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Delineation of metals and radionuclides bioconcentration in eggs of seabream *Sparus aurata* and effect of environmental pCO_2

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ABSTRACT

Considered as the most vulnerable ontogenic stages to environmental stressors, the early-life stages of fish paid a peculiar attention with respect to their vulnerability to metal and radionuclides contamination. Concomitantly, the increasing anthropogenic CO_2 release in the atmosphere will cause major change of the seawater chemistry that could affect the trace elements and radionuclides bioconcentration efficiencies by marine organisms. The aim of this work was to 1) delineate the uptake behaviours of Ag, Am, Cd, Co and Zn in seabream eggs during 65 h of development and retention by newly hatched and 7 h-old larvae maintained in clean seawater, respectively, and 2) investigate the effects of elevated pCO_2 on the bioconcentration efficiencies of these elements in eggs. Besides differing in terms of maximal concentration factors values, the uptake kinetics showed element-specific patterns with Am being linearly bioconcentrated and Co and Zn showing a saturation state equilibrium. The ^{110m}Ag and ^{109}Cd uptake kinetics shared a two-phases pattern being best described by a saturation equation during the first 24 h of development, and then an exponential loss of accumulated elements although the radiotracer concentrations in the surrounding water remained constant. At hatching time, the radioactivity of ^{110m}Ag was the highest among radiotracers detected in the larvae. After 7 h in depuration conditions, 60% of this metal was still detected whereas ^{241}Am, ^{60}Co and ^{65}Zn were almost totally lost, suggesting an efficient incorporation of Ag in the embryo during the egg development. Finally, this study brought first qualitative data on the effect of pCO_2/pH on metal bioconcentration in eggs, raising the need to unravel chemical and biological processes to predict a potential shift of the toxicity of environmental contamination of fish early life stages with future ocean change.

1. Introduction

The occurrence of trace elements, including the radionuclides, in the marine environment results from their increasing widespread from human activities (e.g. industries, wastewater treatment, nuclear power plant and accidents, etc,.), their persistence in the environment considering also medium and long lived radionuclides (e.g. ^{241}Am with $T_{1/2}$ = 426 y), and their progressive dispersion through oceanic current (Periáñez et al., 2016). These elements are generally considered as contaminants in the oceans and they are also known to accumulate in marine organisms (e.g. Bustamante et al., 2006; Lacoue-Labarthe et al., 2010; Metian et al., 2016; Miramand and Guary, 1981). There, they can lead toxic effects through their chemical interactions with cellular components and/or through the tissue irradiation from accumulated radionuclides. Being considered as the most vulnerable ontogenic stages to environmental stressors (Pörtner and Farrell, 2008), the early-life stages of fish paid a peculiar attention with respect to their vulnerability to trace elements contamination (e.g. Jeffree et al., 2006; Rombough and Garside, 1982 and references hereafter). Nevertheless, papers reporting data on the uptake and accumulation of metallic elements and radionuclide in whole eggs and embryo dated back to four-five decades (e.g. Beattie and Pascoe, 1978; Kimura and Honda, 1977; Shazili and Pascoe, 1986), the main part focusing on cultured fish species, such as Salmonid (e.g. Peterson et al., 1985; Rombough, 1985; Rombough and Garside, 1982) and to a lesser concerned marine family, such as Clupeid (Von Westernhagen et al., 1974, 1975, 1981, 1979). Since this period, this thematic was strayed off course by scientific communities, leading to a lack of information especially regarding marine cultured species.

Through metal concentration measurements in embryo, radiotracers

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uptake kinetics delineation, or sensitivity endpoints, many previous works highlighted the shielding but metal-dependant properties of the egg chorion that limit the element accumulation in the developing fish embryo (e.g. Beattie and Pascoe, 1978; Kimura and Honda, 1977; Shazili and Pascoe, 1986). For example, the embryo of the steelhead trout (*Salmo gairdneri*) was shown to be more resistant to Cd, Pb, and Zn but significantly less resistant to Ag, Cu and Hg when the egg capsule was removed than with the capsule intact (Rombough, 1985). The eggshell membrane forms an elastic and physical protective barrier around the embryo. It is known being permeable to water, ions and small molecules, but not macromolecules (Alderdice, 1988; Rudy and Potts, 1969). Regarding trace element, the accumulation efficiencies of cations strongly varies according the metal but also the development stage (Rombough, 1985; Shazili and Pascoe, 1986). Moreover, the environmental factors have been also identified as modulator of the metal uptake efficiency or toxicity on eggs. Thus, Von Westernhagen et al. (1975) clearly demonstrated that lowering salinity increased the Cd accumulation in marine fish eggs (*Clupea harengus*, *Belone belone* and *Pleuronectes flesus*). In addition, the temperature increased the toxicity of Cd on embryos of *Pseudopleuronectes americanus* (Voyer et al., 1977).

Less is known on the effect of pH and/or pCO_2 on the trace elements accumulation in fish eggs. Nevertheless, this question rose from the expected decrease of seawater pH during the current century caused by the increasing anthropogenic CO_2 release in the atmosphere. The seawater pH already declined of 0.1 unit since the industrial revolution and is expected to decline by 0.06–0.32 units by the end of century (Ciais et al., 2013), resulting in an unprecedented change of seawater chemistry equilibrium since the last 800,000 years (Zeebe and Ridgwell, 2011). This "ocean acidification" phenomenon will therefore occur in a background of chronic local contamination by dissolved inorganic contaminants (i.e. metals and radionuclides) in coastal area sheltering the major part of marine biodiversity and resources. First, the change of pH and seawater chemistry caused by the increased CO_2 modifies the speciation of metals/radionuclides and therefore their bioavailability for marine organisms (Millero et al., 2009). Secondly, the animal physiology or metabolism affected by increasing pCO_2 (Pörtner, 2008; Pörtner and Farrell, 2008) could modify the bioconcentration efficiencies through upregulated ionic processes especially in active organisms (Lacoue-Labarthe et al., 2009). Considering the early life stages, the embryos of fish protected by a chorionic egg envelope were generally less sensitive than larvae, which are directly in contact with waterborne contaminants (e.g. Lavolpe et al., 2004; Van Leeuwen et al., 1985). Nevertheless, the shielding efficiency of the envelope, known as being metal-specific (Von Westernhagen, 1988) could be affected by increasing pCO_2 with consequences on the bioconcentration capacities in embryos as demonstrated in the cephalopods eggs (Lacoue-Labarthe et al., 2012).

The gilthead seabream *Sparus aurata* is a temperate fish widely distributed over the North-Eastern Atlantic Ocean and the Mediterranean Sea. This coastal species is subjected to recreational and professional fishing due to its high tasting and economic value. Moreover, this is a major resource for aquaculture (FAO, 2017) with more than 150,000 tonnes produced in 2014 in 17 countries for a worth *ca.* US$ 785 million (GFCM, 2013) in the Mediterranean Sea. Cultured in high densities conditions, farmed seabreams are usually exposed to elevated metabolic pCO_2, due to respiration, well above the predicted levels expected for the end of the century (~10,000 µatm). Usually, no apparent detrimental implications are observed on fish health, as food is abundant and fish are protected against diseases and predators (Ellis et al., 2017), but less is evident for early-life stages that developed in hypercarbic conditions.

In this context, the aim of this work was to 1) delineate the uptake behaviours of Ag, Am, Cd, Co and Zn in seabream eggs during 65 h of development and the elements retention by newly hatched and 7 h-old larvae maintained in clean seawater, respectively, and 2) to investigate the effects of elevated pCO_2 on the bioconcentration efficiencies of these elements. The range of pHs values was chosen to encompass the expected change of pH until the end of the century, i.e. ~7.75, and a worst value, i.e. 7.5, to highlight the effect of hypercarbia on the bioconcentration processes.

2. Materials and methods

2.1. Experimental procedure

Fertilized eggs of seabream, *Sparus aurata,* were collected few minutes (*ca* 30 min) after spawning of genitors at the premises of the fish farm "Cannes Aquaculture" in Monaco and immediately placed in 10 l bottle filled with filtered (0.22 µm) and oxygenated (1%) seawater before transfer to the IAEA-EL Radioecology Laboratory for radiotracers exposure.

2.1.1. Experiment 1: Delineation of accumulation behaviours of radionuclides in seabream eggs

Freshly collected eggs were numbered before being placed with a density of 5000 eggs l^{-1} in three 20 l polycarbonate tanks (Nalgene®) placed in a 300 l tank kept at constant temperature (17 ± 1 °C). The eggs were suspended in 10 l of seawater (1 µm filtered and UV sterilized Mediterranean seawater; constantly aerated closed circuit; 38 p.s.u.; light/dark cycle: 12 h/12 h) and maintained gently agitated. Then, the 1-h old eggs were exposed to dissolved ^{110m}Ag, ^{241}Am, ^{109}Cd, ^{60}Co and ^{65}Zn during the *ca.* 65 h of development until hatching time (see Table 1 for the radiotracer concentrations). The radioactivity in eggs was then followed along the development course: 1) the eggs were manually agitated to ensure a homogenous distribution of eggs in seawater, 2) one sample per tank (n = 3) of egg suspension of 50 ml (~approx. 150 eggs) and 100 ml (~approx. 500 eggs) was collected after 10 min, 1, 2 h and 3, 18, 24, 42, 48 h of exposure, respectively. After 65 h of development, before the first larvae hatched, 200 ml of egg suspension (~approx. 1000 eggs) were sampled. The eggs were filtered on 1.0 µm pre-weighted polycarbonate membrane, resuspended and rinsed with non-radiolabelled seawater. Before resuspension and rinsing, 50 ml of filtrate seawater were sampled and pooled to obtain 150 ml volume for seawater radioactivity counting. The filters and eggs were then weighted, placed in a plastic tube with 5 ml of HNO_3 2N to facilitate a homogeneous repartition of radioisotopes in the solution volume and radiocounted as described below.

As soon as the first eggs started to hatch (after 65 h of incubation), the remaining eggs have been then immediately placed in depuration conditions to follow the loss of radiotracers in young larvae. Basically, 3 L of radiolabelled seawater containing the contaminated eggs have been transferred in clean tanks and immediately mixed with 8 l of non-radiolabelled seawater (1 µm filtered and UV sterilized Mediterranean seawater; 38 p.s.u.; 17 ± 1 °C; light/dark cycle: 12 h/12 h) and left in open circuit with a water renewal of 10 l. h^{-1}. Thirty minutes and 7 h after this transfer, larvae were collected, rinsed with clean seawater, weighted, placed in a plastic tube, euthanized by placing them at −20 °C, and then mixed with 5 ml of HNO_3 2N before counting. The losses of ^{110m}Ag, ^{241}Am, ^{60}Co and ^{65}Zn have been expressed as the percentage of remaining activities counted in larvae sampled after 7 h in depuration condition compared to those sampled 30 min after the transfer in clean seawater. This comparison allows at qualitatively evaluating the retention or loss of trace elements accumulated in larvae following exposure of egg during the embryonic development. The data regarding ^{109}Cd were not kept as the activities recorded in larvae after 7 h in non-radiolabelled conditions were below the detection limit.

2.1.2. Experiment 2: Effect of pCO_2 on metal accumulation in seabream eggs

Few months later, 1-h old eggs have been again collected from other seabream genitors as described above, and immediately placed in four pH conditions (i.e. four 20 l tanks filled with 10 l of seawater; 5000 eggs