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**Limited effects of increased CO<sub>2</sub> and temperature on metal and radionuclide bioaccumulation in a sessile invertebrate, the oyster**

*Crassostreagigas*

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## ABSTRACT

This study investigated the combined effects of reduced pH and increased temperature on the capacities of the Pacific cupped oyster *Crassostrea gigas* bioconcentrate radionuclide and metals. Oysters were exposed to dissolved radiotracers ( $^{110m}\text{Ag}$ ,  $^{241}\text{Am}$ ,  $^{109}\text{Cd}$ ,  $^{57}\text{Co}$ ,  $^{54}\text{Mn}$ , and  $^{65}\text{Zn}$ ) at three pH (7.5, 7.8, 8.1) and two temperatures (21°C and 24°C) under controlled laboratory conditions. Although calcifying organisms are recognized as particularly vulnerable to ocean acidification, the oyster did not accumulate differently the studied metals when exposed under the different pH conditions. However, temperature alone or in combination with pH somewhat altered the bioaccumulation of the studied elements. At pH 7.5, Cd was accumulated with an uptake rate constant two-fold higher at 24°C than 21°C. Bioaccumulation of Mn was significantly affected by an interactive effect between seawater pH and temperature, with a decreased uptake rate at pH 7.5 when temperature increased ( $27 \pm 1$  vs.  $17 \pm 1 \text{ d}^{-1}$  at 21°C and 24°C, respectively). Retention of Co and Mn tended also to decrease at the same pH with decreasing temperature. Neither pH nor temperature affected strongly the elements distribution between shell and soft tissues. Significant effects of pH were found on the bio-accessibility of Mn, Zn and  $^{241}\text{Am}$  during experimental *in vitro* simulation of human digestion.

**Keywords:** Ocean acidification, bioaccumulation, Pacific oyster, metal, radiotracer, *in vitro* digestion

## INTRODUCTION

The increase of partial pressure of CO<sub>2</sub> ( $p\text{CO}_2$ ) in the atmosphere due to anthropogenic activities is well recognized as a driver of major global changes such as the elevation of atmospheric temperature. The global mean surface air temperature is projected to increase by 1 to 3.7°C by the end of the century (IPCC, 2013). Accordingly the temperature of ocean will increase by 0.6°C to 2.0°C for the top 100 metres (IPCC, 2013). Additionally, the ocean is a major sink for atmospheric CO<sub>2</sub> (it absorbs ~25 % of the anthropogenic emissions) and thus the increase in  $p\text{CO}_2$  in the atmosphere implies an increase in  $p\text{CO}_2$  in surface ocean waters, that causes the “other” CO<sub>2</sub> problem (Doney et al., 2009), better known as “ocean acidification”.

Marine organisms can be affected by ocean acidification through (1) limitation of available carbonates, mainly affecting calcifying organisms, (2) the increase in H<sup>+</sup> ions in the water resulting in decreasing pH –i.e. acidification of the surrounding environment– and (3) an increase in  $p\text{CO}_2$  within their body (hypercapnia). In this context, attention should be paid to vulnerable species, which have key role(s) in the food chains, or are of high economic interest for aquaculture or fisheries.

The Pacific cupped oyster *Crassostrea gigas* is the most farmed oyster species in the world (FAO, 2014), with an annual production of 0.609 million tonnes for ca. US\$ 1.3 billion (2012 data). Consequently, many researchers focused on this marine resource and recently investigated, among others, the responses of the Pacific cupped oyster to ocean acidification. Several studies assessed growth, calcification, development and survival of oysters under realistic low pH and low carbonate concentrations conditions (for a review, see Gazeau et al., 2013). A particular attention has been paid to the early life stages of the oyster, which are considered as the most vulnerable and thereby as a bottleneck for population dynamics (Dupont et al., 2010). Overall, reduced shell size and thickness, developmental

retardation and increased mortality are the most common effects of elevated  $p\text{CO}_2$  on *Crassostrea gigas* (e.g. Parker et al., 2010; Gazeau et al., 2011; Barton et al., 2012). Intraspecific variations have been highlighted, and it has been suggested that some populations could be pre-adapted to ocean acidification (Parker et al., 2010; Parker et al., 2011; Timmins-Schiffman et al., 2013).

The elevated  $p\text{CO}_2$  in seawater is expected to affect the metabolism through disturbances of acid-base regulation and respiration (Pörtner et al., 2004). It is admitted that active organisms, such as fish, cephalopods and crustaceans have naturally the required physiological machinery to deal with elevated extracellular  $p\text{CO}_2$  (Melzner et al., 2009). In contrast, sessile organisms, such as oysters, are weak acid-base regulators and, in light of the current knowledge, tend to increase their energy demand to poorly compensate the body acidosis (Parker et al., 2013). This energy budget change is exacerbated when seawater temperature increases (Lannig et al., 2010) and could affect other performances such as somatic growth and survival (Beniash et al., 2010).

Mining, fossil fuel combustion, industrial activities, and uncontrolled discharges are common sources of trace element and radionuclide releases in the environment and are major contributors to the contamination of coastal marine ecosystems over the last century. Widespread in the environment, these contaminants are accumulated in organisms, and potentially bio-magnified along the food chains (Zhou et al., 2008). Excessive accumulation of essential (e.g. Co, Mn or Zn) or non-essential (e.g. Ag,  $^{241}\text{Am}$ , Cd) elements in organisms can induce toxic effects (Rainbow, 2002). Since bioaccumulation of metals in biota depends on 1) the bioavailability of the element determined by physicochemical conditions of the environment, 2) the biological traits of organisms (e.g. metabolism, feeding strategy), and 3) the element properties with respect to biological tissues (e.g. ligand affinities, biological function), it appears pertinent to investigate the effect of ocean acidification on metal

bioaccumulation capacities. Indeed pH and seawater chemistry changes caused by increased  $p\text{CO}_2$  affect chemical speciation of metals and therefore their bioavailability to organisms (Millero et al., 2009), especially for elements that form strong complexes with carbonates (e.g. Am - Choppin, 2006). Furthermore, increased temperature or hypercapnia (as discussed above) can influence the bioaccumulation of metals (White and Rainbow, 1984; Houllbrèque et al., 2011), and can thus enhance their toxicity (e.g. Pascal et al., 2010; Roberts et al., 2013).

This study aims at assessing the bioaccumulation behaviours of radionuclides/metals in a sessile species of economic importance, the Pacific cupped oyster, *Crassostrea gigas*, usually cultured in coastal areas subjected to metal contamination and high  $p\text{CO}_2$  variations (e.g. Green et al., 2009). Radiotracer techniques were used to determine uptake and depuration parameters of 6 elements during experimental exposures of oysters to dissolved radiotracers ( $^{110\text{m}}\text{Ag}$ ,  $^{241}\text{Am}$ ,  $^{109}\text{Cd}$ ,  $^{57}\text{Co}$ ,  $^{54}\text{Mn}$ , and  $^{65}\text{Zn}$ ) at three pH (7.5, 7.8, 8.1) and two temperatures (21°C and 24°C) under controlled laboratory conditions (Warnau and Bustamante, 2007). *In vitro* human digestion simulations (Versantvoort et al., 2005) were also carried out to determine the bio-accessible fraction of metals and radionuclides in oysters that would be ingested by human (Metian et al., 2009a).

## MATERIALS AND METHODS

### Organisms, acclimation and experimental conditions

One hundred and sixty-two adult oysters *Crassostrea gigas* ( $17.71 \pm 3.36$  g wet wt) were purchased at the “Satmar” farm in Oleron Island, Charentes-Maritimes, France, in January 2013. At the IAEA-EL premises, the oysters were randomly distributed among six 70-L glass aquaria and acclimated for 2 weeks in 0.45- $\mu\text{m}$  filtered, UV-sterilized natural seawater (aerated open circuit 70-L aquarium; seawater flux:  $70 \text{ L h}^{-1}$ ; salinity: 38 p.s.u.; light/dark cycle: 12 h/12 h). Then, the pH and the temperature were progressively modified over one week until targeted values (see experimental procedure below) and acclimation to these targeted conditions was allowed for at least 15 days prior to the radiotracers exposure. During the whole acclimation period, oysters were fed daily with a mixt algal diet composed of *Isochrysis galbana* and *Skeletonema costatum*.

The oysters were maintained under controlled temperature and pH in a crossed experimental design (2 temperatures x 3 pH levels). Due to technical constraints (mainly in term of radiotracer cost and waste water management) only one 70-L tank could be dedicated per  $p\text{CO}_2$  – temperature condition. In order to avoid other variation in other factors (e.g. light) the tanks were randomly interspersed on the bench. In addition seawater was very frequently renewed (every one to two days) and tanks were cleaned at each seawater renewal to prevent any “tank” effect due to the development of different biomasses or to the accumulation of detritus such as pseudo faeces, or bacterial proliferation, which might affect the metabolism of oysters or the bioavailability of the chemicals. The 3 selected pH values were 8.10 (~400  $\mu\text{atm}$ ), 7.80 (~800  $\mu\text{atm}$ ), 7.50 (~1800  $\mu\text{atm}$ ) and the two temperatures were 21°C and 24°C in accordance to the projected values over the next two centuries (Orr et al., 2005; Solomon et al., 2007; current condition: pH 8.1 – 21°C; estimated conditions by 2100: pH 7.8 - 24°C; estimated conditions by 2200: pH 7.5 – 24°C). The pH was controlled in each aquarium to

within  $\pm 0.05$  pH unit with a continuous pH-stat system (IKS, Aquastar©) that bubbled pure CO<sub>2</sub> into the tanks that were continuously aerated with CO<sub>2</sub>-free air (see Lacoue-Labarthe et al., 2012). The pH values of the pH-stat system were calibrated every week from measurements of pH on the total scale, using Tris/HCl buffer solution with a salinity of 38 p.s.u. and prepared according to Dickson et al. (Dickson et al., 2007). The  $p\text{CO}_2$  was determined from pH and total alkalinity using the R package seacarb (Gattuso et al., 2015).

### **Radiotracer exposures.**

The oysters were exposed for 21 days to dissolved radiotracers: <sup>110m</sup>Ag [as <sup>110m</sup>AgNO<sub>3</sub>; T<sub>1/2</sub> = 250 d], <sup>241</sup>Am [as <sup>241</sup>AmNO<sub>3</sub>; T<sub>1/2</sub> = 432 y], <sup>109</sup>Cd [as <sup>109</sup>CdCl<sub>2</sub>; T<sub>1/2</sub> = 464 d], <sup>57</sup>Co [as <sup>57</sup>CoCl<sub>2</sub>; T<sub>1/2</sub> = 272 d], <sup>54</sup>Mn [as <sup>54</sup>MnCl<sub>2</sub>; T<sub>1/2</sub> = 312 d] and <sup>65</sup>Zn [as <sup>65</sup>ZnCl<sub>2</sub>; T<sub>1/2</sub> = 244 d]. Uptake kinetics were followed during that period, after which seawater was no longer spiked in order to assess the metal retention capacities of the oysters.

During the exposure period, the seawater of each 70-L tank was spiked with typically 5  $\mu\text{L}$  of radioactive stock solution (<sup>110m</sup>Ag, dissolved in 0.1 M HCl, <sup>241</sup>Am in 1 M HCl, <sup>109</sup>Cd in 0.5 M HCl, <sup>57</sup>Co 0.1 M in HCl, <sup>54</sup>Mn in 0.5 M HCl and <sup>65</sup>Zn in 0.5 M HCl). Seawater and spikes were renewed daily during the first week and then every second day to maintain good water quality and radiotracer concentrations as constant as possible (Metian et al., 2008) as metal could be removed from seawater medium through absorption (in organisms) or adsorption on surface (e.g. aquarium glasses). For water renewal, each tank was emptied and then, immediately slowly refilled (30 min for  $\sim 60$  L) with new seawater at the required temperature. The slow refill allowed for immediate regulation of the targeted  $p\text{CO}_2$  level by the IKS system. Before renewal of the seawater, the oysters were fed with the algal diet for 30



minutes, after which time the new seawater was spiked with the required volumes of the radioactive stock solutions.

Activity of the radiotracers in seawater was checked before and after each spike renewal, yielding for the time-integrated activities in seawater for the 6 radiotracers shown in Table 1.

Five oysters from each aquarium were identified (tagged), weighted and whole-body radioanalyzed alive (same individuals each time) daily during the first week, and then every second day in order to follow the radiotracers uptake kinetics. During the counting sessions, oysters were held out of the seawater as briefly as possible (typically 10 to 20 minutes). At the end of the 21-d exposure period, 3 individuals (not belonging to the tag-identified batch) from each tank were collected and dissected. Shell and all soft tissues were separated and radioanalyzed in order to assess the distribution of radiotracers between these two compartments.

The remaining exposed oysters were placed in non-contaminated conditions (open-circuit 70-L tank with same salinity, temperature, pH and light: dark conditions as previously indicated) for 38 days. Flowing seawater was adjusted in order to maintain pH values as constant as possible (seawater flux:  $70 \text{ L h}^{-1}$ ). The five tag-identified oysters in each tank were radioanalyzed every day during the first week and then every second day in order to follow the depuration kinetics of the radiotracers. Three individuals from each tank were collected at the end of the depuration period and dissected for radio-analysis as described above.

### ***In vitro* digestion simulation.**

At the end of the exposure and depuration periods, three oysters were collected in each tank in order to perform *in vitro* simulated digestion (Versantvoort et al., 2005) in order to assess the bioaccessible fraction of elements for human consumers of oysters. The method consists of a three-step procedure simulating quite closely the human digestion processes. Homogenized oyster tissues were step by step exposed to artificial saliva, gastric juice and mixture of

duodenal juice, bile and NaHCO<sub>3</sub>(chemicals and enzymes were purchased from Sigma®). Following the *in vitro* digestion, the resulting chyme was centrifuged and the radiotracers activities in supernatant, considered as the bioaccessible element fraction, were counted. The detailed procedure has been previously described by Metian and colleague(Metian et al., 2009a).

### **Radioanalyses and data treatments**

The radiotracers were counted using a high-resolution  $\gamma$ -spectrometry system consisting of five coaxial Germanium (N- or P-type) detectors (EGNC 33-195-R, Canberra® and Eurysis®) connected to a multi-channel analyzer and a computer equipped with a spectra analysis software (Interwinner® 6). The radioactivity of the samples (whole-body oysters, seawater, soft tissues, and supernatant and pellets) was determined by comparison with standards of known activities and appropriate geometries and was corrected for background and physical decay of the radiotracers. The counting time was adjusted to obtain a propagated counting error of less than 5% (Rodriguez y Baena et al., 2006).

The uptake of radiotracer was expressed as change in concentration factors (CF), which is the ratio between radiotracer activity in the whole-body oyster (Bq g<sup>-1</sup>) and the time-integrated radiotracer activity in seawater (Bq g<sup>-1</sup>) over time. Uptake kinetics were fitted using either a linear equation (Eq. 1) or a saturation exponential equation (Eq. 2):

$$CF_t = k_u t \quad (\text{Eq. 1})$$

$$CF_t = CF_{ss} (1 - e^{-k_e t}) \quad (\text{Eq. 2})$$

where CF<sub>t</sub> and CF<sub>ss</sub>(CF<sub>ss</sub> = k<sub>u</sub> / k<sub>e</sub>) are the concentration factors at time t (d) and at steady-state, respectively, and k<sub>e</sub> and k<sub>u</sub> are the biological depuration and uptake rate constants (d<sup>-1</sup>), respectively(Whicker and Schultz, 1982).

Radiotracer depuration kinetics were expressed in terms of change in percentage of remaining

activity (i.e., radioactivity at time  $t$  divided by the initial radioactivity measured in the individual at the beginning of the depuration period \* 100) with time. The depuration kinetics were fitted by a simple exponential model (Eq. 3):

$$A_t = A_0 e^{-k_e t} \text{ (Eq. 3)}$$

where  $A_t$  and  $A_0$  are the remaining activities at time  $t$  (d) and 0, respectively,  $k_e$  is the biological depuration rate constant ( $d^{-1}$ ) (Warnau et al., 1996). The biological half-life (d) of the radiotracer can then be calculated according to the relation (Eq. 4):

$$T_{b1/2} = \ln 2 / k_e \text{ (Eq. 4)}$$

All statistics and graphics were performed using R freeware (R Core Team, 2014). Model constants and their statistics were estimated by iterative adjustment of the model using linear and non-linear mixed effect models (*lme* and *nlme* functions from the package “nlme”; (Pinheiro et al., 2014), in which individual oyster identity had been considered a random factor. Marginal  $R^2$  representing the variance explained by the fixed factor (*i.e.* time) has been applied to linear models using the *r.squaredGLMM* function (package “MuMIn”; Bartoń, 2014). Comparison of uptake and elimination constant rates among the different pH/temperature conditions was performed using two-way ANOVA on  $k_u$  and  $k_e$  calculated for each individual oyster (the best fitting model obtained for the entire set of oysters was applied to individuals). A Chi-square test was used to compare bioaccessible fractions of metals among the different oyster groups. The level of significance for statistical analyses was always set at  $\alpha = 0.05$ .

## RESULTS

During the whole experimental period (*i.e.* three and five weeks of uptake and depuration phase, respectively) where the oysters were exposed to six different conditions (combinations of 2 temperatures and 3 pH; see Materials and Methods), limited growth of individual (< 1%) was measured and mortality was relatively high (18.5-29.6% according to tank) although no connexion between mortality and specific exposure condition could be detected. Specific mortality of oysters with condition of exposure were the following: 18.5% for 7.5 (pH) - 21°C (temperature), 26% for 7.8 - 21°C, 26% for 8.1 - 21°C, 29.6% for 7.5 - 24°C, 18.5% for 7.8 - 24°C, and 26% for 8.1 - 24°C.

The whole-body bioaccumulation kinetics of  $^{110m}\text{Ag}$ ,  $^{241}\text{Am}$ ,  $^{109}\text{Cd}$ ,  $^{57}\text{Co}$ ,  $^{54}\text{Mn}$ , and  $^{65}\text{Zn}$  in oysters and their respective model parameters are shown in Figure 1 and Table 2. Uptake of all elements except  $^{241}\text{Am}$  were best fitted by linear models. The actinide displayed an exponential uptake pattern, tending to reach a steady-state value ranging between 360 and 470 according to pH – temperature conditions (Table 2) after 3 weeks of exposure. At the end of the exposure period and whatever the pH/temperature conditions, the estimated uptake rate constant ( $k_u$ ) allowed ranking radiotracers taken up linearly according to their bioaccumulation capacities:  $^{110m}\text{Ag} > ^{65}\text{Zn} > ^{54}\text{Mn} > ^{109}\text{Cd} \sim ^{57}\text{Co}$ .

At the end of the exposure period, oysters were placed in non-contaminating conditions and depuration of radiotracers was followed for 37 days (Figure S1). The whole-body depuration kinetics of all elements were best fitted by a one-compartment exponential model (Table 3). However it was not possible to determine accurately the depuration kinetic parameters for  $^{109}\text{Cd}$  as the activity rapidly dropped below the detection limits of gamma spectrometers. All radiotracers were efficiently retained by *Crassostrea gigas* ( $T_{b1/2}$  between 50 and > 600 days).

Statistical analyses carried out on individual uptake rate constants ( $k_u$ ; two-way ANOVA;

Table 4) revealed that neither pH nor temperature significantly affected the bioaccumulation of metals in oysters (uptake and depuration biokinetics) except  $^{109}\text{Cd}$  and  $^{54}\text{Mn}$ . The highest temperature affected Cd accumulation (Figure 1, Table 4), which displayed an uptake rate constant at pH 7.5 two-fold higher at  $24^\circ\text{C}$  than at  $20^\circ\text{C}$  ( $23 \pm 1$  vs.  $11 \pm 1 \text{ d}^{-1}$ , respectively; Table 4, interactive effect pH x temperature:  $p < 0.1$ ). In the case of Mn,  $k_u$  was significantly affected by an interactive effect between seawater pH and temperature, with a decreased uptake rate constant at pH 7.5 when temperature increased ( $27 \pm 1$  vs.  $17 \pm 1 \text{ d}^{-1}$  at  $21^\circ\text{C}$  and  $24^\circ\text{C}$ , respectively; see Figure 1 and Table 4). Regarding the depuration rate constant of  $^{241}\text{Am}$ , a significant interactive effect was observed at pH 7.8 where  $T_{b1/2}$  was two-fold lower at  $24^\circ\text{C}$  than at  $21^\circ\text{C}$  (Tukey test;  $p = 0.049$ ; Table 4). Retention of Co and Mn tended also to decrease at the same pH when comparing warmer with colder temperatures (Table 4; pH x temperature;  $p < 0.1$ ).

When looking at the distribution of the radiotracers between shells and soft tissues (Table 5),  $^{241}\text{Am}$ ,  $^{57}\text{Co}$  and  $^{54}\text{Mn}$  were mainly associated with the shells ( $> 94\%$ ), whereas  $^{109}\text{Cd}$  was equally distributed between the two compartments and  $^{110\text{m}}\text{Ag}$  and  $^{65}\text{Zn}$  were mainly found in soft tissues (76-98% and 67-92%, respectively). It is also noteworthy that  $^{65}\text{Zn}$  fraction in soft tissues increased between the end of uptake period and the end of depuration period, at all pH and temperature conditions, suggesting a faster depuration of Zn associated with shells than that with soft tissues when non-contaminating conditions were restored. Neither pH nor temperature affected strongly distribution of metals and radionuclide between shells and soft tissues, except that the fraction of  $^{110\text{m}}\text{Ag}$  associated with soft tissues at the end of the uptake phase were lower in oysters maintained at  $24^\circ\text{C}$  when compared to those kept at  $21^\circ\text{C}$  ( $p < 0.05$ , Mann Whitney U test; Table 5). Finally, the radiotracer CFs reached in the oyster shells at the end of the uptake phase were not affected significantly by  $p\text{CO}_2$  conditions (Table S1).

The *in vitro* digestion simulations revealed that the bioaccessible fraction of metals and radionuclide varied from 30% ( $^{241}\text{Am}$  in oyster maintained at 24°C and pH 7.8) to more than 90% ( $^{57}\text{Co}$  in all oysters; Figure 2). Significant effects of pH were highlighted for  $^{241}\text{Am}$ ,  $^{109}\text{Cd}$ ,  $^{54}\text{Mn}$  and  $^{65}\text{Zn}$  (Table 6). For these elements, the bioaccessible fraction at the end of the uptake phase tended to increase with decreasing pH except at pH 8.1 – 24°C. At the end of the depuration phase, a similar trend was observed in oysters maintained at 24°C whereas a decrease in bioaccessibility with decreasing pH was noted for the 21°C-maintained oysters. Nevertheless, these results must be considered with caution due to the low number of oysters analysed per condition (n = 3).

## DISCUSSION

Contamination of coastal waters is a worldwide concern. Ocean acidification and thus modified chemistry of seawater may change the way contaminants will interact with marine biota. For example, it affects the chemical speciation of cations, especially those that form complexes with carbonates and potentially increases their bioavailability for biota (e.g. Byrne et al., 1988) by increasing the free-ion form in acidified conditions. Among the elements considered in the present study, only  $^{241}\text{Am}$  is known as being able to bind to carbonates but with limited change in speciation in the tested pH range, largely dominated by hydroxyl-Am form (Choppin, 2006). According to previous work and metal speciation model as a function of seawater pH, metals that are mainly bound to chloride (such as Ag and Cd) or those that are predominantly found in the free ion form (e.g. Co, Mn, Zn) are not strongly affected by decreasing pH. Indeed, the free metal ion concentrations of these elements increased from only a few percent when pH decreased from 8.1 to 7.5 (Lacoue-Labarthe et al., 2009; Millero et al., 2009). We thus assume that, for the seawater pH range tested in the context of ocean acidification, bioaccumulation would be less affected by change in metal speciation itself than by resulting change in the biological mechanisms of metal uptake and/or elimination.

In the current study, we examined the bioaccumulation patterns of six metals and one actinide in the Pacific cupped oyster *Crassostrea gigas* under elevated conditions of temperature and  $p\text{CO}_2$ , using the levels projected for the end of the current century. Like other sessile invertebrates living in estuarine, intertidal and subtidal zones, *C. gigas* is expected to be exposed to broad ranges of pH and temperatures in their habitats, potentially leading to an adaptation to a wide range of abiotic conditions (Lannig et al., 2010). For example, metabolic depression is an adaptive mechanism used by shelled intertidal molluscs to preserve energy during low tides. This physiological plasticity is time-limited and might not be adapted to long-term, continuous exposure to hypercapnic conditions. In the present study, the higher mortality

observed at the lowest pH and highest temperature may suggest that energetic balance were pushed to the edge when both stressors were combined (Lannig et al., 2010).

The distribution of radiotracers between soft tissues and shells was in accordance with previous studies:  $^{241}\text{Am}$ , Co, Mn were mainly adsorbed on the calcareous structures whereas Cd, Zn and Ag fraction associated with soft tissues was gradually increasing during the depuration period (Metian et al., 2009b; Hédouin et al., 2010; Metian et al., 2011). CFs of  $^{241}\text{Am}$ , Co and Mn in shells at the end of the uptake phase were not significantly affected by seawater  $p\text{CO}_2$  conditions, implying that the adsorption capacity of shells is not strongly affected by decreasing pH for these metals. This might be explained by the fact that 1) increasing protons concentration resulting from decreasing pH does not significantly compete with metallic cations for shell binding sites and/or 2) the binding sites are not affected by hypercapnia in the conditions tested (shells of *C. gigas* -mainly made of calcite- are considered less vulnerable to calcareous dissolution than aragonitic shells; (Gazeau et al., 2007).

The main result of our study is that no effect of increasing  $p\text{CO}_2$  and temperature has been detected on Ag, Cd and Zn bioaccumulation capacities in *C. gigas* soft tissues. Only few studies have investigated so far the impact of warming and hypercapnia on metal accumulation and/or their effects on marine organisms (Lacoue-Labarthe et al., 2009; Pascal et al., 2010; Lacoue-Labarthe et al., 2011; Lacoue-Labarthe et al., 2012; Ivanina et al., 2013; Roberts et al., 2013; Ivanina et al., 2014) and contrasting responses (metal- or species-dependent) were observed. For instance, Cd accumulation was lowered in cuttlefish embryos and Cd toxicity decreased in benthic copepod with decreasing  $p\text{CO}_2$  (Lacoue-Labarthe et al., 2009; Pascal et al., 2010) presumably due to increasing competition between metallic cations and protons for biological binding sites. Similarly, a lower Cd uptake was observed in mantle cells of clams *Mercenaria mercenaria*, supposedly due to a decrease in transmembrane channel activities with metabolic depression of clams in short-term hypercapnic conditions (Ivanina et al., 2013). In



contrast, we noted in this study a higher Cd uptake in *C. gigas* when oysters were exposed to pH 7.5 (~1700  $\mu\text{atm}$ ) and 24°C for three weeks. Similar observation of enhanced Cd bioaccumulation in *C. virginica* maintained at pH 7.8 (~ 800  $\mu\text{atm}$ ) during 4-5 weeks were reported by Götze and co-authors (Götze et al., 2014). The mechanistic explanation of this Cd bioaccumulation modulation remains unclear. However it is noteworthy that, in contrast to others bivalves that maintain a comparatively constant internal pH by decreasing their metabolic rates and/or dissolving their shell (Berge et al., 2006), *C. gigas* was shown to have elevated standard metabolic rate under hypercapnia when exposed to temperature stress (Lannig et al., 2010). This in turn could increase Cd ion uptake in oyster tissues.

Interestingly, it has been previously shown that the non-essential element Ag is more efficiently accumulated, up to 2.5 fold, in embryos and larvae of cuttlefish and squid when exposed to elevated  $p\text{CO}_2$  ranged from 600 to 1500  $\mu\text{atm}$  (Lacoue-Labarthe et al., 2009; Lacoue-Labarthe et al., 2011). This higher Ag uptake might be due to the Na mimetic properties of this element and caused by the enhanced acid-base regulation combined to the ionic regulation machinery. Indeed the  $\text{Na}^+/\text{H}^+$ -exchanger, an ionic channel, is expected involved in Ag uptake (Webb and Wood, 2000; Grosell and Wood, 2001) in mobile organisms when they are exposed to environmental hypercapnia (Melzner et al., 2009; Hu et al., 2011; Hu et al., 2014). In oysters, no significant change of Ag uptake was observed in soft tissues as a consequence of change in seawater  $p\text{CO}_2$ . This is congruent with the less-developed ion-exchange machinery in sessile invertebrates, and their subsequent limited ion regulation capacities (Lannig et al., 2010; Gazeau et al., 2013). This limited physiological capacity to compensate environmental acidosis might also explain the absence of modulation of the Zn bioaccumulation in soft tissues according to  $p\text{CO}_2$  conditions. Indeed, Zn is a co-factor of the carbonic anhydrase (CA) that catalyses the conversion of  $\text{CO}_2$  to bicarbonate and vice versa, and that is involved in the acid-base regulation and calcification in shelled mollusc (Gazeau et

al., 2013). Beniash and co-authors (2010) have reported an increase in CA expression levels in the mantle of *C. virginica* exposed to 3500  $\mu\text{atm}$ , but the latter partial pressure was much higher than those tested in the present study.

Oysters are a very important marine resource and constitute a major source of animal protein for humans. They are also rich in essential elements such as Fe and Zn (Tacon and Metian, 2013). Preliminary experiments were carried out to determine whether lower pH and higher temperatures may affect metal and radionuclide bioaccessibility to human consumers. Results indicated that  $^{241}\text{Am}$ , Mn, and Zn bioaccessibility tended to be enhanced when oysters were exposed to high temperature and lower pH. Although these results have to be considered with caution, they raise the question of the possible impact of environmental hypercapnia on metal detoxification mechanisms in oysters and subsequent subcellular storage of elements. Oysters are well known to accumulate efficiently trace elements such as Zn in soft tissues in the form of metabolically inert, metal-rich granules (e.g. Wallace and Luoma, 2003). These Mg/Ca carbonate granules are known to have the physiological role of buffering the extracellular pH (Viarengo and Nott, 1993) and might thus be easily dissolved when oysters are experiencing hypercapnia. In this context, the increased seawater  $p\text{CO}_2$  is susceptible to affect the metal detoxification strategies and thus the Trophically Available Fraction (TAM; Wallace and Luoma, 2003) that drives the bioaccessibility and transfer of metals to higher trophic levels.

Finally, the present study brought new data on the potential impact of ocean acidification on metal accumulation in a marine resource of high economic importance and raised the question of the seafood safety that could be worsened by future ocean conditions. Despite the limited number of oysters, the experimental design offered enough statistical power (with power = 90%,  $n=5$ , standard deviation = sd max in Table 2) to detect a putative 2-fold Ag uptake increase and a 3-fold Cd uptake decrease and corroborate previous observations made in active (mobile) organisms (Lacoue-labarthe et al., 2009, 2011). We therefore assume that our

results bring a new insight on the potential effect of environmental factors such as combined  $p\text{CO}_2$  and temperature on the transfer and integration of metals in coastal organisms.

However, our experimental approach(oysters were acclimated to temperature and  $p\text{CO}_2$  conditions for two weeks before the experiments) implies that the responses observed with respect to metal accumulation efficiency reflected oyster physiological acclimation performances. They are therefore relevant to a coastal or estuarine context where high and short-term variations of these factors do occur(Melzner et al., 2013). Hence our results do not allow figuring out what could be the impact of ocean acidification and warming over longer timescale, during which adaptation processes will set up. Further studies should be carried out to highlight finer modulations of metal uptake at tissue and subcellular levels, taking into account the high variability of responses between oyster populations (Parker et al., 2011), and the adaptive capacities of organisms facing long-term major changes.

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## References

- Barton, A., Hales, B., Waldbusser, G. G., Langdon, C., and Feely, R. A. 2012. The Pacific oyster, *Crassostrea gigas*, shows negative correlation to naturally elevated carbon dioxide levels: Implications for near-term ocean acidification effects. *Limnology and Oceanography*, 57: 698-710.
- Bartoń, K. 2014. MuMIn: Multi-Model Inference. R package version 1.12.1.
- Beniash, E., Ivanina, A., Lieb, N. S., Kurochkin, I., and Sokolova, I. M. 2010. Elevated level of carbon dioxide affects metabolism and shell formation in oysters *Crassostrea virginica*. *Marine Ecology Progress Series*, 419: 95-108.
- Berge, J. A., Bjerkgeng, B., Pettersen, O., Schaanning, M. T., and Øxnevad, S. 2006. Effects of increased sea water concentrations of CO<sub>2</sub> on growth of the bivalve *Mytilus edulis* L. *Chemosphere*, 62: 681-687.
- Byrne, R. H., Kump, L. R., and Cantrell, K. J. 1988. The influence of temperature and pH on trace metal speciation in seawater. *Marine Chemistry*, 25: 163-181.
- Choppin, G. R. 2006. Actinide speciation in aquatic systems. *Marine Chemistry*, 99: 83-92.
- Dickson, A. G., Sabine, C. L., and Christian, J. R. 2007. Determination of the pH of sea water using a glass / reference electrode cell. *In* Guide to best practices for ocean CO<sub>2</sub> measurements, pp. 1-7. Ed. by A. G. Dickson, C. L. Sabine, and J. R. Christian. PICES Special Publication 3.
- Doney, S. C., Fabry, V. J., Feely, R. A., and Kleypas, J. 2009. Ocean acidification: the other CO<sub>2</sub> problem. *Annual Review of Marine Science*, 1: 169-192.
- Dupont, S., Dorey, N., and Thorndyke, M. 2010. What meta-analysis can tell us about vulnerability of marine biodiversity to ocean acidification? *Estuarine Coastal and Shelf Science*, 89: 182-185.
- Gattuso, J.-P., Epitalon, J.-M., and Lavigne, H. 2015. seacarb: Seawater Carbonate Chemistry. R package version 3.0.5.
- Gazeau, F., Gattuso, J.-P., Greaves, M., Elderfield, H., Peene, J., Heip, C. H. R., and Middelburg, J. J. 2011. Effect of carbonate chemistry alteration on the early embryonic development of the Pacific oyster (*Crassostrea gigas*). *PLoS ONE*, 6: e23010.

- Gazeau, F., Parker, L. M., Comeau, S., Gattuso, J.-P., O'Connor, W. A., Martin, S., Pörtner, H.-O., et al. 2013. Impacts of ocean acidification on marine shelled molluscs. *Marine Biology* 160: 2207–2245.
- Gazeau, F., Quiblier, C., Jansen, J. M., Gattuso, J. P., Middelburg, J. J., and Heip, C. H. R. 2007. Impact of elevated CO<sub>2</sub> on shellfish calcification. *Geophysical Research Letters*, 34.
- Götze, S., Matoo, O. B., Beniash, E., Saborowski, R., and Sokolova, I. M. 2014. Interactive effects of CO<sub>2</sub> and trace metals on the proteasome activity and cellular stress response of marine bivalves *Crassostrea virginica* and *Mercenaria mercenaria*. *Aquatic Toxicology*, 149: 65-82.
- Green, M. A., Waldbusser, G. G., Reilly, S. L., Emerson, K., and O'Donnella, S. 2009. Death by dissolution: Sediment saturation state as a mortality factor for juvenile bivalves. *Limnology and Oceanography*, 54: 1037-1047.
- Grosell, M., and Wood, C. M. 2001. Branchial versus intestinal silver toxicity and uptake in the marine teleost *Parophrys vetulus*. *Journal of Comparative Physiology*, 171B: 585-594.
- Hédouin, L., Metian, M., Teyssié, J.-L., Fichez, R., and Warnau, M. 2010. Delineation of heavy metal contamination pathways (seawater, food and sediment) in tropical oysters from New Caledonia using radiotracer techniques. *Marine Pollution Bulletin*, 61: 542-553.
- Houlbrèque, F., Rodolfo-Metalpa, R., Jeffree, R., Oberhänsli, F., Teyssié, J.-L., Boisson, F., Al-Trabeen, K., et al. 2011. Effects of increased pCO<sub>2</sub> on zinc uptake and calcification in the tropical coral *Stylophora pistillata*. *Coral Reefs*, 31: 101-109.
- Hu, M. Y., Guh, Y.-J., Stumpp, M., Lee, J.-R., Chen, R.-D., Sung, P.-H., Chen, Y.-C., et al. 2014. Branchial NH<sub>4</sub><sup>+</sup>-dependent acid–base transport mechanisms and energy metabolism of squid (*Sepioteuthis lessoniana*) affected by seawater acidification. *Frontiers in Zoology*, 11: 55.
- Hu, M. Y., Tseng, Y.-C., Stumpp, M., Gutowska, M. A., Kiko, R., Lucassen, M., and Melzner, F. 2011. Elevated seawater PCO<sub>2</sub> differentially affects branchial acid-base transporters over the course of development in the cephalopod *Sepia officinalis*. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 300: R1100-R1114.
- IPCC 2013. Climate Change 2013: The Physical Science Basis. *In* Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.
- Ivanina, A. V., Beniash, E., Etkorn, M., Meyers, T. B., Ringwood, A. H., and Sokolova, I. M. 2013. Short-term acute hypercapnia affects cellular responses to trace metals in the hard clams *Mercenariamercenaria*. *Aquatic Toxicology*, 140-141: 123-133.
- Ivanina, A. V., Hawkins, C., and Sokolova, I. M. 2014. Immunomodulation by the interactive effects of cadmium and hypercapnia in marine bivalves *Crassostreavirginica* and *Mercenariamercenaria*. *Fish & Shellfish Immunology*, 37: 299-312.
- Lacoue-Labarthe, T., Martin, S., Oberhänsli, F., Teyssié, J.-L., Jeffree, R., Gattuso, J.-P., and Bustamante, P. 2012. Temperature and pCO<sub>2</sub> effect on the bioaccumulation of radionuclides and trace elements in the eggs of the common cuttlefish *Sepia officinalis* *Journal of Experimental Marine Biology and Ecology*, 413: 45-49.
- Lacoue-Labarthe, T., Martin, S., Oberhänsli, F., Teyssié, J. L., Markich, S. J., Jeffree, R., and Bustamante, P. 2009. Effects of increased pCO<sub>2</sub> and temperature on trace element (Ag, Cd and Zn) bioaccumulation in the eggs of the common cuttlefish, *Sepia officinalis*. *Biogeosciences*, 6: 2561-2573.
- Lacoue-Labarthe, T., Réveillac, E., Oberhänsli, F., Teyssié, J.-L., Jeffree, R., and Gattuso, J.-P. 2011. Effects of ocean acidification on trace element accumulation in the early-life stages of squid *Loligo vulgaris*. *Aquatic Toxicology*, 105: 166-176.
- Lannig, G., Eilers, S., Pörtner, H. O., Sokolova, I. M., and Bock, C. 2010. Impact of ocean acidification on energy metabolism of oyster, *Crassostreagigas* - changes in metabolic pathways and thermal response. *Marine Drugs*, 8: 2318-2339.
- Melzner, F., Gutowska, M. A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M. C., Bleich, M., et al. 2009. Physiological basis for high CO<sub>2</sub> tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? *Biogeosciences*, 6: 2313-2331.

- Melzner, F., Thomsen, J., Koeve, W., Oschlies, A., Gutowska, M. A., Bange, H. W., Hansen, H. P., et al. 2013. Future ocean acidification will be amplified by hypoxia in coastal habitats. *Marine Biology* 160: 1875-1888.
- Metian, M., Bustamante, P., Cosson, R. P., Hédouin, L., and Warnau, M. 2008. Investigation of Ag in the king scallop *Pectenmaximus* using field and laboratory approaches. *Journal of Experimental Marine Biology and Ecology*, 367: 53-60.
- Metian, M., Charbonnier, L., Oberhänsli, F., Bustamante, P., Jeffree, R., Amiard, J. C., and Warnau, M. 2009a. Assessment of metal, metalloid, and radionuclide bioaccessibility from mussels to human consumers, using centrifugation and simulated digestion methods coupled with radiotracer techniques. *Ecotoxicology and Environmental Safety*, 72: 1499-1502.
- Metian, M., Warnau, M., Hédouin, L., and Bustamante, P. 2009b. Bioaccumulation of essential metals (Co, Mn and Zn) in the king scallop *Pectenmaximus*: seawater, food and sediment exposures. *Marine Biology* 156: 2063-2075.
- Metian, M., Warnau, M., Teyssié, J.-L., and Bustamante, P. 2011. Characterization of <sup>241</sup>Am and <sup>134</sup>Cs bioaccumulation in the king scallop *Pectenmaximus*: investigation via three exposure pathways. *Journal of Environmental Radioactivity*, 102: 543-550.
- Millero, F. J., Woosley, R., Ditrolo, B., and Waters, J. 2009. Effect of ocean acidification on the speciation of metals in seawater. *Oceanography*, 22: 72-85.
- Parker, L. M., Ross, P. M., and O'Connor, W. A. 2010. Comparing the effect of elevated *p*CO<sub>2</sub> and temperature on the fertilization and early development of two species of oysters. *Marine Biology* 157: 2435-2452.
- Parker, L. M., Ross, P. M., and O'Connor, W. A. 2011. Populations of the Sydney rock oyster, *Saccostreaglomerata*, vary in response to ocean acidification. *Marine Biology* 158: 689-697.
- Parker, L. M., Ross, P. M., O'Connor, W. A., Pörtner, H.-O., Scanes, E., and Wright, J. M. 2013. Predicting the response of molluscs to the impact of ocean acidification. *Biology*, 2: 651-692.
- Pascal, P.-Y., Fleeger, J. W., Galvez, F., and Carman, K. R. 2010. The toxicological interaction between ocean acidity and metals in coastal meiobenthic copepods. *Marine Pollution Bulletin*, 60: 2201-2208.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team 2014. {nlme}: Linear and Nonlinear Mixed Effects Models. R package version 3.1-118.
- Pörtner, H. O., Langenbuch, M., and Reipschläger, A. 2004. Biological impact of elevated ocean CO<sub>2</sub> concentrations: lessons from animal physiology and earth history. *Journal of Oceanography*, 60: 705-718.
- R Core Team 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rainbow, P. S. 2002. Trace metal concentrations in aquatic invertebrates: why and so what? *Environmental Pollution*, 120: 497-507.
- Roberts, D. A., Birchenough, S. R., Lewis, C., Sanders, M., Bolam, T., and Sheahan, D. 2013. Ocean acidification increases the toxicity of contaminated sediments. *Global Change Biology*, 19: 340-351.
- Rodriguez y Baena, A. M., Metian, M., Teyssie, J. L., De Broyer, C., and Warnau, M. 2006. Experimental evidence for <sup>234</sup>Th bioaccumulation in three Antarctic crustaceans: Potential implications for particle flux studies. *Marine Chemistry*, 100: 354-365.
- Tacon, A. G. J., and Metian, M. 2013. Fish Matters: Importance of Aquatic Foods in Human Nutrition and Global Food Supply. *Reviews in Fisheries Science*, 21: 22-38.
- Timmins-Schiffman, E., O'Donnell, M. J., Friedman, C. S., and Roberts, S. B. 2013. Elevated *p*CO<sub>2</sub> causes developmental delay in early larval Pacific oysters, *Crassostrea gigas*. *Marine Biology* 160: 1973-1982.
- Versantvoort, C. H. M., Oomen, A. G., Van de Kamp, E., Rompelberg, C. J. M., and Sips, A. J. A. M. 2005. Applicability of an in vitro digestion model in assessing the bioaccessibility of mycotoxins from food. *Food and Chemical Toxicology*, 43: 31-40.

- Viarengo, A., and Nott, J. A. 1993. Mechanisms of heavy metal cation homeostasis in marine invertebrates. *Comparative Biochemistry and Physiology*, 104C: 355-372.
- Wallace, W. G., and Luoma, S. N. 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. II. Significance of trophically available metal (TAM). *Marine Ecology Progress Series*, 257: 125-137.
- Warnau, M., and Bustamante, P. 2007. Radiotracer techniques: A unique tool in marine ecotoxicological studies. *Environmental Bioindicators*, 2: 217-218.
- Warnau, M., Teyslié, J.-L., and Fowler, S. W. 1996. Biokinetics of selected heavy metals and radionuclides in the common Mediterranean echinoid *Paracentrotus lividus*: sea water and food exposures. *Marine Ecology Progress Series*, 141: 83-94.
- Webb, N. A., and Wood, C. M. 2000. Bioaccumulation and distribution of silver in four marine teleosts and two marine elasmobranchs: influence of exposure duration, concentration, and salinity. *Aquatic Toxicology*, 49: 111-129.
- Whicker, F. W., and Schultz, V. 1982. *Radioecology : nuclear energy and the environment*, CRC Press, Boca Raton, FL.
- White, S. L., and Rainbow, P. S. 1984. Regulation of zinc concentration by *Palaemon elegans* (Crustacea:Decapoda): zinc flux and effects of temperature, zinc concentration and moulting. *Marine Ecology Progress Series*, 16: 135-147.
- Zhou, Q., Zhang, J., Fu, J., Shi, J., and Jiang, G. 2008. Biomonitoring: An appealing tool for assessment of metal pollution in the aquatic ecosystem. *Analytica Chimica Acta*, 606: 135-150.

**Table 1.** Carbonate system parameters during the uptake and depuration phase of the experiment on the bioaccumulation of metal and radionuclide in oyster exposed. The indicative average partial pressure of CO<sub>2</sub> (*p*CO<sub>2</sub>) were calculated for a seawater salinity of 38 and a total alkalinity of 2540 μmol.kg<sup>-1</sup> average.

Experiment phase	Temperature (°C)	pH <sub>T</sub>	<i>p</i> CO <sub>2</sub> (μatm)	<sup>110m</sup> Ag (Bq.ml <sup>-1</sup> )	<sup>241</sup> Am (Bq.ml <sup>-1</sup> )	<sup>109</sup> Cd (Bq.ml <sup>-1</sup> )	<sup>57</sup> Co (Bq.ml <sup>-1</sup> )	<sup>54</sup> Mn (Bq.ml <sup>-1</sup> )	<sup>65</sup> Zn (Bq.ml <sup>-1</sup> )
<i>Uptake</i>	20.9 ± 0.9	7.43 ± 0.03	2161 ± 177	0.06 ± 0.05	0.15 ± 0.08	0.28 ± 0.05	0.44 ± 0.09	0.25 ± 0.08	0.38 ± 0.12
	20.8 ± 0.9	7.79 ± 0.03	854 ± 54	0.06 ± 0.05	0.14 ± 0.07	0.25 ± 0.02	0.43 ± 0.09	0.21 ± 0.10	0.37 ± 0.13
	20.8 ± 0.8	8.03 ± 0.02	451 ± 27	0.06 ± 0.04	0.13 ± 0.07	0.26 ± 0.03	0.44 ± 0.09	0.19 ± 0.11	0.36 ± 0.12
	23.7 ± 0.2	7.52 ± 0.03	1732 ± 125	0.07 ± 0.03	0.14 ± 0.07	0.27 ± 0.03	0.44 ± 0.08	0.23 ± 0.09	0.39 ± 0.11
	24.0 ± 0.1	7.88 ± 0.08	699 ± 157	0.07 ± 0.04	0.14 ± 0.08	0.25 ± 0.03	0.42 ± 0.11	0.18 ± 0.12	0.38 ± 0.11
	24.0 ± 0.1	8.08 ± 0.05	396 ± 63	0.07 ± 0.04	0.15 ± 0.08	0.28 ± 0.05	0.45 ± 0.09	0.19 ± 0.12	0.38 ± 0.12
<i>Loss</i>	21.6 ± 0.1	7.45 ± 0.04	2023 ± 180	-	-	-	-	-	-
	21.6 ± 0.1	7.85 ± 0.03	742 ± 61	-	-	-	-	-	-
	21.2 ± 0.2	8.03 ± 0.01	444 ± 16	-	-	-	-	-	-
	24.0 ± 0.2	7.54 ± 0.11	1689 ± 355	-	-	-	-	-	-
	23.8 ± 0.1	7.79 ± 0.07	867 ± 149	-	-	-	-	-	-
	24.0 ± 0.1	8.06 ± 0.04	413 ± 50	-	-	-	-	-	-



1 **Table 2.** Parameters of the uptake kinetics of  $^{110m}\text{Ag}$ ,  $^{241}\text{Am}$ ,  $^{109}\text{Cd}$ ,  $^{57}\text{Co}$ ,  $^{54}\text{Mn}$ ,  $^{65}\text{Zn}$  in oysters exposed for 21 days to dissolved radiotracers under three pHs and two  
 2 temperatures conditions.  
 3

Element	Temp (°C)	pH	Model	$k_u \pm \text{SE}$	$k_e \pm \text{SE}$	$\text{CF}_{ss} \pm \text{SE}$	Marginal $R^2$	$\text{CF}_{21d}$	4
$^{110m}\text{Ag}$	21	7.5	L	$123.0 \pm 6.4$	-	-	0.755	$2661 \pm 1003$	5
		7.8	L	$118.2 \pm 7.9$	-	-	0.644	$2549 \pm 1273$	6
		8.1	L	$146.5 \pm 8.2$	-	-	0.719	$3141 \pm 1198$	7
	24	7.5	L	$68.2 \pm 6.2$	-	-	0.443	$1587 \pm 1092$	8
		7.8	L	$146.1 \pm 11.0$	-	-	0.610	$2844 \pm 1410$	9
		8.1	L	$110.5 \pm 9.2$	-	-	0.556	$2236 \pm 1409$	10
$^{241}\text{Am}$	21	7.5	E	$30.4 \pm 5.3$	$0.078 \pm 0.011$	$390 \pm 39$	-	$281 \pm 513$	11
		7.8	E	$31.9 \pm 5.5$	$0.078 \pm 0.011$	$407 \pm 39$	-	$330 \pm 484$	12
		8.1	E	$30.2 \pm 4.7$	$0.074 \pm 0.008$	$408 \pm 48$	-	$329 \pm 515$	13
	24	7.5	E	$45.7 \pm 8.0$	$0.127 \pm 0.019$	$362 \pm 32$	-	$311 \pm 1027$	14
		7.8	E	$24.8 \pm 5.3$	$0.052 \pm 0.010$	$474 \pm 33$	-	$314 \pm 718$	15
		8.1	E	$30.3 \pm 4.1$	$0.085 \pm 0.008$	$357 \pm 33$	-	$315 \pm 489$	16
$^{109}\text{Cd}$	21	7.5	L	$12.5 \pm 0.5$	-	-	0.887	$272 \pm 320$	17
		7.8	L	$13.2 \pm 0.8$	-	-	0.726	$272 \pm 642$	18
		8.1	L	$10.5 \pm 0.8$	-	-	0.642	$209 \pm 703$	19
	24	7.5	L	$22.8 \pm 1.0$	-	-	0.852	$504 \pm 1204$	20
		7.8	L	$10.2 \pm 1.1$	-	-	0.502	$216 \pm 1005$	21
		8.1	L	$14.7 \pm 1.2$	-	-	0.545	$295 \pm 1026$	22
$^{57}\text{Co}$	21	7.5	L	$16.9 \pm 0.6$	-	-	0.895	$332 \pm 828$	23
		7.8	L	$12.6 \pm 0.6$	-	-	0.827	$266 \pm 729$	24
		8.1	L	$10.7 \pm 0.6$	-	-	0.753	$226 \pm 850$	25
	24	7.5	L	$12.0 \pm 0.7$	-	-	0.725	$244 \pm 1042$	26
		7.8	L	$14.9 \pm 0.3$	-	-	0.966	$305 \pm 383$	27
		8.1	L	$12.4 \pm 0.4$	-	-	0.882	$261 \pm 584$	28
								29	
								30	
								31	
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								35	

36 **Table 2** (continued)

37

Element	Temp (°C)	pH	Model	$k_u \pm SE$	$k_e \pm SE$	$CF_{ss} \pm SE$	Marginal $R^2$	$CF_{21d}$	
<sup>54</sup> Mn	21	7.5	L	$26.8 \pm 0.9$	-	-	0.900	$518 \pm 124$	
		7.8	L	$22.5 \pm 0.9$	-	-	0.829	$468 \pm 140$	
		8.1	L	$18.1 \pm 0.8$	-	-	0.792	$391 \pm 133$	
	24	7.5	L	$16.7 \pm 0.8$	-	-	0.796	$341 \pm 114$	
		7.8	L	$22.8 \pm 0.7$	-	-	0.917	$473 \pm 76$	
		8.1	L	$20.1 \pm 0.6$	-	-	0.900	$430 \pm 76$	
	<sup>65</sup> Zn	21	7.5	L	$34.7 \pm 1.0$	-	-	0.921	$740 \pm 122$
			7.8	L	$32.8 \pm 1.2$	-	-	0.876	$671 \pm 144$
			8.1	L	$39.2 \pm 1.1$	-	-	0.922	$842 \pm 116$
24		7.5	L	$52.1 \pm 3.0$	-	-	0.763	$1160 \pm 426$	
		7.8	L	$30.5 \pm 2.0$	-	-	0.665	$641 \pm 282$	
		8.1	L	$48.3 \pm 2.9$	-	-	0.657	$996 \pm 385$	

48 E: exponential model, L: Linear model;  $k_u$ : uptake rate  $d^{-1}$ ,  $k_e$ : elimination rate  $d^{-1}$ ; SE: standard error;  $CF_{ss}$ : steady state concentration factor; Marginal  $R^2$ : marginal  
 49 coefficient of determination

50 **Table 3.** Parameters of the depuration kinetics of  $^{110m}\text{Ag}$ ,  $^{241}\text{Am}$ ,  $^{57}\text{Co}$ ,  $^{54}\text{Mn}$ , and  $^{65}\text{Zn}$  in oysters previously  
 51 exposed for 21 days to dissolved radiotracers under 3 pHs and 2 temperatures conditions and maintained for 37  
 52 days in non-contaminated conditions.

Element	Temp (°C)	pH	$A_0 \pm \text{SE}$ (%)	$k_{el}$	$T_{b/2} \pm \text{SE}$ (d)
$^{54}\text{Mn}$	21	7.5	$98.2 \pm 1.4$	$0.007 \pm 0.001^{***}$	$97 \pm 13$
		7.8	$101.0 \pm 1.4$	$0.006 \pm 0.001^{***}$	$119 \pm 19$
		8.1	$99.3 \pm 1.2$	$0.006 \pm 0.001^{***}$	$118 \pm 16$
	24	7.5	$96.3 \pm 2.5$	$0.003 \pm 0.002$	$217 \pm 121$
		7.8	$99.9 \pm 0.9$	$0.013 \pm 0.001^{***}$	$56 \pm 3$
		8.1	$97.8 \pm 1.3$	$0.005 \pm 0.001^{***}$	$110 \pm 18$
$^{57}\text{Co}$	21	7.5	$99.7 \pm 1.7$	$0.006 \pm 0.001^{***}$	$120 \pm 24$
		7.8	$99.0 \pm 1.6$	$0.005 \pm 0.001^{***}$	$132 \pm 26$
		8.1	$99.0 \pm 1.7$	$0.007 \pm 0.001^{***}$	$95 \pm 15$
	24	7.5	$98.9 \pm 2.6$	$0.003 \pm 0.002$	$236 \pm 143$
		7.8	$100.4 \pm 1.3$	$0.013 \pm 0.001^{***}$	$55 \pm 4$
		8.1	$97.3 \pm 1.6$	$0.006 \pm 0.001^{***}$	$115 \pm 21$
$^{65}\text{Zn}$	21	7.5	$97.2 \pm 1.5$	$0.007 \pm 0.001^{***}$	$100 \pm 16$
		7.8	$99.6 \pm 2.5$	$0.008 \pm 0.002^{***}$	$92 \pm 21$
		8.1	$99.6 \pm 1.2$	$0.005 \pm 0.001^{***}$	$153 \pm 26$
	24	7.5	$97.4 \pm 3.7$	$0.006 \pm 0.003^*$	$125 \pm 59$
		7.8	$95.8 \pm 1.5$	$0.005 \pm 0.001^{***}$	$149 \pm 34$
		8.1	$98.8 \pm 1.3$	$0.005 \pm 0.001^{***}$	$134 \pm 22$
$^{110m}\text{Ag}$	21	7.5	$99.9 \pm 1.7$	$0.005 \pm 0.001^{***}$	$137 \pm 31$
		7.8	$102.1 \pm 2.0$	$0.006 \pm 0.001^{***}$	$110 \pm 22$
		8.1	$101.8 \pm 1.2$	$0.003 \pm 0.001^{***}$	$260 \pm 72$
	24	7.5	$92.3 \pm 4.6$	$0.001 \pm 0.003$	$613 \pm 1173$
		7.8	$98.9 \pm 1.5$	$0.003 \pm 0.001$	$273 \pm 102$
		8.1	$100.9 \pm 1.1$	$0.004 \pm 0.001^{***}$	$185 \pm 33$
$^{241}\text{Am}$	21	7.5	$95.1 \pm 1.5$	$0.012 \pm 0.001^{***}$	$56 \pm 5$
		7.8	$97.8 \pm 1.9$	$0.009 \pm 0.001^{***}$	$77 \pm 11$
		8.1	$96.7 \pm 1.6$	$0.011 \pm 0.001^{***}$	$61 \pm 6$
	24	7.5	$98.0 \pm 2.5$	$0.006 \pm 0.001^{***}$	$107 \pm 30$
		7.8	$97.1 \pm 2.0$	$0.016 \pm 0.002^{***}$	$42 \pm 4$
		8.1	$92.8 \pm 3.3$	$0.007 \pm 0.002^{***}$	$88 \pm 27$

53 All kinetics were best fitted by a one-compartment exponential model:  $A_0$ : assimilation efficiency; SE: standard  
 54 error;  $T_{b/2}$ : biological half-life (d),  $k_{el}$ : depuration rate constant;  $R^2$ : regression coefficient. Significant differences  
 55 of  $k_{el}$  are indicated by \*( $p < 0.05$ ), \*\*( $p < 0.01$ ), or \*\*\*( $p < 0.005$ )

56 **Table 4.** Two-way ANOVA parameters testing the effects of three pH (7.5, 7.8 and 8.1) and two temperatures (21 and 24°C) on the uptake kinetic parameters,  
 57 i.e.  $k_u$  (see Figures 1 and Table 1), and on the loss kinetics parameters, i.e.  $k_e$  (see Figures 2 and Table 2) for all elements

Parameter	pH			Temperature			pH X Temperature		
	<i>df</i>	MS	F	<i>df</i>	MS	F	<i>df</i>	MS	F
<sup>110m</sup> Ag - $k_u$	2	3042	0.816	1	2018	0.541	2	8735	1.172
<sup>241</sup> Am - $k_u$	2	235	2.144	1	303	2.772	2	199	1.817
<sup>109</sup> Cd - $k_u$	2	64	2.713 <sup>○</sup>	1	143	6.061*	2	65	2.735 <sup>○</sup>
<sup>57</sup> Co - $k_u$	2	22	1.682	1	0.05	0.004	2	38	2.860 <sup>○</sup>
<sup>54</sup> Mn - $k_u$	2	32	1.192	1	26	0.969	2	132	4.835*
<sup>65</sup> Zn - $k_u$	2	380	1.847	1	522	2.537	2	207	1.006
<sup>110m</sup> Ag - $k_{el}$	2	1.6 10 <sup>-3</sup>	1.787	1	1.1 10 <sup>-3</sup>	1.282	2	1.4 10 <sup>-3</sup>	1.551
<sup>241</sup> Am - $k_{el}$	2	2.8 10 <sup>-5</sup>	2.070	1	1.1e-6	0.081	2	9.9 10 <sup>-5</sup>	7.367**
<sup>57</sup> Co - $k_{el}$	2	1.4 10 <sup>-5</sup>	0.785	1	8.3 10 <sup>-5</sup>	4.620*	2	4.8 10 <sup>-5</sup>	2.714 <sup>○</sup>
<sup>54</sup> Mn - $k_{el}$	2	4.2 10 <sup>-5</sup>	2.215	1	6.7 10 <sup>-5</sup>	3.529	2	3.3 10 <sup>-5</sup>	1.762 <sup>○</sup>
<sup>65</sup> Zn - $k_{el}$	2	1.1 10 <sup>-4</sup>	1.848	1	2.0 10 <sup>-5</sup>	0.344	2	5.7 10 <sup>-5</sup>	0.972

58 *df* = degree of freedom; MS = mean squares. Probability levels for significant effects:  $p < 0.01$  (\*\*),  $p < 0.05$  (\*),  $p < 0.1$  (○).

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62 **Table 5.** <sup>54</sup>Mn, <sup>57</sup>Co, <sup>65</sup>Zn, <sup>110m</sup>Ag, <sup>109</sup>Cd, and <sup>241</sup>Am distribution (%; mean ± SD) among soft tissue and shell of oysters exposed for 21 days of the uptake (n=3) and 37 days of  
 63 depuration phase (n=5) to dissolved radiotracers at three different pH and two temperatures

Parameters	Compartment	Weight	<sup>54</sup> Mn	<sup>57</sup> Co	<sup>65</sup> Zn	<sup>110m</sup> Ag	<sup>109</sup> Cd	<sup>241</sup> Am	
21 °C x pH 7.5	End of uptake	Soft tissue	1.4 ± 0.3	0.8 ± 0.2	2.1 ± 0.7	82.9 ± 1.7	95.7 ± 1.9	50.0 ± 13.0	5.7 ± 1.5
		Shell	9.7 ± 2.2	99.2 ± 0.2	97.9 ± 0.7	17.1 ± 1.7	4.3 ± 1.9	50.0 ± 13.0	94.3 ± 1.5
	End of depuration	Soft tissue	1.9 ± 0.5	0.4 ± 0.1	0.8 ± 0.4	91.5 ± 3.6	97.8 ± 1.8	-	2.9 ± 1.2
		Shell	11.2 ± 2.3	99.6 ± 0.1	99.2 ± 0.4	8.5 ± 3.6	2.2 ± 1.8	-	97.1 ± 1.2
21 °C x pH 7.8	End of uptake	Soft tissue	1.3 ± 0.2	0.4 ± 0.1	1.5 ± 0.5	84.6 ± 4.9	97.5 ± 0.4	48.0 ± 19.4	3.1 ± 1.4
		Shell	8.9 ± 0.9	99.6 ± 0.1	98.5 ± 0.5	15.4 ± 4.9	2.5 ± 0.4	52.0 ± 19.4	96.9 ± 1.4
	End of depuration	Soft tissue	1.9 ± 0.9	0.4 ± 0.1	1.0 ± 0.3	89.2 ± 7.6	96.1 ± 3.4	-	2.1 ± 0.7
		Shell	9.7 ± 3.0	99.6 ± 0.1	99.0 ± 0.3	10.8 ± 7.6	3.9 ± 3.4	-	97.9 ± 0.7
21 °C x pH 8.1	End of uptake	Soft tissue	1.8 ± 0.4	0.8 ± 0.4	1.8 ± 0.7	78.4 ± 9.2	97.3 ± 1.5	43.3 ± 15.3	3.5 ± 1.2
		Shell	11.5 ± 1.1	99.2 ± 0.4	98.2 ± 0.7	21.6 ± 9.2	2.7 ± 1.5	56.7 ± 15.3	96.5 ± 1.2
	End of depuration	Soft tissue	2.0 ± 0.8	0.5 ± 0.3	1.0 ± 0.7	91.1 ± 6.3	96.9 ± 1.9	-	1.5 ± 0.4
		Shell	10.8 ± 3.4	99.5 ± 0.3	99.0 ± 0.7	8.9 ± 6.3	3.1 ± 1.9	-	98.5 ± 0.4
24 °C x pH 7.5	End of uptake	Soft tissue	1.7 ± 0.2	0.9 ± 0.2	1.8 ± 0.2	76.3 ± 9.2	90.7 ± 5.4	68.8 ± 15.8	4.2 ± 0.7
		Shell	10.7 ± 2.7	99.1 ± 0.2	98.2 ± 0.2	23.7 ± 9.2	9.3 ± 5.4	31.2 ± 15.8	95.8 ± 0.7
	End of depuration	Soft tissue	1.6 ± 0.6	0.7 ± 0.4	1.4 ± 0.6	86.5 ± 11.5	94.7 ± 5.1	-	5.1 ± 2.3
		Shell	11.9 ± 2.0	99.3 ± 0.4	98.6 ± 0.6	13.5 ± 11.5	5.3 ± 5.1	-	94.9 ± 2.3
24 °C x pH 7.8	End of uptake	Soft tissue	1.2 ± 0.1	0.4 ± 0.1	0.9 ± 0.2	73.2 ± 3.5	90.8 ± 0.7	52.7 ± 2.2	1.4 ± 0.4
		Shell	8.9 ± 1.2	99.6 ± 0.1	99.1 ± 0.2	26.8 ± 3.5	9.2 ± 0.7	47.3 ± 2.2	98.6 ± 0.4
	End of depuration	Soft tissue	1.1 ± 0.2	0.7 ± 0.3	1.7 ± 0.9	84.2 ± 13.6	92.8 ± 9.2	-	2.7 ± 1.4
		Shell	9.0 ± 1.6	99.3 ± 0.3	98.3 ± 0.9	15.8 ± 13.6	7.2 ± 9.2	-	97.3 ± 1.4
24 °C x pH 8.1	End of uptake	Soft tissue	1.4 ± 0.7	0.9 ± 0.7	1.7 ± 1.3	66.6 ± 16.5	75.8 ± 16.5	63.9 ± 10.2	8.4 ± 10.6
		Shell	9.1 ± 0.3	99.1 ± 0.1	98.3 ± 1.3	33.4 ± 16.5	24.2 ± 16.5	36.1 ± 10.2	91.6 ± 10.6
	End of depuration	Soft tissue	1.3 ± 0.5	0.7 ± 0.4	1.2 ± 0.5	89.7 ± 6.7	96.8 ± 1.9	-	2.4 ± 1.5
		Shell	9.9 ± 1.3	99.3 ± 0.4	98.8 ± 0.5	10.3 ± 6.7	3.2 ± 1.9	-	97.6 ± 1.5

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65 **Table 6.** Chi-square test on the effects of three pH levels on bioaccessible fraction of metals in oyster tissue  
 66 following *in vitro* digestion.

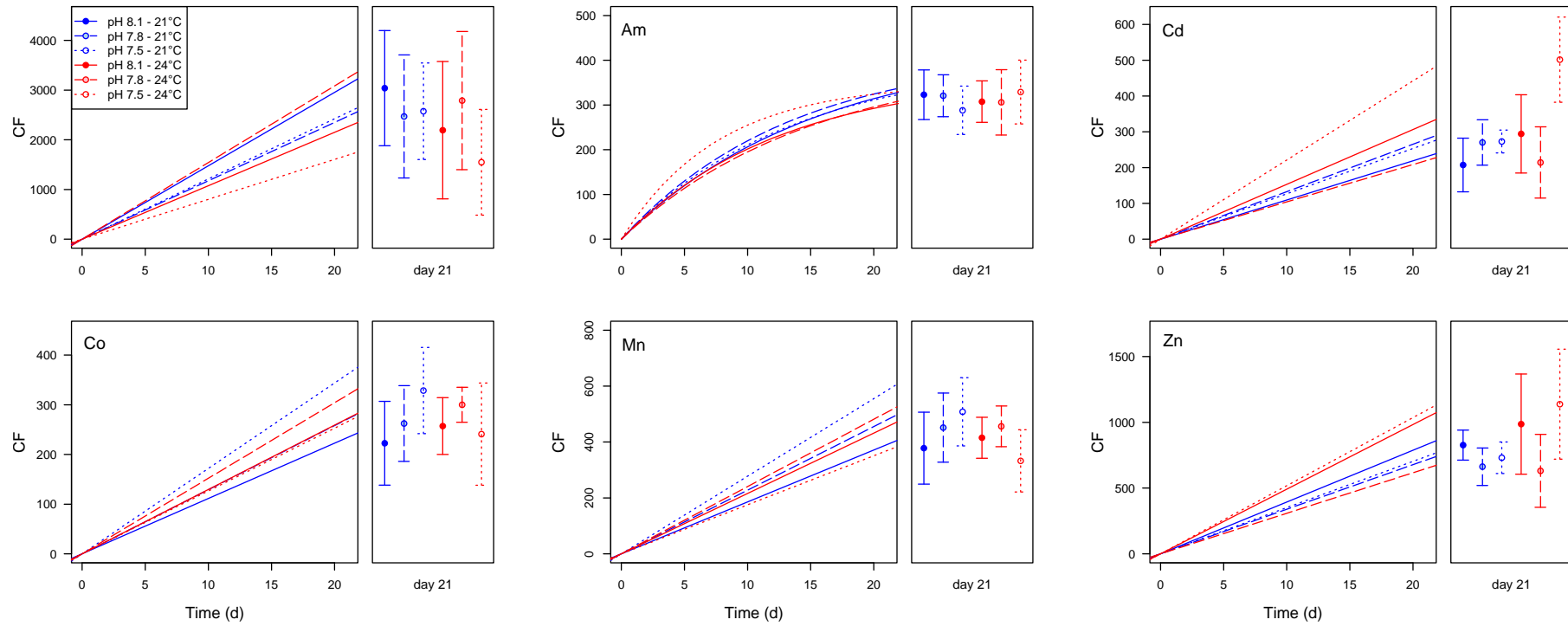
Digestion time	Temperature	<sup>54</sup> Mn	<sup>57</sup> Co	<sup>65</sup> Zn	<sup>110m</sup> Ag	<sup>109</sup> Cd	<sup>241</sup> Am
End of the uptake	21 °C	8.71*	2.35	6.55*	0.78	3.96	1.69
	24 °C	19.44***	0.10	17.13***	0.30	12.13**	22.77***
End of the loss	21 °C	19.15***	1.87	14.57***	1.78	-	3.12
	24 °C	7.72*	0.74	16.21***	0.28	-	27.51***

74 df (degree of freedom): 2

75 \* p <0.05 \*\* p <0.01 \*\*\* p <0.001

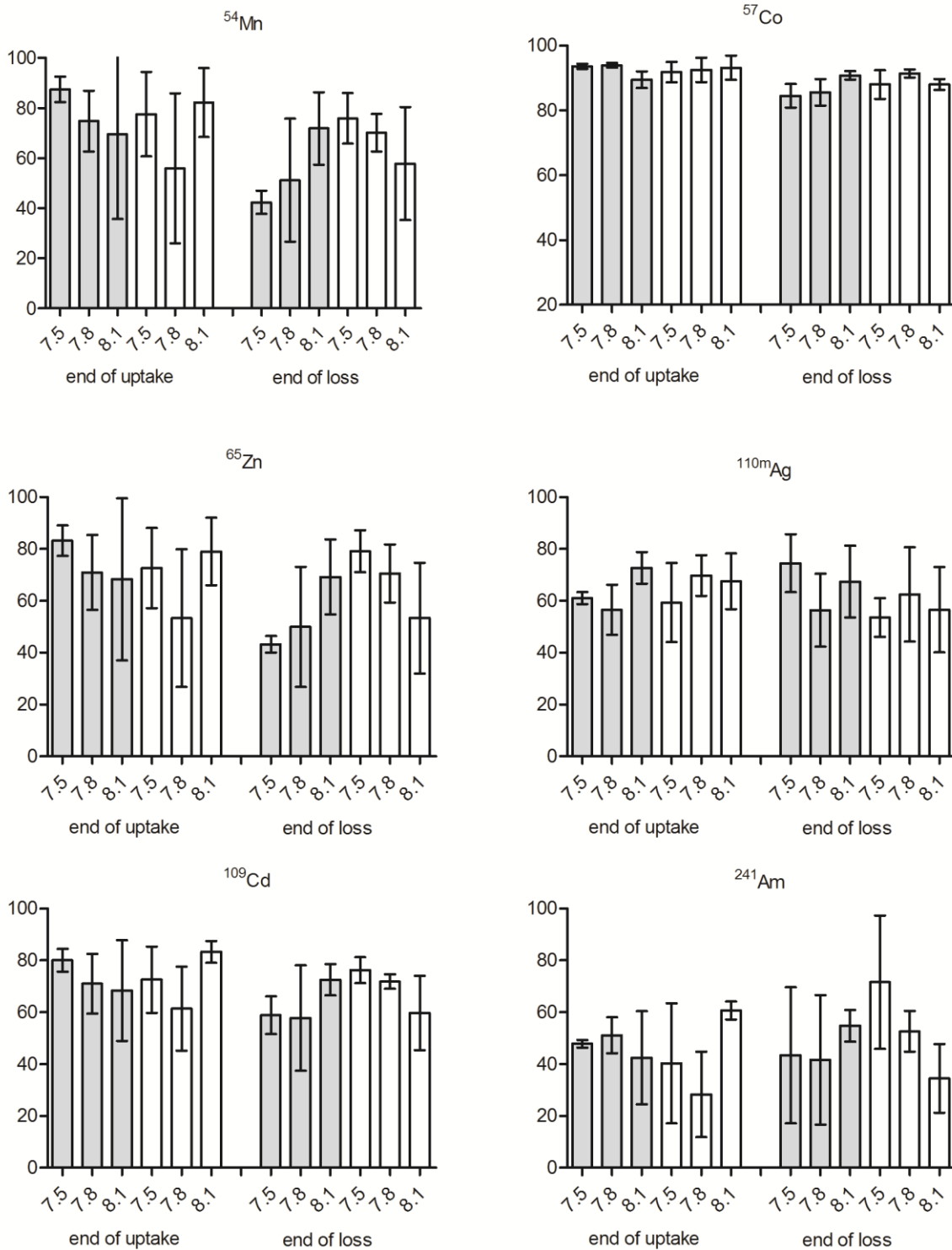
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81 Figure 1. *Crassostrea gigas*. Uptake kinetics of  $^{110m}\text{Ag}$ ,  $^{241}\text{Am}$ ,  $^{109}\text{Cd}$ ,  $^{57}\text{Co}$ ,  $^{54}\text{Mn}$ ,  $^{65}\text{Zn}$  (CF) in the oysters exposed to dissolved radiotracers at three different  
82 pHs – pH 8.1, pH 7.8, and pH 7.5, and two temperatures (21°C in blue and 24°C in red). Points are omitted for clarity and only kinetics models are delineated  
83 along the uptake phase. Mean  $\pm$  SD of CF are plotted for the day 24 at the end of the uptake. Parameters of models and statistics are reported in Table 2 and 4,  
84 respectively.



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86 Figure 2: Radiotracer bioaccessibility (% , mean  $\pm$  SD, n=3) from oyster soft tissues to human consumers  
 87 assessed using the simulated *invitro* digestion method (gray: 21 °C, white: 24 °C)

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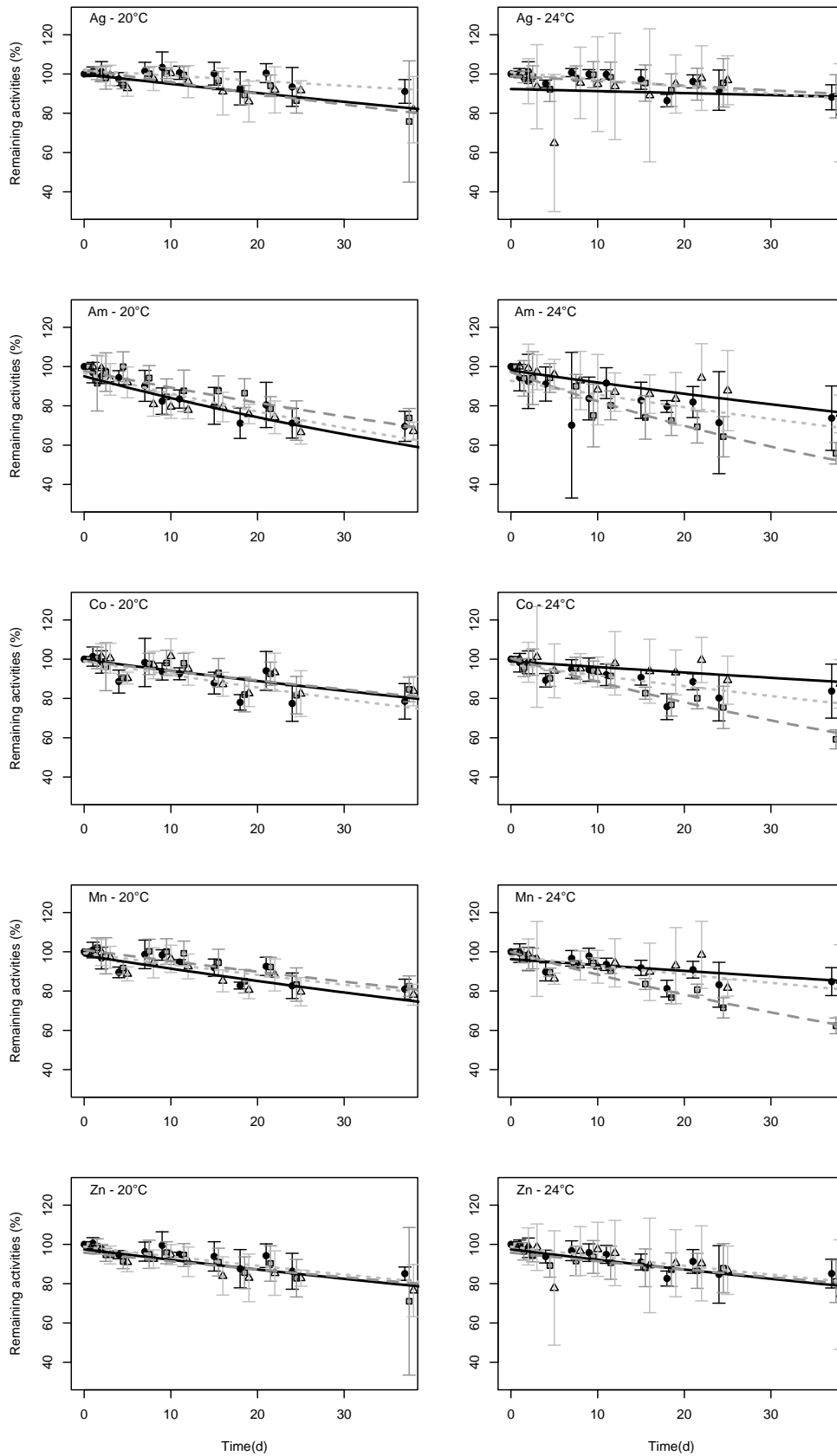


89 Table S1. CF values in the shell at the end of uptake period

pH/Temp	<sup>54</sup> Mn	<sup>60</sup> Co	<sup>65</sup> Zn	<sup>110m</sup> Ag	<sup>109</sup> Cd	<sup>241</sup> Am
7.5/20	923 ± 379	589 ± 269	264 ± 116	116 ± 20	143 ± 59	644 ± 168
7.8/20	886 ± 39	464 ± 58	188 ± 34	76 ± 45	117 ± 67	802 ± 43
8.1/20	1247 ± 276	704 ± 219	264 ± 39	98 ± 15	109 ± 66	835 ± 118
7.5/24	709 ± 76	473 ± 74	216 ± 102	76 ± 55	65 ± 18	627 ± 156
7.8/24	1031 ± 73	723 ± 23	256 ± 15	73 ± 6	110 ± 20	644 ± 65
8.1/24	1149 ± 9	607 ± 36	381 ± 116	239 ± 205	85 ± 45	815 ± 51

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93 **Figure S1.**  $^{110m}\text{Ag}$ ,  $^{241}\text{Am}$ ,  $^{57}\text{Co}$ ,  $^{54}\text{Mn}$  and  $^{65}\text{Zn}$  loss kinetics (CF; mean  $\pm$  SD; n = 5) in the oysters  
 94 during the depuration phase following exposure to dissolved radiotracers at three different pH – pH

95 8.10 (full line- black), pH 7.85 (dashed line - grey), pH 7.60 (dotted line - lightgrey), and two  
96 temperatures (21°C on the left side and 24°C on the right side) for 37 days.

97