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Protein expression from zooplankton communities in a metal contaminated NW mediterranean coastal ecosystem

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ABSTRACT

Bidimensional and monodimensional polyacrylamide gel electrophoresis were used to study protein expression from zooplankton collected in thirteen stations of Toulon Bay (NW Mediterranean). In this ecosystem, Little Bay showed higher trace metal concentrations (13.5–23.8 nM for Cu, 0.73–1.24 nM for Pb, 27.8–58.7 nM for Zn) than Large Bay (Cu 2.2–15.6 nM; Pb 0.19–0.78 nM; Zn 9.0–38.8 nM). Trace metals positively correlated ($p < 0.05$) with expression of four zooplankton proteins (MW in kDa/pI: 25.0/5.6; 48.8/4.1; 38.2/4.4; 38.3/5.8) and with biomass of *Oithona nana*, predominant copepod in Little Bay. Sequencing by LC–MS/MS putatively provided zooplankton identity of these proteins: they were cytoskeleton actin, except one protein that was the chaperone calreticulin. We suggest that actin and calreticulin could be regarded as zooplankton markers of metal stress and be involved in a possible tolerance of *O. nana* to contamination, contributing to its development in a marine perturbed ecosystem.

1. Introduction

Recently, many authors have mentioned the relevance of investigating the new field of marine proteomics (Johnson and Browman, 2007; Lopez, 2007; Nunn and Timperman, 2007). This field presents two coexisting approaches: the first involves analysing proteins from microorganisms cultured under specific environmental conditions, the second involves the recovery and analysis of proteins directly sampled from the marine environment (Nunn and Timperman, 2007). According to the first approach, some studies have been carried out on pure cultures of marine microorganisms such as the bacterium *Pseudomonas fluorescens* (Poirier et al., 2008), the cyanobacterium *Prochlorococcus marinus* (Pandhal et al., 2007), the phytoplankton *Alexandrium* (Chan et al., 2005, 2006; Lee and Lo, 2008; Wang et al., 2008), *Prorocentrum* (Chan et al., 2002, 2004), *Lingulodinium polyedrum* (Akimoto et al., 2004), and the zooplankton *Calanus finmarchicus* (Hansen et al., 2007). According to the second approach, proteomic studies have taken place in marine environments to sample targeted organisms, such as the zooplankton species *Acartia tonsa* (Tartarotti and Torres, 2009) and *Eurytemora affinis* (Kimmel and Bradley, 2001), as the fish *Paralichthys olivaceus* (Ling et al., 2009; Zhu et al., 2006) or the bivalve *Mytilus* (Manduzio et al., 2005; Mosquera et al., 2003; Ronzitti et al., 2008).

However, it may be assumed that the many species composing a high-complexity community constitute a metaorganism, in which metaproteome shifts could be regarded as a functional response to the dynamic changes affecting the environment (Lacerda et al.,

Abbreviations: AFDW, ash-free dry weight; BSA, bovine serum albumin; 2-DE, bidimensional polyacrylamide gel electrophoresis; DOC, dissolved organic carbon; DOM, dissolved organic matter; DW, dry weight; ER, endoplasmic reticulum; HSP, heat shock protein; HPLC, high performance liquid chromatography; IPG, immobilized pH gradient; IEF, isoelectric focusing; pI, isolectric point; LC–MS/MS, liquid chromatography tandem mass spectrometry; MW, molecular weight; 1-DE, monodimensional polyacrylamide gel electrophoresis; NCBI, National Centre for Biotechnology Information; POM, particulate organic matter; ROS, reactive oxygen species; SDS–PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis.

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2007; Wilmes and Bond, 2004). In this way, very recent studies have focused on the metaproteomics characterization of the dissolved organic matter (DOM) and the particulate organic matter (POM) sampled in the South China Sea (Dong et al., 2010; Wang et al., 2011).

Proteomics provides an excellent tool to analyse changes in protein expression in response to contaminant exposure (Andacht and Winn, 2006). Now, coastal marine ecosystems are increasingly exposed to contamination, among which pollution by metals is a serious threat due to their persistence and toxicity towards marine organisms (Huang et al., 2005). Although zooplankton represent a point of entry of contaminants into the food web, through grazing on phytoplankton, and being themselves an important prey for fish (Hansen et al., 2007), few studies have assessed effects of contaminants on zooplankton communities.

Here, we present the first metaproteomics study characterising protein expression from zooplankton communities in a coastal marine ecosystem contaminated by trace metals. We used monodimensional gel electrophoresis (1-DE) and bidimensional polyacrylamide gel electrophoresis (2-DE) to detect shifts in protein expression, from zooplankton sampled in thirteen stations of Toulon Bay (France). We then related the protein shifts to the structure of the zooplankton communities, and to the trace metal concentrations measured in this ecosystem. Our goal was to understand how the zooplankton protein expression changed according to the metal contamination of a marine natural environment. This was to (i) define zooplankton protein markers of metal stresses, (ii) identify stress proteins potentially involved in the tolerance of some zooplankton species to contamination, (iii) understand the potential contribution of some proteins in the changes in zooplankton diversity recurrently observed in the perturbed Toulon Bay (Jamet et al., 2001, 2005; Richard and Jamet, 2001).

2. Material and methods

2.1. Sampling site

On June 11th 2009, seawater and zooplankton have been sampled in thirteen stations – S_1 to S_{13} – of Toulon Bay, on the NW Mediterranean French coast (Fig. 1). Thanks to a sea wall, Toulon Bay is divided in two smaller bays: Large Bay (LaB) in the south-east (maximal depth: 17 m), with sampling stations S_1–S_9, and Little Bay (LiB) in the north-west (maximal depth: 12 m), with sampling stations S_{10}–S_{13}.

LiB, semi-closed with a surface area of 11 km^2, harbours major commercial traffic as well as a military port (French Navy). Through the Las river, the first river crossing Toulon city, anthropogenic inputs from the urbanized Toulon area (population density = approximatively 600 000 inhabitants) flow into LiB. This ecosystem is significantly polluted, as shown by the high trace metal concentrations measured in *Mytilus* during the 'Mussel Watch Programme' carried out by the Réseau d'Observation de la Contamination Chimique: 7.26 mg Pb per kg of dry weight (4.4 times the national media of 1.65 mg per kg of dry weight) and 0.54 mg Hg per kg of dry weight (3.6 times the national media of 0.15 mg per kg of dry weight) (Ifremer, 2010). In LiB, the macrophyte *Posidonia oceanica* has disappeared for thirty years (Bernard et al., 2001). The proliferation of harmful phytoplankton species *Dinophysis* and *Pseudo-nitzschia* occurs at some periods of the year (Ifremer, 2010). The dominance and high abundance of the Cyclopoid Copepod *Oithona nana* have been previously reported there, resulting in lower zooplankton diversity (Jamet et al., 2005).

Unlike LiB, LaB is connected to the open-sea, the renewal of its water masses being provided by the deep northwest to southeast current, and then by the Liguro-Provençal drift. LaB receives anthropogenic inputs carried by the Eygoutier, the second river passing through Toulon. Compared to the Las, the Eygoutier is regularly dry in summer, suggesting that it contributes less to the pollution of LaB. A recent study reported that sediments were less impacted by metals in LaB than in LiB (Tessier et al., 2011). Great meadows of *P. oceanica* (surface = 325 ha) and low occurrences of harmful phytoplankton species confirm that LaB is less affected by anthropogenic activities than LiB.

2.2. Sampling procedures

All samplings were carried out in LaB (S_1–S_9) and in LiB (S_{10}–S_{13}) (Fig. 1) on June 11th 2009.

Zooplankton samples were collected using a nylon net (Hydro-Bios, model Apstein) with 90 μm mesh (0.5 m mouth diameter, 2.5 m length), equipped with a flowmeter (Hydro-Bios, model 438 110). Each zooplankton sample had a volume ranging from 1.0 to 1.6 L and came from a filtration of volumes comprised between 7.5 and 10.7 m^3 of seawater.

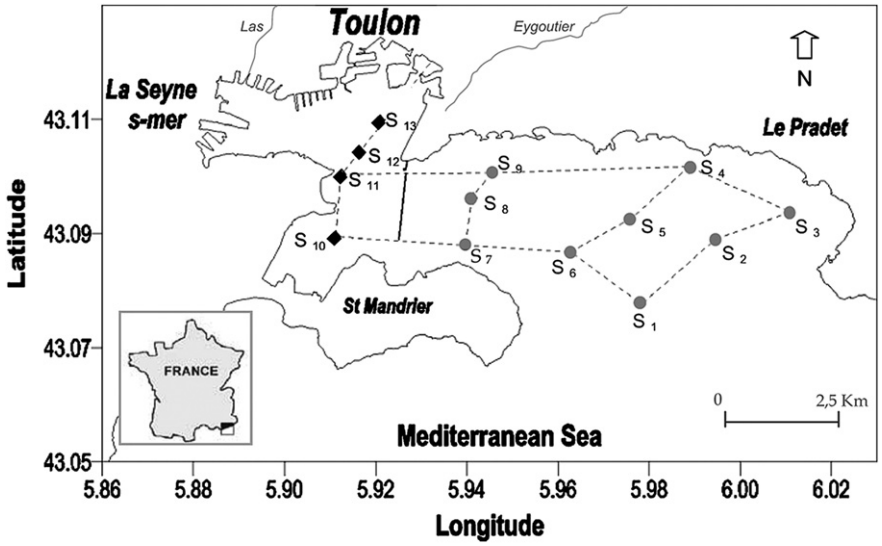

Fig. 1. Location of the S_1–S_9 sampling stations in the Large Bay of Toulon (●), and the S_{10}–S_{13} stations in the Little Bay of Toulon (◆).