



## Trace metal concentrations in post-hatching cuttlefish *Sepia officinalis* and consequences of dissolved zinc exposure



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### ARTICLE INFO

#### Article history:

Received 11 September 2014

Received in revised form 31 October 2014

Accepted 13 November 2014

Available online 28 November 2014

#### Keywords:

Mollusk cephalopod

Juvenile stage

Zinc

Metal subcellular distribution

Oxidative stress

*Sepia officinalis*

### ABSTRACT

In this study, we investigated the changes of 13 trace metal and metalloid concentrations (i.e. Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, V, Zn) and their subcellular fractionation in juvenile cuttlefish *Sepia officinalis* reared in controlled conditions between hatching and 2 months post-hatching. In parallel, metallothionein concentrations were determined. Our results highlighted contrasting changes of studied metals. Indeed, As and Fe concentrations measured in hatchlings suggested a maternal transfer of these elements in cuttlefish. The non-essential elements Ag and Cd presented the highest accumulation during our study, correlated with the digestive gland maturation. During the 6 first weeks of study, soluble fractions of most of essential trace metals (i.e. Co, Cr, Cu, Fe, Se, Zn) slowly increased consistently with the progressive needs of cuttlefish metabolism during this period.

In order to determine for the first time in a cephalopod how metal concentrations and their subcellular distributions are impacted when the animals are trace metal-exposed, we studied previously described parameters in juveniles exposed to dissolved Zn at environmental (i.e. 50  $\mu\text{g l}^{-1}$ ) and sublethal (i.e. 200  $\mu\text{g l}^{-1}$ ) levels. Moreover, oxidative stress (i.e. glutathione S-transferase (GST), superoxide dismutase (SOD) and catalase activities, and lipid peroxidation (LPO)) was assessed in digestive gland and gills after 1 and 2 months exposures. Our results highlighted no or low ability of this stage of life to regulate dissolved Zn accumulation during the studied period, consistently with high sensitivity of this organism. Notably, Zn exposures caused a concentration-dependent Mn depletion in juvenile cuttlefish, and an increase of soluble fraction of Ag, Cd, Cu without accumulation modifications, suggesting substitution of these elements (i.e. Mn, Ag, Cd, Cu) by Zn. In parallel, metallothionein concentrations decreased in individuals most exposed to Zn. Finally, no perturbations in oxidative stress management were detected in gills, whereas modifications of GST, SOD and catalase activity levels were recorded in digestive gland, resulting in an increase of LPO content after a 6-week exposure to low Zn concentration. Altogether, these perturbations are consistent with previously described high sensitivity of juvenile cuttlefish towards Zn. Our results underlined the need to study deeply contamination impact on this animal at this stage of life.

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**Abbreviations:** CDNB, 1-chloro-2,4-dinitrobenzene; DPP, differential pulse polarographic analysis; GSH, reduced glutathione; GST, glutathione S-transferase; HMWP, high molecular weight proteins; LPO, lipid peroxidation; MDA, malondialdehyde; MT, metallothionein; PBS, phosphate buffered saline; ROS, reactive oxygen species; SOD, superoxide dismutase.

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<http://dx.doi.org/10.1016/j.aquatox.2014.11.012>

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## 1. Introduction

Mollusk cephalopods are known for their importance in trophic marine ecosystems both as predator and prey (Chouvelon et al., 2011; Clarke, 1996). The economic importance of their fisheries has recently grown worldwide to compensate for finfish stock depletion (Jereb et al., 2005), whereas abundant literature underlines their sensitivity to a wide range of environmental parameters (reviewed in Pierce et al., 2008). Despite growing anthropogenic pressures on marine ecosystems, few studies have investigated the potential physiological impacts of contaminants on these organisms (Di Poi et al., 2013, 2014; Lacoue-Labarthe et al., 2009c, 2010a; Sen and Sunlu, 2007). Yet, early life stages (i.e. eggs and juveniles) of economically important cephalopods are likely to be impacted by anthropogenic contaminants because they develop in coastal areas where these compounds are found at relatively high concentrations (Colas, 2011; Pierce et al., 2010). This is the case of the common cuttlefish *Sepia officinalis*, which is characterized by inshore migration during spring and summer months to reproduce and spawn, and juvenile residence in coastal areas from 1 to 2 months post-hatching to take advantage of prey abundance (Bloor et al., 2013; Boucaud-Camou and Boismery, 1991; Le Goff and Daguzan, 1991). In spite of the shielding properties of the eggshell against metals (Bustamante et al., 2002b, 2004, 2006c; Lacoue-Labarthe et al., 2008b, 2009b, 2010b, 2011), cuttlefish embryos are impacted by low metal concentration exposure (Lacoue-Labarthe et al., 2009a, 2010a). In addition, accumulation in soft tissues of waterborne Ag, Cd, and Zn followed by relatively long-term retention was reported in juveniles (Bustamante et al., 2002b, 2004). Surprisingly little is known about the effects of such trace metal exposure on juvenile cuttlefish physiology (Lacoue-Labarthe et al., 2009a, 2010a).

Despite a short life cycle of 1–2 years, *Sepia officinalis* (as other cephalopods) is known to accumulate various contaminants (e.g. Ansari et al., 2012; Bustamante et al., 1998, 2006a,b; Danis et al., 2005; Miramand and Bentley, 1992). Bioaccumulation of trace metals such as Ag, Cd, Cu, Fe and Zn has been reported in the digestive gland, sometimes at very high concentrations (e.g. Bustamante et al., 2006a; Decleir et al., 1978; Martin and Flegel, 1975; Miramand and Bentley, 1992; Schipp and Hevert, 1978). Notably, trace metal accumulation starts after hatching and takes place through the entire life cycle, yet tissue-specific accumulation depends on digestive gland maturation (Miramand et al., 2006). While several studies have highlighted the essential role of the digestive gland in the detoxification of trace metal elements in *Sepia officinalis* and other cephalopods (Bustamante et al., 2002b, 2004, 2008; Lacoue-Labarthe et al., 2009c; Martin and Flegel, 1975; Miramand and Bentley, 1992; Raimundo et al., 2010, 2014), no study has investigated subcellular fractionation of metals at juvenile stages during maturation of this organ (i.e. the first month post-hatching).

Detoxification mechanisms of marine invertebrates mainly involve the precipitation of metals into insoluble concretions and the binding to cytosolic proteins, both being interconnected (Wallace et al., 2003; Wang and Rainbow, 2010). Metallothioneins (MTs) are one class of these metal-binding proteins, characterized by low molecular weight, high cysteine content and heat stability. They play a role in the homeostasis of the essential metals, Cu and Zn, but can be induced by various other metals (e.g. Ag, Cd, Hg) that they bind (Amiard et al., 2006; Cosson, 1991; Wang and Rainbow, 2010). In cephalopods, subcellular distribution of metals in the digestive gland appears to be species- and element-specific, but most studies have reported mainly association of Ag, Fe, Mn, Pb and Zn with the insoluble fraction, while Cd, Co and Cu are mainly associated with the cytosolic fraction (Bustamante et al., 2002a, 2006a; Finger and Smith, 1987; Tanaka et al., 1983). Cu is also the main element found associated with MTs in the digestive gland of

several cephalopod species (Bustamante et al., 2006a; Finger and Smith, 1987; Tanaka et al., 1983), whereas results about Ag- and Cd-MT association remain controversial (Bustamante et al., 2002a, 2006a; Finger and Smith, 1987; Tanaka et al., 1983). To date, the MT involvement in the homeostasis of the cephalopod juvenile stage has been investigated once in the squid *Loligo forbesii* without highlighting its significant role in Cu and Zn management (Craig and Overnell, 2003).

Although Zn is an essential nutrient of living organisms – it is a component of more than 300 enzymes and other proteins (McCall et al., 2000), some studies have highlighted its toxicity in marine organisms at concentrations of  $100 \mu\text{g l}^{-1}$  and above (e.g. Amado Filho et al., 1997; Brereton et al., 1973; Nadella et al., 2013; Tellis et al., 2014; Watling, 1982). In European marine waters, dissolved Zn is relatively concentrated, regularly measured near  $10 \mu\text{g l}^{-1}$  (Lachambre and Fisson, 2007a; Sheahan et al., 2007), and worldwide, some studies reported concentrations reaching several hundred  $\mu\text{g Zn l}^{-1}$  in anthropically impacted bays, gulfs and estuaries (Amado Filho et al., 1997; Liu and Wang, 2013; Srinivasa Reddy et al., 2005). Zn is also one of the most concentrated metals in the digestive gland of cuttlefish (Bustamante et al., 2006a; Miramand and Bentley, 1992; Miramand et al., 2006; Rjeibi et al., 2014). Its effects on the uptake of other metals, their subcellular distribution, and MT concentrations have been highlighted in bivalve mollusks (Blackmore and Wang, 2002; Hennig, 1986; Lemoine et al., 2000; Liu and Wang, 2013; Shi and Wang, 2004). For instance, Zn exposure induced an increase in Zn uptake as well as that of Cd and Cu in the bivalve *Crassostrea hongkongensis* (Liu and Wang, 2013). To the best of our knowledge, such responses have not been assayed in cephalopods. Finally, trace metals are known for their ability to induce reactive oxygen species (ROS) production, causing oxidative stress as previously described in bivalves (e.g. Company et al., 2004; Funes et al., 2006; Geret and Cosson, 2002; Geret et al., 2002), gastropods (Chandran et al., 2005; Malanga et al., 2004; Radwan et al., 2010) and the cephalopod *Octopus vulgaris* (Semedo et al., 2012). To avoid or counteract ROS effects, organisms use a set of enzymes including glutathione S-transferases (EC 2.5.1.18, GST), superoxide dismutase (EC 1.15.1.1, SOD) and catalase (EC 1.11.1.6). Their activities have been extensively used as biomarkers of oxidative stress, and correlated to the presence of metals in invertebrates (e.g. Regoli and Principato, 1995; Semedo et al., 2012; Vlahogianni et al., 2007). The imbalance between production and removal of ROS may result in lipid peroxidation (LPO), a biomarker often used to evaluate the degree of oxidative stress (e.g. Di Salvatore et al., 2013; Radwan et al., 2010; Vlahogianni et al., 2007; Zielinski and Pörtner, 2000).

In order to better describe and understand metal regulation in juvenile cuttlefish and point out the effect of physiological modifications on the bioaccumulation and detoxification processes associated with digestive gland maturation, we quantified the presence, changes of the concentrations and the subcellular distributions of 13 elements (Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, V, and Zn) in juvenile cuttlefish during the period corresponding to their coastal life stage (i.e. first 2 months post-hatching). The concentration of MTs was also assayed and correlated with metal concentration modifications. Concurrently, these parameters were measured under Zn-exposure, in addition to oxidative stress parameters (GST, SOD, catalase, and LPO) in the digestive gland and in the gills which are the tissue directly in contact with food and waterborne trace elements, respectively.

## 2. Materials and methods

### 2.1. Animals

Cuttlefish eggs were collected from traps set along the Calvados coast (Basse-Normandie, France) during summer 2012. All rearing

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