

Limited effects of increased CO2 and temperature on metal and radionuclide bioaccumulation in a sessile invertebrate, the oyster Crassostrea gigas

Murat Belivermis, M. Warnau, Marc Metian, François Oberhänsli, Jean-Louis Teyssié, Thomas Lacoue-Labarthe

▶ **To cite this version:**

Murat Belivermis, M. Warnau, Marc Metian, François Oberhänsli, Jean-Louis Teyssié, et al.. Limited effects of increased CO2 and temperature on metal and radionuclide bioaccumulation in a sessile invertebrate, the oyster Crassostrea gigas. ICES Journal of Marine Science, 2016, 73, 10.1093/icesjms/fsv236 . hal-01253916

HAL Id: hal-01253916
https://hal.science/hal-01253916

Submitted on 11 Jan 2016

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Limited effects of increased CO_2 and temperature on metal and radionuclide bioaccumulation in a sessile invertebrate, the oyster *Crassostreagigas*

Murat Belivermiş[1,¥], Michel Warnau[2], Marc Metian[2], François Oberhänsli[2], Jean-Louis Teyssié[2], Thomas Lacoue-Labarthe[2,3,*]

[1] Department of Biology, Faculty of Science, Istanbul University, 34134 Vezneciler, Istanbul, Turkey

[2] International Atomic Energy Agency - Environment Laboratories, 4 Quai Antoine I[er], MC 98000 Monaco, Monaco

[3] Littoral Environnement et Sociétés, UMR 7266 CNRS - Université de La Rochelle, 2 rue Olympe de Gouges, 17000 La Rochelle, France.

[¥]Dr Murat Belivermiş, Department of Biology,Faculty of Science, Istanbul University,34134 Vezneciler,Istanbul, Turkey, Telephone: +90 212 455 57 00, belmurat@istanbul.edu.tr

* Corresponding author: Thomas Lacoue-Labarthe
Telephone: +33 5 46 45 83 88
E-mail:tlacouel@univ-lr.fr

ABSTRACT

This study investigated the combined effects of reduced pH and increasedtemperature on the capacities of the Pacific cupped oyster *Crassostrea gigas*to bioconcentrateradionuclide and metals . Oysters were exposed to dissolved radiotracers (^{110m}Ag, ^{241}Am,^{109}Cd,^{57}Co, ^{54}Mn, and^{65}Zn)atthree pH (7.5, 7.8, 8.1) and two temperatures (21°C and 24°C)undercontrolled 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 ± 1 *vs.* 17 ± 1 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 affectedstronglythe elements distribution between shell and soft tissues. Significant effects of pH were foundon the bio-accessibility of Mn, Zn and ^{241}Amduring 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_2 (pCO_2) in the atmosphere due to anthropogenic activities is well recognized as a driver ofmajor global changes such as theelevation ofatmospheric temperature. The global mean surface air temperature is projected to increase by 1 to3.7°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_2 (it absorbs~25 % of the anthropogenic emissions) and thusthe increase inpCO_2in the atmosphere implies an increase inpCO_2 in surface ocean waters, that causesthe "other" CO_2 problem (Doney et al., 2009), better known as"ocean acidification".

Marine organisms can be affectedby ocean acidificationthrough(1) limitation of available carbonates, mainly affecting calcifying organisms, (2) the increase in H^+ ions in the water resulting in decreasing pH –i.e. acidification of the surrounding environment– and (3) an increase in pCO_2within their body (hypercapnia).In this context,attention should be paid tovulnerablespecies, which have key role(s) in the food chains, or are of high economic interest for aquaculture or fisheries.

The Pacific cupped oyster *Crassostrea giga*s is the most farmedoyster 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 oystersunder realistic low pH and low carbonate concentrations conditions(for a review, see Gazeau et al., 2013). A particular attention has beenpaid tothe early life stages of the oyster, which are considered as the most vulnerable and therebyas a bottleneck for population dynamics(Dupont et al., 2010). Overall, reduced shell size and thickness, developmental

retardation and increased mortalityare the most common effectsof elevated pCO_2on *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 suggestedthat some populations could bepre-adapted to ocean acidification(Parker et al., 2010; Parker et al., 2011; Timmins-Schiffman et al., 2013).

The elevatedpCO_2 in seawater is expected to affect the metabolism though disturbances of acid-base regulation and respiration (Pörtner et al., 2004). It is admitted that active organisms, such as fish, cephalopods and crustaceanshave naturally the required physiological machinery to deal with elevated extracellular pCO_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) andcould 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 environmentandare major contributorsto the contamination of coastal marine ecosystemsover the last century.Widespread in the environment, these contaminants are accumulated in organisms, and potentially bio-magnifiedalong the food chains(Zhou et al., 2008).Excessive accumulationof essential (*e.g.* Co, Mn or Zn) or non-essential (*e.g.*Ag, ^{241}Am, Cd) elements in organismscan 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 pCO_2affect chemicalspeciation 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 influencethebioaccumulation of metals(White and Rainbow, 1984; Houlbrèque et al., 2011),and can thus enhancetheirtoxicity(e.g. Pascal et al., 2010; Roberts et al., 2013).

This study aimsat assessing the bioaccumulationbehaviours of radionuclides/metals in a sessile species of economic importance, the Pacific cupped oyster,*Crassostrea gigas*,usually cultured in coastal areassubjected to metal contamination and high pCO_2 variations (e.g. Green et al., 2009).Radiotracer techniqueswereused to determine uptake and depuration parameters of 6 elements during experimental exposuresof oysters to dissolved radiotracers (^{110m}Ag, ^{241}Am, ^{109}Cd, ^{57}Co, ^{54}Mn, and ^{65}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 todeterminethe bio-accessible fraction of metals and radionuclides in oysters that would be ingested by human (Metian et al., 2009a).

MATERIALS AND METHODS

Organisms, acclimationand experimentalconditions

One hundred and sixty-two adult oysters *Crassostrea gigas*(17.71 ± 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 randomlydistributed among six 70-L glass aquaria and acclimated for 2 weeks in0.45-µm filtered,UV-sterilized natural seawater (aerated open circuit 70-L aquarium; seawater flux: 70 L h^{-1}; salinity: 38 p.s.u.; light/dark cycle:12 h/12 h).Then, the pH and the temperature were progressively modified overone 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 dietcomposed 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 one70-L tank could be dedicated per pCO_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 3selected pH values were8.10 (~400 µatm), 7.80 (~800 µatm), 7.50 (~1800 µatm) andthe two temperatures were 21°C and 24°C in accordance to the projected values over the next twocenturies (Orr et al., 2005; Solomon et al., 2007; currentcondition: 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 ± 0.05 pH unit with a continuous pH-stat system (IKS, Aquastar©) that bubbled pure CO_2 into the tanksthat were continuously aerated with CO_2-free air (see Lacoue-Labarthe et al., 2012). The pH values of the pH-stat system were calibratedevery 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 pCO_2 was determined from pH and total alkalinity using the R package seacarb (Gattuso et al., 2015).

Radiotracer exposures.

Theoysters wereexposedfor 21 days to dissolved radiotracers:^{110m}Ag [as $^{110m}AgNO_3$; $T_{½}$ = 250 d], ^{241}Am [as $^{241}AmNO_3$; $T_{½}$ = 432 y], ^{109}Cd [as $^{109}CdCl_2$; $T_{½}$ = 464 d],^{57}Co [as $^{57}CoCl_2$; $T_{½}$ = 272 d],^{54}Mn [as $^{54}MnCl_2$; $T_{½}$ = 312 d] and^{65}Zn [as $^{65}ZnCl_2$; $T_{½}$ = 244 d].Uptake kinetics were followed during that period, after which seawater was no longer spikedin order to assess the metal retention capacities of the oysters.

During the exposure period, the seawater of each 70-L tankwas spiked with typically 5 µL ofradioactive stock solution (^{110m}Ag, dissolved in 0.1M HCl, ^{241}Am in 1M HCl, ^{109}Cd in 0.5 M HCl, ^{57}Co 0.1 M in HCl, ^{54}Mn in 0.5 M HCl and ^{65}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 ~ 60 L) with new seawater at the required temperature. The slow refill allowedfor immediate regulation of the targeted pCO_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 wasspiked 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 radiotracersshown 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 andall soft tissues were separated and radio-analyzed in order to assess the distribution of radiotracers between these two compartments.

The remaining exposedoysters were placed in non-contaminated conditions (open-circuit 70-L tankwith 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 Lh^{-1}). The five tag-identifiedoystersin 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 orderto assess the bioaccessible fraction of elements for human consumers of oysters. The method consists of a three-step proceduresimulating quiet 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 $NaHCO_3$(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 γ-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) wasdetermined 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^{-1}) and the time-integrated radiotracer activity in seawater (Bq g^{-1}) over time. Uptake kinetics were fitted using either a linear equation (Eq. 1) or a saturation exponential equation (Eq. 2):

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

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

where CF_t and CF_{ss}($CF_{ss} = k_u / k_e$) are the concentration factors at time t (d) and at steady-state, respectively, and k_e and k_u are the biological depuration and uptake rate constants (d^{-1}),respectively(Whicker and Schultz, 1982).

Radiotracer depuration kinetics wereexpressed 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 werefitted bya simple exponentialmodel (Eq. 3):

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

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) ofthe 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 modelsusing linear and non-linear mixed effect models (*lme* and *nlme* functions from the package "nlme";(Pinheiro et al., 2014), in whichindividual oyster identity hadbeen 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 eliminationconstant ratesamongthe 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 conditioncould be detected. Specific mortality of oysters with condition of exposure werethe 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-bodybioaccumulation kinetics of ^{110m}Ag, ^{241}Am, ^{109}Cd, ^{57}Co, ^{54}Mn, and ^{65}Zn in oysters and their respective model parameters are shownin Figure 1 and Table 2. Uptake of all elements except^{241}Am were best fitted by linear models.The actinide displayed an exponential uptake pattern, tending to reach a steady-state value rangingbetween 360 and470 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 linearlyaccording to their bioaccumulation capacities: ^{110m}Ag >^{65}Zn >^{54}Mn >^{109}Cd ~^{57}Co.

At the end ofthe exposure period, oysters were placed in non-contaminating conditions and depuration of radiotracers wasfollowed 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}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}Cd and ^{54}Mn. The highest temperature affectedCd accumulation(Figure 1,Table 4), which displayed an uptake rate constant at pH 7.5 two-fold higherat 24°C than at 20°C (23 ± 1 vs. 11 ± 1 d^{-1}, respectively; Table 4, interactive effectpHx 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 ± 1 *vs.* 17 ± 1 d^{-1}at 21°C and 24°C, respectively; see Figure 1 and Table 4).Regarding the depuration rate constant of ^{241}Am, a significant interactive effectwas observed at pH 7.8 where $T_{b1/2}$was two-fold lower at 24°C than at 21°C (Tukey test; $p = 0.049$;Table 4). Retention of Co and Mntendedalso to decrease at the same pH when comparing warmerwith colder temperatures (Table 4; pHx temperature; $p < 0.1$).

When looking atthe distribution of the radiotracers betweenshells and soft tissues (Table 5), ^{241}Am, ^{57}Co and ^{54}Mn were mainlyassociatedwiththe shells (> 94%), whereas ^{109}Cd wasequally distributed between the two compartments and ^{110m}Ag and ^{65}Zn were mainly found in soft tissues (76-98% and 67-92%, respectively). It is also noteworthy that ^{65}Zn fraction in soft tissues increased between the end of uptake period and the end of depurationperiod, at all pH and temperature conditions, suggesting a fasterdepuration of Zn associated with shellsthanthat withsoft tissues when non-contaminatingconditions were restored.Neither pH nor temperature affectedstrongly distribution ofmetals and radionuclide betweenshells and soft tissues, except that the fraction of ^{110m}Ag associated with soft tissues at the end of the uptake phasewere lower in oysters maintained at 24°C when compared to those keptat 21°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 pCO_2 conditions (Table S1).

The *in vitro* digestion simulations revealed that the bioaccessible fraction of metals and radionuclide varied from 30% (^{241}Am in oyster maintained at 24°C and pH 7.8) to more than 90% (^{57}Co in all oysters; Figure 2). Significant effects of pH were highlighted for ^{241}Am,^{109}Cd,^{54}Mn and ^{65}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 bioacessibilitywith decreasing pH was noted for the21°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 maychangethe 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 theelements considered in the present study, only ^{241}Am is known as being able to bind to carbonates but with limited change inspeciation 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 andCd)or those that are predominantly found in the free ion form (*e.g.* Co, Mn, Zn) arenot strongly affected by decreasing pH. Indeed, the free metal ion concentrations of these elements increasedfrom only few percent when pH decreased from 8.1 to 7.5 (Lacoue-Labarthe et al., 2009; Millero et al., 2009).We thus assume that, forthe seawater pH range tested in the context of ocean acidification, bioaccumulation wouldbe less affected by change in metal speciationitself than byresulting change in the biological mechanisms of metal uptake and/or elimination.

In the current study, we examined the bioaccumulation patterns of sixmetals and oneactinidein the Pacific cupped oyster *Crassostrea gigas*under elevated conditions of temperature and pCO_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*usesto 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 stressorswere combined(Lannig et al., 2010).

The distribution of radiotracers between soft tissues andshells was in accordance with previous studies: ^{241}Am, Co, Mn were mainly adsorbed on the calcareous structures whereas Cd, Zn and Ag fraction associated with soft tissueswas gradually increasing during the depuration period (Metian et al., 2009b; Hédouin et al., 2010; Metian et al., 2011). CFs of^{241}Am, Co and Mnin shellsat the end of the uptake phase were not significantly affected by seawater pCO_2 conditions, implying that the adsorptioncapacity of shellsis not strongly affected by decreasing pH for these metals. This might be explained by the fact that 1) increasing protons concentration resulting from decreasing pHdoes not significantly compete with metallic cations for shell bindingsites and/or 2) the binding sites are not affected by hypercapnia in the conditions tested (shellsof *C. gigas* -mainly made of calcite- are considered less vulnerable tocalcareous dissolution than aragonitic shells;(Gazeau et al., 2007).

The main result of our study is that no effect of increasing pCO_2andtemperature has been detected on Ag,Cd and Znbioaccumulation capacities in *C.gigas* soft tissues. Only few studies have investigated so far the impact of warming and hypercapnia on metal accumulation and/ortheir 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 accumulationwas lowered in cuttlefish embryosand Cd toxicity decreased in benthic copepod with decreasing pCO_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 intransmembranechannel 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 µatm) and 24°C for three weeks. Similar observation of enhanced Cd bioaccumulation in *C. virginica* maintained at pH 7.8 (~ 800 µatm) 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 is turn couldincrease Cd ion uptake in oyster tissues.

Interestingly, it has been previously shownthat the non-essential element Ag is more efficiently accumulated, up to 2.5 fold, in embryos and larvae of cuttlefish and squidwhen exposed to elevated pCO_2 ranged from 600 to 1500 µatm(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. Indeedthe Na^+/H^+-exchanger,an ionic channel, isexpected involved in Ag uptake (Webb and Wood, 2000; Grosell and Wood, 2001) in mobileorganisms 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 tissuesas a consequence of change in seawater pCO_2. This is congruent with the less-developed ion-exchange machinery in sessile invertebrates, and their subsequentlimited 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 topCO_2 conditions. Indeed, Zn is a co-factor of the carbonic anhydrase (CA) that catalyses the conversion of 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 inCA expression levels in the mantle of *C. virginica* exposed to 3500 µatm, but the latter partial pressure was much higher than those tested in the present study.

Oysters area 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 werecarried outto determine whetherlower pH and higher temperatures may affect metal and radionuclide bioaccessibilty to human consumers. Results indicated that^{241}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 ofthe 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 pCO_2 is susceptible to affect the metal detoxificationstrategiesand 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 worsen 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 pCO_2 and temperature on the transfer and integration of metals in coastal organisms.

However, our experimental approach(oysters were acclimated totemperature and pCO_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.

Acknowledgements

The IAEA is grateful to the Government of the Principality of Monaco for the support provided to its Environment Laboratories.Murat Belivermiş was supported by a post-doctoral scholarshipprovided byCouncil of Higher Education of Turkish Republic. Michel Warnau is an Honorary Senior Research Associate of the National Fund for Scientific Research (NFSR, Belgium).Authors are grateful to Florian Rivello, Kevin Calabro, andMarie-Yasmine Dechraouifor theirtechnical assistance in the laboratory.

References

Barton, A., Hales, B., Waldbusser, G. G., Langdon, C., and Feely, R. A. 2012. The Pacific oyster, *Crassostreagigas*, 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., Bjerkeng, B., Pettersen, O., Schaanning, M. T., and Øxnevad, S. 2006. Effects of increased sea water concentrations of CO_2 on growth of the bivalve *Mytilusedulis* 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 CO2 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_2 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., Peeene, 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_2 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_2 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_2 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_4^+-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_2 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., Etzkorn, 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_2 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., Teyssie, J. L., Markich, S. J., Jeffree, R., and Bustamante, P. 2009. Effects of increased pCO_2 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_2 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 ^{241}Am and ^{134}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 pCO_2 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_2 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 ^{234}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 pCO_2 causes developmental delay in early larval Pacific oysters, *Crassostreagigas*. 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., Teyssié, J.-L., and Fowler, S. W. 1996. Biokinetics of selected heavy metals and radionuclides in the common Mediterranean echinoid *Paracentrotuslividus*: 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 *Palaemonelegans* (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_2 (pCO_2) were calculated for a seawater salinity of 38 and a total alkalinity of 2540 µmol.kg^{-1} average.

Experiment phase	Temperature (°C)	pH_T	pCO_2 (µatm)	^{110m}Ag (Bq.ml^{-1})	^{241}Am (Bq.ml^{-1})	^{109}Cd (Bq.ml^{-1})	^{57}Co (Bq.ml^{-1})	^{54}Mn (Bq.ml^{-1})	^{65}Zn (Bq.ml^{-1})
Uptake	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
Loss	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*	-	-	-	-	-	-

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

Element	Temp (°C)	pH	Model	k_u ± SE	k_e ± SE	CF_{ss} ± SE	Marginal R^2	CF_{21d}
^{110m}Ag	21	7.5	L	123.0 ± 6.4	-	-	0.755	2661 ± 1003
		7.8	L	118.2 ± 7.9	-	-	0.644	2549 ± 1278
		8.1	L	146.5 ± 8.2	-	-	0.719	3141 ± 1198
	24	7.5	L	68.2 ± 6.2	-	-	0.443	1587 ± 1092
		7.8	L	146.1 ± 11.0	-	-	0.610	2844 ± 1419
		8.1	L	110.5 ± 9.2	-	-	0.556	2236 ± 1409
^{241}Am	21	7.5	E	30.4 ± 5.3	0.078 ± 0.011	390 ± 39	-	281 ± 53
		7.8	E	31.9 ± 5.5	0.078 ± 0.011	407 ± 39	-	330 ± 48
		8.1	E	30.2 ± 4.7	0.074 ± 0.008	408 ± 48	-	329 ± 56
	24	7.5	E	45.7 ± 8.0	0.127 ± 0.019	362 ± 32	-	311 ± 102
		7.8	E	24.8 ± 5.3	0.052 ± 0.010	474 ± 33	-	314 ± 75
		8.1	E	30.3 ± 4.1	0.085 ± 0.008	357 ± 33	-	315 ± 48
^{109}Cd	21	7.5	L	12.5 ± 0.5	-	-	0.887	272 ± 32
		7.8	L	13.2 ± 0.8	-	-	0.726	272 ± 64
		8.1	L	10.5 ± 0.8	-	-	0.642	209 ± 70
	24	7.5	L	22.8 ± 1.0	-	-	0.852	504 ± 124
		7.8	L	10.2 ± 1.1	-	-	0.502	216 ± 100
		8.1	L	14.7 ± 1.2	-	-	0.545	295 ± 109
^{57}Co	21	7.5	L	16.9 ± 0.6	-	-	0.895	332 ± 88
		7.8	L	12.6 ± 0.6	-	-	0.827	266 ± 77
		8.1	L	10.7 ± 0.6	-	-	0.753	226 ± 86
	24	7.5	L	12.0 ± 0.7	-	-	0.725	244 ± 104
		7.8	L	14.9 ± 0.3	-	-	0.966	305 ± 36
		8.1	L	12.4 ± 0.4	-	-	0.882	261 ± 58

Table 2 (continued)

Element	Temp (°C)	pH	Model	$k_u \pm SE$	$k_e \pm SE$	$CF_{ss} \pm SE$	Marginal R^2	CF_{21d}
^{54}Mn	21	7.5	L	26.8 ± 0.9	-	-	0.900	518 ± 124
		7.8	L	22.5 ± 0.9	-	-	0.829	468 ± 148
		8.1	L	18.1 ± 0.8	-	-	0.792	391 ± 133
	24	7.5	L	16.7 ± 0.8	-	-	0.796	341 ± 114
		7.8	L	22.8 ± 0.7	-	-	0.917	473 ± 76
		8.1	L	20.1 ± 0.6	-	-	0.900	430 ± 76
^{65}Zn	21	7.5	L	34.7 ± 1.0	-	-	0.921	740 ± 122
		7.8	L	32.8 ± 1.2	-	-	0.876	671 ± 144
		8.1	L	39.2 ± 1.1	-	-	0.922	842 ± 116
	24	7.5	L	52.1 ± 3.0	-	-	0.763	1160 ± 426
		7.8	L	30.5 ± 2.0	-	-	0.665	641 ± 282
		8.1	L	48.3 ± 2.9	-	-	0.657	996 ± 385

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 coefficient of determination

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

Element	Temp (°C)	pH	A_{0l}± SE (%)	k_{el}	$T_{b½}$ ± SE(d)
^{54}Mn	21	7.5	98.2 ± 1.4	0.007 ± 0.001***	97 ± 13
		7.8	101.0 ± 1.4	0.006 ± 0.001***	119 ± 19
		8.1	99.3 ± 1.2	0.006 ± 0.001***	118 ± 16
	24	7.5	96.3 ± 2.5	0.003 ± 0.002	217 ± 121
		7.8	99.9 ± 0.9	0.013 ± 0.001***	56 ± 3
		8.1	97.8 ± 1.3	0.005 ± 0.001***	110 ± 18
^{57}Co	21	7.5	99.7 ± 1.7	0.006 ± 0.001***	120 ± 24
		7.8	99.0 ± 1.6	0.005 ± 0.001***	132 ± 26
		8.1	99.0 ± 1.7	0.007 ± 0.001***	95 ± 15
	24	7.5	98.9 ± 2.6	0.003 ± 0.002	236 ± 143
		7.8	100.4 ± 1.3	0.013 ± 0.001***	55 ± 4
		8.1	97.3 ± 1.6	0.006 ± 0.001***	115 ± 21
^{65}Zn	21	7.5	97.2 ± 1.5	0.007 ± 0.001***	100 ± 16
		7.8	99.6 ± 2.5	0.008 ± 0.002***	92 ± 21
		8.1	99.6 ± 1.2	0.005 ± 0.001***	153 ± 26
	24	7.5	97.4 ± 3.7	0.006 ± 0.003*	125 ± 59
		7.8	95.8 ± 1.5	0.005 ± 0.001***	149 ± 34
		8.1	98.8 ± 1.3	0.005 ± 0.001***	134 ± 22
^{110m}Ag	21	7.5	99.9 ± 1.7	0.005 ± 0.001***	137 ± 31
		7.8	102.1 ± 2.0	0.006 ± 0.001***	110 ± 22
		8.1	101.8 ± 1.2	0.003 ± 0.001***	260 ± 72
	24	7.5	92.3 ± 4.6	0.001 ± 0.003	613 ± 1173
		7.8	98.9 ± 1.5	0.003 ± 0.001	273 ± 102
		8.1	100.9 ± 1.1	0.004 ± 0.001***	185 ± 33
^{241}Am	21	7.5	95.1 ± 1.5	0.012 ± 0.001***	56 ± 5
		7.8	97.8 ± 1.9	0.009 ± 0.001***	77 ± 11
		8.1	96.7 ± 1.6	0.011 ± 0.001***	61 ± 6
	24	7.5	98.0 ± 2.5	0.006 ± 0.001 ***	107 ± 30
		7.8	97.1 ± 2.0	0.016 ± 0.002***	42 ± 4
		8.1	92.8 ± 3.3	0.007 ± 0.002***	88 ± 27

All kinetics were best fitted by a one-compartment exponential model: A_0: assimilation efficiency; SE: standard error; $T_{b½}$: biological half-life (d), k_e: depuration rate constant; R^2: regression coefficient.Significant differences of k_e are indicated by *($p<0.05$), **($p<0.01$), or ***($p<0.005$)

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

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

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

Parameters		Compartments	Weight	^{54}Mn	^{57}Co	^{65}Zn	^{110m}Ag	^{109}Cd	^{241}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

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

Digestion time	Temperature	^{54}Mn	^{57}Co	^{65}Zn	^{110m}Ag	^{109}Cd	^{241}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***

df (degree of freedom): 2

* p <0.05 ** p <0.01 *** p <0.001

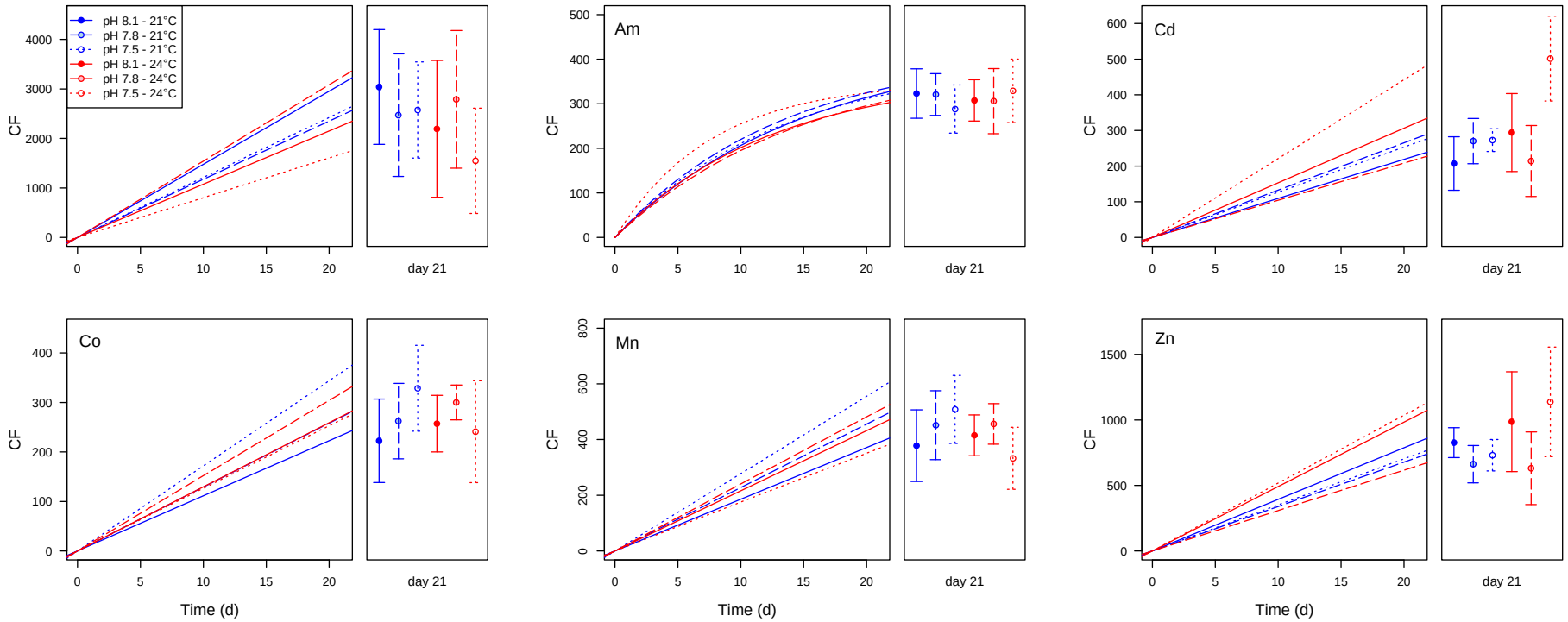


Figure 1. *Crassostrea gigas*. Uptake kinetics of ^{110m}Ag, ^{241}Am, ^{109}Cd, ^{57}Co, ^{54}Mn, ^{65}Zn (CF) in the oysters exposed to dissolved radiotracers at three different pHs – pH 8.1, pH 7.8, and pH 7.5, and two temperatures (21°C in blue and 24°C in red). Points are omitted for clarity and only kinetics models are delineated along the uptake phase. Mean ± 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, respectively.

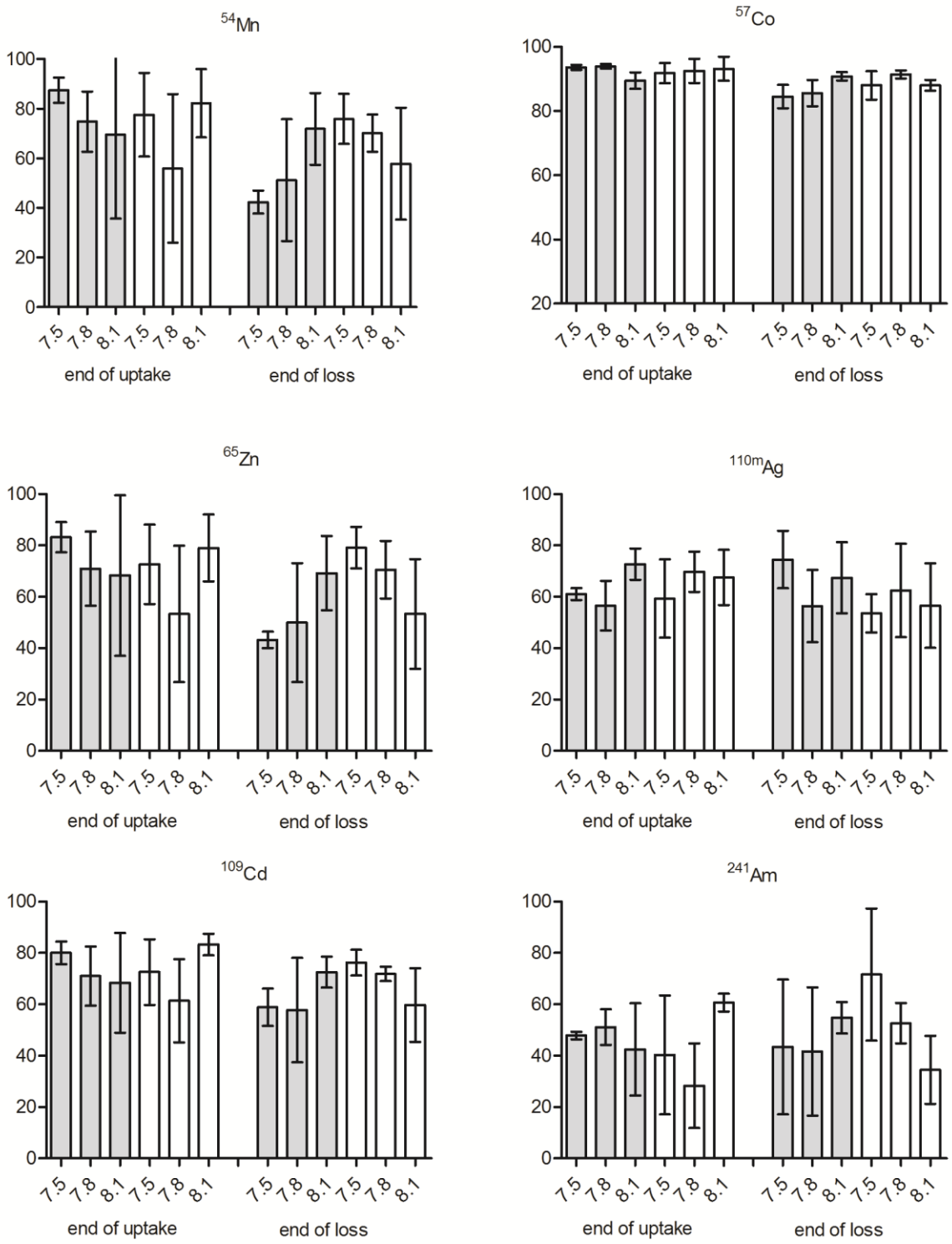


Figure 2: Radiotracer bioaccessibility (%, mean ± SD, n=3) from oyster soft tissues to human consumers assessed using the simulated *invitro* digestion method (gray: 21 °C, white: 24 °C)

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

pH/Temp	^{54}Mn	^{60}Co	^{65}Zn	^{110m}Ag	^{109}Cd	^{241}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

90

91

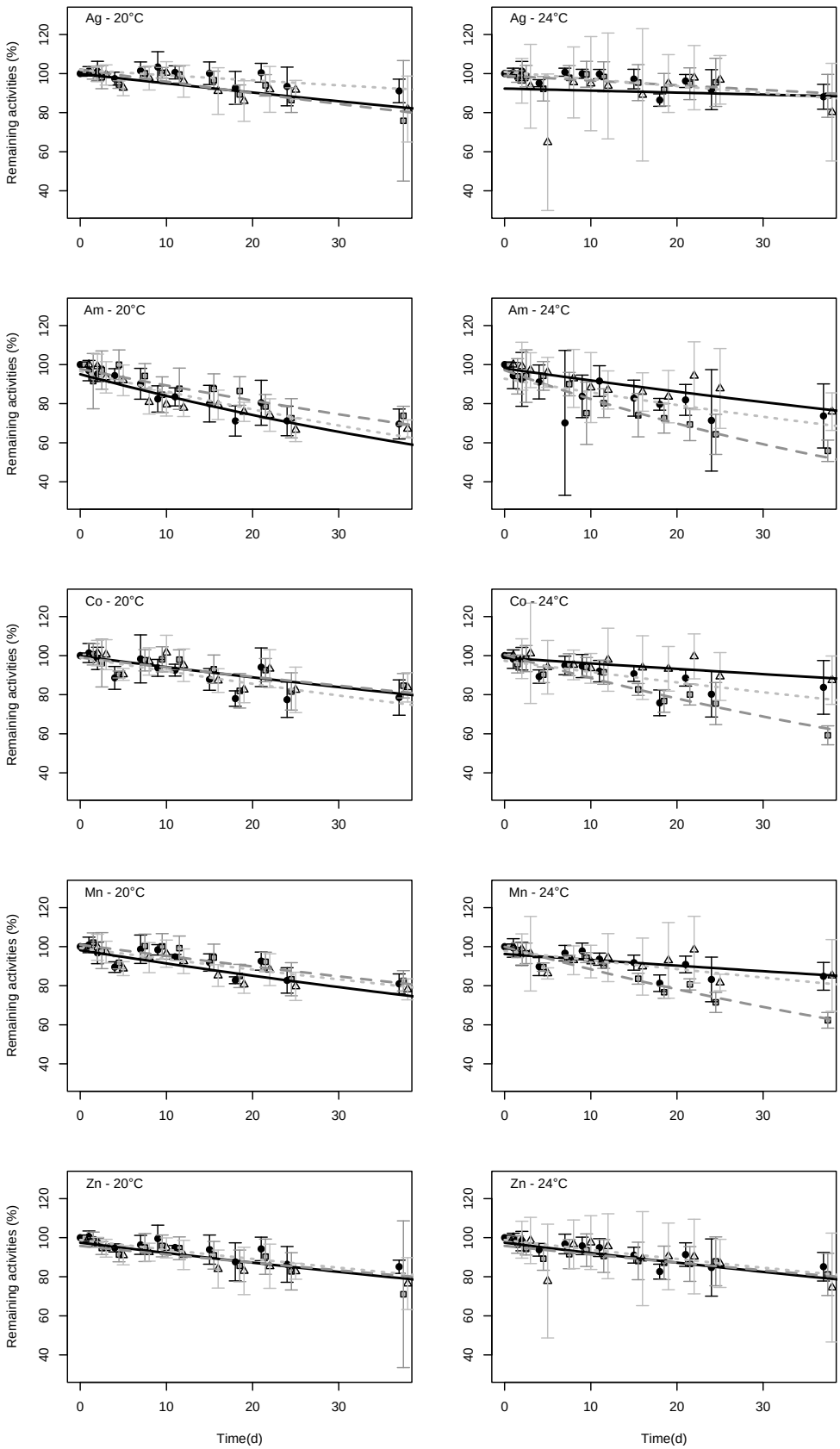


Figure S1. ^{110m}Ag, ^{241}Am, ^{57}Co, ^{54}Mn and ^{65}Zn loss kinetics (CF; mean ± SD; n = 5) in the oysters during the depuration phase following exposure to dissolved radiotracers at three different pH – pH

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