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Effects of depuration on metal levels and health status of bivalve molluscs

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ABSTRACT

Depuration has been mandatory to eliminate microorganisms in bivalve molluscs, but not to eliminate toxic chemicals, since there is still shortage of scientific evidence about the efficacy of depuration to decrease the levels of toxic chemicals in bivalves. This study aims to assess the effects of depuration on the levels of toxic, macro and trace elements in three bivalve species (*Ruditapes philippinarum*-RP, *Mytilus galloprovincialis*-MG and *Scrobicularia plana*-SP), taking into account their condition. During depuration, a significant reduction of elements occurred in RP (Fe, Pb, Cu, Rb, Br, Hg, Cd and As), MG (Cl, Sr, Fe, Pb and Br) and SP (Fe, Pb, Cu and Rb), while an increase was registered for other elements in RP (Cl and Sr), MG (S and Zn) and SP (Cl, Zn, Br and Sr). After two days of depuration, Pb in SP had decreased to levels below the permissible limits, thus allowing this species to be acceptable for human consumption as far as toxic elements are concerned. SP revealed the highest glycogen levels compared to the other species indicating different physiological requirements. However, in this species glycogen levels significantly decreased after two days of depuration and mortality increased. In contrast, in MG and RP glycogen and mortality did not vary significantly during depuration. Therefore, SP should be only depurated for a maximum period of two days. Overall, the results of this study are particularly useful to retailers to ensure high quality bivalve products for consumers. Additionally, glycogen can be used as a suitable biomarker of healthy status of bivalve species during depuration.

1. Introduction

Chemical pollution in shellfish-growing waters is a worldwide problem in coastal areas (Almeida & Soares, 2012). Bivalve molluscs (e.g. mussels, oysters and clams) can take up contaminants from sediments, suspended particulate materials, water column and also food sources (Laffon, Rábade, Pásaro, & Méndez, 2006; Livingstone, 1993). They have been widely used for many years as bioindicator (sentinel) organisms in monitoring of chemical pollutants and biomonitoring (estimation of environmental quality) in aquatic ecosystems. This is particularly due to their sedentary nature or immobility, filter-feeding activity, low metabolism, contact with sediments, suitable size for biochemical analysis, wide distribution in marine, estuarine and freshwater environments, practicality in collection, ability to bioaccumulate pollutants and high tolerance to chemical exposure due to a remarkably active immune system (Emmanouil, Kypriotakis, Kungolos, & Machera, 2008; Gupta & Singh, 2011; Waykar & Deshmukh, 2012; Zuykov, Pelletier, & Harper, 2013). Among environmental contaminants, toxic elements are a main concern due to their harmful effects on organisms and ability to bioaccumulate in aquatic ecosystems (Censi et al., 2006). Several bivalve species live in estuaries that are subjected to several anthropogenic pressures, thus being exposed to high levels of toxic elements (Förstner & Wittmann, 1979). The incorporation rate of contaminants in bivalves depends on biotic factors (e.g. species, age, sex, soft-body weight, gametogenesis and physiological status) and abiotic factors (e.g. availability of contaminants in the environment, filtration rate, temperature, salinity, pH,

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chemical species and interaction with other elements) (Fernández-Tajes et al., 2011; Phillips, 1980).

Elements are separated into two categories: essential and non-essential. Essential elements, such as Cu, Fe, Mn, Sr, Se and Zn, have defined biological functions in organisms (Simkiss, 1981; Williams, 1981), whereas non-essential elements, such as Hg, Pb, Cd and As seem not to participate in any metabolic functions (Dallinger, 1995; Suzuki & Suzuki, 1996). The latter elements are among the main toxic elements found in water bodies and may accumulate in bivalve species at high concentrations that can reach several orders of magnitude above those in the environment (Fang, Cheung, & Wong, 2003; Zuykov et al., 2013) and may be biomagnified in the food chain to levels that cause physiological impairment at higher trophic levels and in consumers (Lemiere et al., 2005; Raposo et al., 2009). The routes of transmission from the environment to humans include the consumption of raw or lightly/extensively cooked shellfish, representing a significant human health hazard to consumers (Lees, Younger, & Dore, 2010). Nonetheless, the European Commission has set Maximum Permissible Limits (MPLs) for toxic elements in edible tissues of bivalve molluscs: 0.5 mg/kg for Hg, 1.0 mg/kg for Cd and 1.5 mg/kg for Pb (EC, 2006). Currently, *Scrobicularia plana* from Tagus estuary is an example of bivalve species declared unfit for human consumption due to the high levels of Pb often found above the MPLs (DR, 2013). Between 2000 and 2010, 6.7% of all notifications from the European Union Rapid Alert System for Food and Feed (RASFF) were recorded for bivalve molluscs contaminated with toxic elements (RASFF, 2012; SARF, 2010).

Depuration is currently mandatory in the EU to diminish pathogenic microorganisms' levels (e.g. *Escherichia coli*) in bivalves harvested for human consumption in polluted waters (B category) in order to ensure healthy and safe products for commercialization (Regulation EC Nos. 853/2004 and 854/2004; EC, 2004a, 2004b). This method consists in maintaining bivalves up to 48 h in sterile seawater (through ozone, UV-light, chlorination or iodophors) with sufficient oxygen and without any feed (Lee, Lovatelli, & Ababouch, 2008). Several factors may influence the efficacy of depuration, such as: the system design, initial water quality, oxygenation and flow rates, salinity, temperature, shellfish-to-water ratios, removal and settlement of faecal material, type and amount of pollutants and the duration (Barile et al., 2009; Cozzi, Suffredini, Ciccaglioni, & Croci, 2009; Lee & Younger, 2002; Manfra & Accornero, 2005). Although, depuration is effective to remove faecal bacterial contaminants from bivalves, the effectiveness of depuration to eliminate chemical contaminants is still poorly understood, despite few studies reported lower levels of some toxic metals in bivalves after depuration (e.g. El-Gamal, 2011).

The physiological and biochemical responses of bivalve species to depuration are also poorly understood. Commonly, condition index (CI) and glycogen content are good physiological/biochemical parameters to assess the health status of bivalves. CI has long been used for biological and commercial purposes (Baird, 1958; Venkataraman & Chari, 1951) and is also recognized as a useful biomarker to reflect the ability of bivalves to withstand adverse natural and/or anthropogenic stressors (Bressan & Marin, 1985; Fernandez-Castro & De Vido de Mattio, 1987; Mann, 1978). Glycogen represents the most prominent carbohydrate stored in marine bivalves and is commonly used as an indicator of their nutritional status (Barber & Blake, 1981).

In this context, the aims of the present study were to evaluate the effects of depuration on the potential accumulation/removal of toxic elements (Hg, Cd, Pb and As) and macro/trace elements (S, Cl, K, Ca, Fe, Zn, Br, Cu, Se, Rb and Sr) in three bivalve species (*Ruditapes philippinarum,* RP, *Mytilus galloprovincialis*, MG and *S. plana*, SP) of economical importance in Southern Europe. These species were selected due to their socio-economic relevance (European production amounted to 423 thousand tonnes in 2012; EUROSTAT, 2014), high demand by consumers (Anacleto, Barrento, Nunes, Rosa, & Marques, 2014), as well as to their different feeding strategies, filtration capacities, and habitats (Akberali & Trueman, 1985). In fact, clams (SP and RP) are burrowing species (Akberali & Davenport, 1981; Jones, Sanford, & Jones, 1993), while mussels (MG) live attached to rocky shores in intertidal areas (FAO, 2014). Additionally, the animal condition of these bivalve species (survival, condition index and glycogen content) was also investigated during depuration.

2. Materials and methods

2.1. Study area and collection of bivalves

Three bivalve species were collected in April 2012 at different sampling sites of Tagus estuary (38°44′ N, 9°08′ W; Fig. 1). It is one of the largest estuaries on the west coast of Europe in the most populated area of Portugal, with a broad shallow bay covering an area of 320 km^2 (Brogueira & Cabeçadas, 2006). The bivalve species were: Japanese carpet shell clam *R. philippinarum*, RP (Adams & Reeve, 1850) harvested near Alcochete ($n = 210$); Mediterranean mussel *M. galloprovincialis*, MG (Lamarck, 1819) collected near Algés ($n = 210$); and peppery furrow shell *S. plana*, SP (Da Costa, 1778) harvested near Sítio das Hortas ($n = 210$; Fig. 1). The bivalves were cleaned and washed from mud and sand with seawater, immediately stored at stable conditions (16 °C) and transported in isothermal insulated boxes to the laboratory (approximately two hours of transport period). Biometric data, i.e. total length (mm), width (mm), height (mm), total weight (g) and edible weight (g) were registered from thirty specimens of each species.

2.1.1. Depuration experiment

Depuration was initiated 2 h after bivalves harvesting simulating the commercial practices commonly used (EC, 2004a), and was conducted in three recirculated tanks (width, 31 cm; length, 31 cm; height, 40 cm) for each species, each containing 15 L of UV sterilized and filtered seawater (0.2 μm) taken from open sea in an area not subjected to any anthropogenic pressure and subjected to strong hydro dynamism, likely having lower levels of chemical contaminants than in Tagus estuary. The flow rate entering the circuit was 2.9 L/min. Each tank contained around 2 kg of bivalves, which corresponds to bivalve densities usual in Portuguese depuration facilities. In order to simulate the usual conditions in these facilities, animals were not fed during the experimental period and seawater was kept with continuous aeration and constant temperature (17.6 °C), salinity (35 g/L) and pH (6.98). Thirty specimens from each species (ten from each tank or replicate) were randomly collected at 0, 2, 4, 6 and 8 days of depuration. Behavioural activity was checked using the criteria described by El-Shenawy (2004) with some modifications. Dead specimens were removed and mortality was recorded. Biometric data were also registered at each sampling day for assessment of condition index. The adductor muscle and mantle (including viscera) were separated. Then pooled samples of adductor muscles and mantles, respectively, were made from the ten specimens from each tank. Samples were freeze–dried for 48 h at −50 °C and low pressure (approximately 10^{-1} atm), homogenized with a grinder, vacuum-packed and stored at −80 °C until further analyses. Biochemical analyses were carried out in triplicate.

2.2. Condition index

The Condition Index (CI) was calculated according to the following equation in wet tissue(Maguire, Fleury, & Burnell, 1999):