



Assessing the effects of seawater temperature and pH on the bioaccumulation of emerging chemical contaminants in marine bivalves



Ana Luísa Maulvault^{a,b,c,*}, Carolina Camacho^a, Vera Barbosa^a, Ricardo Alves^a, Patrícia Anacleto^{a,b,c}, Fabiola Fogaça^d, Christiaan Kwadijk^e, Michiel Kotterman^e, Sara C. Cunha^f, José O. Fernandes^f, Rie R. Rasmussen^g, Jens J. Sloth^g, Óscar Aznar-Alemany^h, Ethel Eljarrat^h, Damià Barceló^h, António Marques^a

^a Division of Aquaculture and Upgrading (DivAV), Portuguese Institute for the Sea and Atmosphere (IPMA, I.P.), Lisbon, Portugal

^b Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Porto, Portugal

^c MARE – Marine and Environmental Sciences Centre, Faculty of Sciences, University of Lisbon (FCUL), Lisboa, Portugal

^d EMBRAPA, Embrapa Meio-Norte, Parnaíba, Brazil

^e Wageningen University & Research, AB Ijmuiden, The Netherlands

^f LAQV-REQUIMTE, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Porto, Portugal

^g National Food Institute, Technical University of Denmark, Søborg, Denmark

^h Water and Soil Quality Research Group, Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research, (IDAEA-CSIC), Barcelona, Spain

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ABSTRACT

Emerging chemical contaminants [e.g. toxic metals speciation, flame retardants (FRs) and perfluorinated compounds (PFCs), among others], that have not been historically recognized as pollutants nor their toxicological hazards, are increasingly more present in the marine environment. Furthermore, the effects of environmental conditions (e.g. temperature and pH) on bioaccumulation and elimination mechanisms of these emerging contaminants in marine biota have been poorly studied until now. In this context, the aim of this study was to assess, for the first time, the effect of warmer seawater temperatures ($\Delta = +4\text{ }^{\circ}\text{C}$) and lower pH levels ($\Delta = -0.4\text{ pH units}$), acting alone or combined, on the bioaccumulation and elimination of emerging FRs (dechloranes 602, 603 and 604, and TBBPA), inorganic arsenic (iAs), and PFCs (PFOA and PFOS) in two estuarine bivalve species (*Mytilus galloprovincialis* and *Ruditapes philippinarum*). Overall, results showed that warming alone or combined with acidification promoted the bioaccumulation of some compounds (i.e. dechloranes 602, 604, TBBPA), but also facilitated the elimination of others (i.e. iAs, TBBPA). Similarly, lower pH also resulted in higher levels of dechloranes, as well as enhanced iAs, PFOA and PFOS elimination. Data also suggests that, when both abiotic stressors are combined, bivalves' capacity to accumulate contaminants may be time-dependent, considering significantly drastic increase observed with Dec 602 and TBBPA, during the last 10 days of exposure, when compared to reference conditions. Such changes in contaminants' bioaccumulation/elimination patterns also suggest a potential increase of human health risks of some compounds, if the climate continues changing as forecasted. Therefore, this first study pointed out the urgent need for further research on the effects of abiotic conditions on emerging contaminants kinetics, to adequately estimate the potential toxicological hazards associated to these compounds and develop recommendations/regulations for their presence in seafood, considering the prevailing environmental conditions expected in tomorrow's ocean.

1. Introduction

The remarkable increase of the human footprint on the planet (i.e. world population constant growth, excessive use of natural resources, and massive production of pollutants), especially since the mid-20th

century, has contributed to one of the greatest environmental concerns of our time: climate change. Climate change effects can already be felt in many regions of the world, and are expected to worsen in the coming 50–100 years, with devastating consequences at ecological and human scales, even if strong efforts are made to maintain greenhouse gas

* Correspondence to: Portuguese Institute for the Sea and Atmosphere, I.P. (IPMA), Lisbon, Portugal.
E-mail address: aluisa@ipma.pt (A.L. Maulvault).

emissions (GHGs) at the current levels (IPCC, 2014). Such changes also affect marine ecosystems, leading to a potential increase of the average seawater surface temperature (up to + 4 °C), as well as to ocean acidification due to increased levels of atmospheric CO₂, which dissolves into the ocean, dropping seawater pH down to 0.4 units in some areas of the globe (according to scenario 8.5 of the Representative Concentration Pathways of GHG concentrations, i.e. RCP 8.5, of the Intergovernmental Panel for Climate Change, IPCC, 2014). Depending on the region, both climate change effects can act independently as a single stressor, promoting deleterious alterations in marine species metabolism, growth, reproduction, among others, or can occur simultaneously (i.e. combined with each other or with other climate stressors, e.g. hypoxia, salinity) representing additional challenges to the resilience of marine ecosystems (e.g. Rosa et al., 2016; Maulvault et al., 2016, 2017; Sampaio et al., 2017).

Marine species are currently chronically surrounded by an array of chemical contaminants, particularly those inhabiting areas that are more vulnerable to anthropogenic impacts (e.g. estuaries and coastal lagoons) (e.g. Bollmann et al., 2012; Maulvault et al., 2015). New chemical substances that have not been historically recognized as pollutants, and for which limited toxicological information is still currently available, are increasingly more present in the marine environment (e.g. Feo et al., 2012; Cunha et al., 2015; Marques et al., 2015; Van der Meersch et al., 2015). These "contaminants of emerging concern" include various compounds from distinct chemical groups, which may occur naturally in the environment [e.g. inorganic arsenic (iAs)] or are exclusively man-made substances [e.g. flame retardants (FRs), perfluorinated compounds (PFCs), pharmaceutical residues, UV-filters and musks, among others] commonly derived from domestic, hospital and industrial effluents, as well as, agriculture and aquaculture activities (e.g. Bollmann et al., 2012; Feo et al., 2012; Maulvault et al., 2015; Van der Meersch et al., 2015). Arsenic is an ubiquitous element that can occur in the aquatic environment in several oxidation states (− 3, 0, + 3 and + 5), although being mostly found in its most toxic forms, i.e. the inorganic ones (AsIII or AsV; Matschullat, 2000). Arsenic concentrations in coastal ecosystems may range from 1 to 20 µg L^{−1} (Smedley and Kinniburgh, 2001), and in biota the highest concentrations are usually found in bivalve species (total As concentrations up to 24 µg g^{−1} dry weight (dw) (Sloth and Julshamn, 2008; Maulvault et al., 2015). Flame retardants (FRs) are persistent contaminants in the environment, particularly accumulating in sediments and biota, since most of them are extremely hydrophobic. Thus, their concentrations in seawater are usually undetectable or within the range of pg L^{−1} (e.g. Bollmann et al., 2012), whereas in marine sediments and biota values are in the order of pg g^{−1} dw and ng g^{−1} lipid weight (lw), respectively (e.g. Feo et al., 2012; Santín et al., 2013; Van der Meersch et al., 2015). Tetra-bromobisphenol A (TBBPA) is one of the most relevant FR widely used by the industry and can be found in river and estuarine sediments, as well as in biota, reaching up to 14 ng g^{−1} of lw (EFSA, 2011; Van der Meersch et al., 2015). As for other types of FRs, based on their known toxic effects, recently, the EU banned or restricted the use of certain compounds (e.g. PBDEs, Mirex), which were replaced by new substances, defined as emerging FRs [e.g. dechloranes (Decs); Feo et al., 2012] for which limited information is available about their levels in marine environments. PFCs (e.g. perfluorooctanesulfonic acid, PFOS, and perfluorooctanoic acid, PFOA) are molecules composed by carbon chains strongly bound to fluorine atoms, which are widely used in various industrial and consumer activities (e.g. stain-resistant coatings for fabrics and carpets, fire-fighting foams and floor polishes, among others). Their strong carbon:fluorine bounds make them extremely resistant to degradation and, therefore, persistent over time in biological compartments. In aquatic environments, PFCs' concentrations can vary from undetectable to values surpassing 100 ng L^{−1} (Flores et al., 2013) and even exceeding 500 ng g^{−1} of wet weight (ww) in aquatic biota (e.g. EFSA, 2008; Van der Meersch et al., 2015).

Chemical contaminants' availability in marine sediments/water

column and toxicity to biota are strongly influenced by environmental drivers, such as temperature, pH, salinity, upwelling and stratification events. Recent literature has intensively described climate change to likely have a direct impact on contaminants' physical-chemical properties and their partitioning among biological compartments (e.g. Noyes et al., 2009; Marques et al., 2010). Moreover, by altering species physiological status and, at the same time, exacerbating many forms of water pollution, climate change can promote deleterious impacts on marine organisms' physiology, hindering them to cope with the presence of chemical contaminants in the same way as they did before (e.g. Marques et al., 2010; Maulvault et al., 2016, 2017; Sampaio et al., 2017). On the other hand, changes in contaminants' uptake, retention and detoxification rates due to climate change may certainly compromise seafood safety, thus rising concerns from the public health point of view (e.g. Marques et al., 2010; Maulvault et al., 2016). Yet, given the limited number of empirical studies, with most available information being based on mechanistic approaches, the interaction between climate change and pollution still requires further understanding.

In this context, the aim of this study was to assess, for the first time, the effect of temperature ($\Delta = +4$ °C) and pH ($\Delta = -0.4$ pH units), acting alone or in combination, on the bioaccumulation and elimination of emerging chemical contaminants (Dec 602, Dec 603, Dec 604, iAs, TBBPA, PFOA and PFOS), using estuarine bivalve species (*Mytilus galloprovincialis* and *Ruditapes philippinarum*) as biological models. The selection of the model species was based on the fact that these species can be considered suitable bioindicators of environmental pollution, since: i) they are filter-feeding and benthic organisms, thus potentially accumulating high levels of chemical contaminants, either dissolved or in suspended particulate forms, often reaching concentrations several orders of magnitude above the surrounding levels (e.g. Maulvault et al., 2015); and ii) they are among the most commercially important seafood species (both wild or farmed), with high consumption frequencies in Europe, thus enabling to establish a link with seafood safety.

2. Materials and methods

2.1. Experimental design

2.1.1. Chemical contaminants

The contaminants used in each exposure trial were purchased from Toronto Research Chemical Inc. (Toronto, ON, Canada) in the case of Dec 602 (C₁₄H₄Cl₁₂O, ≥ 98%), Dec 603 (C₁₇H₈Cl₁₂, ≥ 98%) and Dec 604 (C₁₃H₄Br₄Cl₆, ≥ 98%), or Sigma-Aldrich (USA) in the case of TBBPA ((CH₃)₂C[C₆H₂(Br)₂OH]₂, 97%), iAs (H₃AsO₄, i.e. As(v) oxide, > 95%), PFOS (heptadecafluorooctanesulfonic acid solution, CF₃(CF₂)₇SO₃H, 10 µg mL^{−1}) and PFOA (CF₃(CF₂)₆COOH, 96%). The remaining reagents used to perform the quantification of each contaminant were of analytical grade or higher, and are given in Section 2.2 throughout the description of the respective methodologies, as well as in Supplementary materials.

2.1.2. Contaminant exposure

To perform contaminant exposure, two different approaches (i.e. exposure routes) were considered taking into account the specific physical-chemical properties of each selected compound: i) Trial I – Exposure via dietary sources through contaminant enriched feed. This exposure was carried out for compounds with hydrophobic behaviour, that are more commonly detected in marine sediments or can potentially be biomagnified along the food chain (due to their long half-lives in animal tissues). Thus, the compounds tested were Dec 602 (water solubility (WS) = 8.49 × 10^{−3} mg mL^{−1}), Dec 603 (WS = 3 × 10^{−2} mg mL^{−1}), Dec 604 (WS = 21 × 10^{−3} mg mL^{−1}; Feo et al., 2012) and TBBPA (WS = 6.3 × 10^{−5} mg mL^{−1}; EU, 2012); and ii) Trial II – Exposure via seawater spiked with contaminant solution. This exposure was carried out for compounds with high water solubility and which are commonly detected in seawater samples. Thus, the compounds

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