 mM EDTA as well as freshly added 1 mM dithiothreitol (DTT), 0.1 mM phenantroline and 0.1 mg/L tripsin inhibitor. The homogenate was centrifuged at 10 000*g* for 30 min at 4 °C and the supernatant (S10) used for biochemical determinations.

2.4. Biochemical determinations

All assays were carried out in triplicate at 25 °C using either undiluted or diluted S10 supernatant, depending on the assay.

2.4.1. Muscle cholinesterase characterisation and determinations

Assay conditions were 1 mM for each substrate: acetylthiocholine iodide (ATC), S-butyrylthiocholine iodide (BTC) and propionylthiocholine iodide (PTC). In each microplate well, 150 µl of 5,5′-dithio-bis-2-nitrobenzoat (DTNB; 270 µM) was mixed with 25 µl of muscle S10, and after 2 min pre-incubation, the reaction was started by adding 50 µl of the substrate. ChE activities were determined using the principle of Ellman et al. (1961) with appropriate modifications for microplates. Reading was performed in triplicate at 405 nm in a microplate reader (TECAN Infinite M200) for 5 min. Activity was expressed in nmol/min/mg prot.

For ChE characterisation, four concentrations were assayed (0.5, 1, 2.5 and 5 mM) for each substrate (ATC, BTC and PTC). The selective inhibitors, eserine (physostigmine) sulphate, which inhibits specific versus non-specific cholinesterases, and BW253c51 (1,5-bis(4-allyldimethyl-ammonimphenyl)penta-3-one dibromide), which inhibits true AChE, were tested in the range 0.64–800 µM, while iso-OMPA (tetraisopropyl pyrophosphoramide), which inhibits BChE, was assayed in the range 0.08–16 mM. In all cases, muscle S10 with the three selective inhibitors was incubated at room temperature for 30 min; 120 µl of S10 was

Table 2
Physico-chemical conditions of the water measured by CTD and considered for *A. antennatus*.

Season	Depth (m)	Water temp. (°C)	Salinity (psu)	Oxygen (mg/L)	Chl *a* (mg/L)	Turbidity (cm^{-1})	Sediment temp. (°C)	Redox pot. (mV)
Winter	800	13.19	38.51	4.11	0.05	0.95	13.60	−35.6
Spring	650	13.29	38.53	5.76	0.02	0.05	15.18	−35.1
Summer	650	13.32	38.54	5.76	0.02	0.02	17.8	−44.5
Autumn	600	13.39	38.55	8.21	0.03	0.47	16.4	−37.2

Sediment parameters were measured by box-core at 6 cm depth and relevant for *N. norvegicus*.