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Ocean acidification modulates the incorporation of radio-labeled heavy metals in the larvae of the Mediterranean sea urchin *Paracentrotus lividus*

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A B S T R A C T

The marine organisms which inhabit the coastline are exposed to a number of anthropogenic pressures that may interact. For instance, the accumulation of toxic metals present in coastal waters is expected to be modified by ocean acidification through e.g. changes in physiological performance and/or elements availability. Changes in bioaccumulation due to lowering pH are likely to be differently affected depending on the nature (essential vs. non-essential) and speciation of each element. The Mediterranean is of high concern for possible cumulative effects due to strong human influences on the coastline.

The aim of this study was to determine the effect of ocean acidification (from pH 8.1 down to −1.0 pH units) on the incorporation kinetics of six trace metals (Mn, Co, Zn, Se, Ag, Cd, Cs) and one radionuclide (^{241}Am) in the larvae of an economically- and ecologically-relevant sea urchin of the Mediterranean coastline: *Paracentrotus lividus*. The radiolabelled metals and radionuclides added in trace concentrations allowed precise tracing of their incorporation in larvae during the first 74 h of their development.

Independently of the expected indirect effect of pH on larval size/developmental rates, *Paracentrotus lividus* larvae exposed to decreasing pHs incorporated significantly more Mn and Ag and slightly less Cd. The incorporation of Co, Cs and ^{241}Am was unchanged, and Zn and Se exhibited complex incorporation behaviors. Studies such as this are necessary prerequisites to the implementation of metal toxicity mitigation policies for the future ocean. We discuss possible reasons and mechanisms for the specific effect of pH on each metals.

1. Introduction

The marine organisms which inhabit the coastline are exposed to a number of anthropogenic pressures. On a local scale, metallic trace elements – originating from activity occurring along river basins (e.g. mine discharge, agriculture, wastewater treatment) – are harmful contaminants of the coastal marine environments and of concern to humans for ocean exploitation (Förstner and Wittmann, 1981). The French Mediterranean coastline, which is strongly influenced by the discharges of the Rhône river that drains industrialized and urban basins, is particularly exposed to metal pollution (UNEP/MAP, 2013). Metals can be highly toxic at low levels, especially to early development stages of marine invertebrates, and they have long been shown to alter (for example) sea urchin gametogenesis, larval growth, morphological development (e.g. Cu: Bougis, 1965; Cd: Pagano et al., 1982 and Filosto et al., 2008; Mn: Pinsino et al., 2010) and biomineralization (reviewed by Matranga et al., 2011).

In parallel, the ocean is facing global changes, a consequence of the increase in anthropogenic carbon dioxide (CO_2) in the atmosphere (280–400 ppm between 1850 and 2013, Etheridge et al., 1996 and Scripps CO_2 Program). On top of a warming (average + 0.1 °C per decade between 1970 and 2010, IPCC, 2013), the surface ocean waters also experience an acidification (average −0.1 pH units - i.e. 30% increase in acidity - between pre-industrial levels and 2000: Caldeira and Wickett, 2008, −0.0044 units per year in the northwestern Mediterranean: Flecha et al., 2015). This ocean acidification is projected to decrease the current average pH of the global oceans (≈8.1) by a further 0.3–0.4 pH units by the end of the 21st century (IPCC, 2013).

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Ocean acidification has been demonstrated to be a major threat for marine organisms, impacting their physiology (e.g. sea urchin larvae disrupted in their acid-base regulation: Stumpp et al., 2011 and feeding: Stumpp et al., 2013, increased metabolism: Dorey et al., 2013 and modulated gene expression, in functions such as immunity: Runcie et al., 2017) as well as their fitness (e.g. negative effect on sea urchin larval growth: Dorey et al., 2013; Martin et al., 2011; Stumpp et al., 2011 and sea urchin gonadal growth: Siikavuopio et al., 2007; Stumpp et al., 2012b: exposure time ≤ 56 days, but see Dupont et al., 2013: no difference after 16 months). Ocean acidification has also been demonstrated to modify larval behavior (e.g. settlement behavior of *Bugula neritina*: Pecquet et al., 2017) and cognitive performances (e.g. sensory capacities and spatial navigation in larvae of fish: Munday et al., 2009) and more largely interactions within communities (e.g. Asnaghi et al., 2013). Early life stages, often considered as bottlenecks for population dynamics, have however been shown to be more resistant than previously thought (Kroeker et al., 2013). For instance, sea urchin larvae are resistant to a seawater acidification down to 7.1, albeit with larval growth being generally reduced when pH is lowered (*Paracentrotus lividus*: Martin et al., 2011, *Strongylocentrotus droebachiensis*: Dorey et al., 2013). Overall, studies show that responses are however species- and stage-specific (Kroeker et al., 2013), with for instance the larvae of another echinoderm, *Ophiotrix fragilis*, experiencing 100% mortality at pH_{NBS} 7.9 (vs. 70% in control pH: Dupont et al., 2008).

The resulting interactions of multiple stressors in the future ocean could lead to non-independent effects (e.g. Harley et al., 2006). The Mediterranean Sea is of high concern for possible cumulative effects (global change, over-fishing, pollution; UNEP/MAP, 2013). Although an important body of works demonstrated that increasing temperature arises metal toxicity (see review by Sokolova and Lannig, 2008) together with an elevation of the organism's metabolism and a modification of its physiology (e.g. feeding, osmoregulation), few studies focused on the effects of ocean acidification on the bioaccumulation and toxicity of metallic contaminants (see review Table 1). Yet, the changes of pH and seawater chemistry caused by increased CO_2 can modify the speciation of metals, because element's speciation is largely dependent on physicochemical parameters (salinity, pH, redox potential: Millero et al., 2009; Stockdale et al., 2016), and therefore their bioavailability for organisms. CO_2-driven acidification has been shown to modulate trace metal accumulation in both cephalopods early-life stages (Lacoue-Labarthe et al., 2009, 2011a) and the mantle cells of clams (Ivanina et al., 2013). A change in bioaccumulation efficiencies could lead to a change of metal toxicity.

Additionally, pH can impact the organism's abilities to deal with metal bioaccumulation and toxicity, as both are likely to interfere with metabolism and more specifically ionoregulation activities (Pörtner et al., 2004; Stumpp et al., 2012a). While sea urchin larvae have been shown to be relatively tolerant to lowered seawater pH - even for values well below the average projections for 2100 -, the effects are strong at the sub-lethal level. Decreasing pH resulted in larval growth delay (but conserved calcification in the form of calcium accumulation and relative arm length: Martin et al., 2011), increased metabolism (e.g. respiration rates increased by 9% per 0.1 pH unit decrease in Dorey et al., 2013) and changes in the expression of traits relating to cell division, metabolism and immune activity (pH 7.7: Runcie et al., 2017). These resulting sub-lethal changes could possibly be linked to the increase in energy demand (Stumpp et al., 2011) in order to maintain intracellular medium, via the use of energy-demanding membrane pumps (Stumpp et al., 2012a). Within these processes, ionic pumps such as -ATPase pumps play a key role by creating electrochemical gradients used by secondary active transporters (Stumpp and Hu, 2017). Yet, in marine organisms, these ion channels represent a major pathway for the entrance of metallic positively-charged ions (e.g. fish: Webb and Wood, 2000). Ocean acidification is therefore likely to increase metal pollutant bioaccumulation in organisms possessing acid-base regulation systems such as the sea urchin larvae. More information on the interactions between pH and metal toxicity is thus required to improve predictive mathematical models and coastal management (Harley et al., 2006).

The aim of this study was to determine the effect of ocean acidification on the incorporation kinetics of six trace metals and one radionuclide (Mn, Co, Zn, Se, Ag, Cd, Cs, ^{241}Am) in an economically- and ecologically-relevant sea urchin of the Mediterranean coastline: *P. lividus*. These elements were chosen for their essential (e.g. Mn, Co, Se and Zn function as catalyst or activators of enzymatic reactions) and non-essential characters (e.g. Ag, Cd and ^{241}Am), their toxicity ("very toxic and relatively accessible" for Co, Se, Zn, Ag and Cd according to Förstner and Wittmann, 1981) and their occurrence in the coastal areas (e.g. ^{241}Am is a toxic and radioactive isotope associated with nuclear waste). The radiolabelled metals and radionuclide added in trace concentrations allowed precise tracing of their incorporation in larvae exposed to six different pH conditions (from pH_T 8.1 down to −1.0 pH units) during the first 74 h of development. We expected to observe decreased accumulation of radionuclides when pH was lowered due to the impaired larval developmental rates and we wished to detangle the more direct effects of pH on bioaccumulation due to e.g. changes in metal speciation, the larval physiology/metabolism and/or impairment of detoxification processes. We also expected differences in how metals would react to pH changes to be based on the nature of the metals (essential vs. non-essential) and their major form in the seawater (e.g. chloride-dominated vs. free form) and hence their expected speciation changes.

2. Material and methods

2.1. Biological material, spawning and experimental conditions

In March and April 2009, specimens of adult sea urchin (*P. lividus*) were collected by divers from subtidal rocky shores (≈ 10 m deep) in the Bay of Villefranche-sur-mer (NW Mediterranean coastline, France). This period corresponds to a few months prior to natural spawning in this area (mid-May to July, Boudouresque and Verlaque, 2001). The adults were maintained in a flow of unfiltered natural seawater (depth = 10 m, salinity = 38) in the Laboratoire d'Océanographie de Villefranche and fed with *Posidonia oceanica* until fertilization (end of April). Three females and one male were opened around the peristome to retrieve gametes from the ripe gonads. Eggs were rinsed three times with filtered seawater (FSW; 0.22 μm, salinity = 38) and pooled together. Sperm was collected dry using a Pasteur pipette and stored at 4 °C in a small tube before use. Within an hour after collection the gametes were transferred to the Radioecology Laboratory (Environment Laboratories of the International Atomic Energy Agency, Monaco) premises for fertilization. A concentrated egg suspension (≈ 4 000 000 eggs) was transferred to six 1-l beakers filled with FSW (20 °C) and diluted sperm (1:50 ratio in FSW) was added to the egg suspension. Eggs were gently agitated for 15 min to optimize fertilization and then distributed to six replicates 4.5-l glass beakers per pH level (N = 36 beakers) filled with radiolabeled FSW (seven radionucleids, detailed below). Embryos (start density of ca. 130 per ml) were grown in aerated FSW at their respective pH levels (pH_T: 7.1, 7.3, 7.5, 7.7, 7.9, 8.1; Table 2).

Natural variability in pH has been described to be broad in certain coastal areas (e.g. Thomsen et al., 2010) and the shift of the pH range as a whole in the future (e.g. Hauri et al., 2012) is highlighted as an important reason to investigate of wide ranges of pH (ΔpH down to −1.0 unit; e.g. Dorey et al., 2013). Yearly surface pH variations are much tamer near our site, with extreme pH values recorded ranging from 7.95 to 8.20 (DYFAMED station in the Ligurian Sea, 50 km from the coast, monthly data from 1995 to 2011: Geri et al., 2014, and SOMLIT Villefranche-sur-Mer Station, 500 m from the shore, April-December 2016, n = 37: http://somlit-db.epoc.u-bordeaux1.fr/bdd.php) as well as in general in the northwestern Mediterranean Sea (e.g. $pH_T = 7.893 \pm 0.007$ near the Strait of Gibraltar in Aug 2012–June