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Bioaccumulation kinetics of copper in *Ruditapes philippinarum* exposed to increasing, continuous and pulsed exposure: Implications for growth

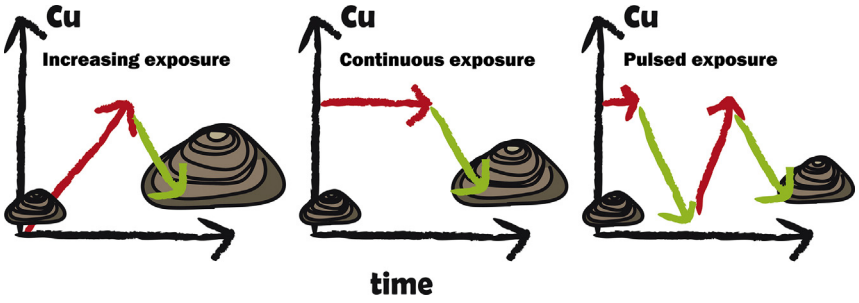
Lígia M.B.M. Santana [a], Julián Blasco [b], Denis M.S. Abessa [c], Olivia Campana [b,*]

[a] Laboratório de Ecotoxicologia Marinha, Instituto de Ciências do Mar (LABOMAR), Universidade Federal do Ceará (UFC), Av. da Abolição, 3207, Meireles, 60165-081 Fortaleza, CE, Brazil
[b] Departamento de Ecología y Gestión Costera - Instituto de Ciencias Marinas de Andalucía (CSIC), Campus Rio San Pedro, 11510 Puerto Real, Cadiz, Spain
[c] Núcleo de Estudos em Poluição e Ecotoxicologia Aquática (NEPEA), Campus Experimental do Litoral Paulista (UNESP), Praça Infante Dom Henrique s/n, Parque Bitaru, 11330-90 São Vicente, SP, Brazil

HIGHLIGHTS

- Growth rate was inversely correlated to the net copper bioaccumulation rate.
- Large stimulatory effect on growth observed during the recovery period.
- Pulsed exposure has a more adverse effect compared to increased or continuous exposures.

GRAPHICAL ABSTRACT



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ABSTRACT

Metal bioaccumulation and toxicity to aquatic organisms depends on factors such as magnitude, duration and frequency of the exposure. The type of the exposure affects the toxicokinetic processes in the organisms. In this study, we carried out 30-day toxicity tests on juveniles of *Ruditapes philippinarum* exposed to increasing, continuous and pulsed exposure. Organisms were exposed to copper-spiked sediments followed by a 10-day recovery period. We assessed the interaction between the kinetics of subcellular copper partitioning and the growth response. Results showed that the growth rate of the bivalve was inversely correlated to the bioaccumulation rate and that sublethal copper concentrations stimulated the detoxification mechanisms inside the organism regardless the type of the exposure. However, a large stimulatory effect on growth was observed during the recovery period, associated with significant negative accumulation rate values and dependent on the type of antecedent exposure. This suggested that on individual and short-term basis pulsed exposures have a more adverse effect compared to increasing or continuous exposure scenarios.

1. Introduction

Aquatic organisms are often exposed to metal contamination and the exposure can be continuous or episodic as a consequence of several activities, e.g. urban or domestic run-off, natural biogeochemical processing, application of agrochemicals, periodic release of industrial waste waters etc. The effect of the exposure will depend on its magnitude, duration and frequency and affect the toxicokinetic processes. These processes regulate the intracellular concentrations of metal ions into the organism, essential for the maintenance of life (George, 1982; Phillips and Rainbow, 1989). Very little is known about the bioaccumulation kinetics of metals and effects on the organisms during intermittent compared to continuous exposures (Amachree et al., 2013).

* Corresponding author.
E-mail address: olivia.campana@icman.csic.es (O. Campana).

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Essential metals, like copper, can be regulated by limiting the metal uptake at the body concentration level, or by metal- and organism-specific accumulation strategies that include active elimination of the metal excess as well as its storage as an inert form (Rainbow, 2002; Rainbow et al., 2015; Vijver et al., 2004). The complexity of the internal metal binding supports the evidence that a tissue-residue approach based on the total tissue concentrations can fail to predict metal toxicity because metal compartmentalization within specific pools, such as extracellular (metal-rich granules) or intracellular (lysosomes, metallothionein-like proteins) structures, makes it possible to sequester metals in a detoxified form. Detoxification strategy typically varies depending on metal, e.g. copper has affinity to nitrogen or sulphur-containing chemical groups (Marigómez et al., 2002; Vijver et al., 2004), and organism but also depends on the field history of the organism itself, which can be subjected to chronic exposure (Giguère et al., 2003; Rainbow et al., 2015).

Characterization and kinetics of the subcellular distribution of metals have significant ecotoxicological implications because they will determine which fraction of metal is metabolically available and contributes to potential toxicity. Toxicity may commence if the metabolically available concentration exceeds the toxic threshold concentration that will occur when the metal influx rate exceeds the combined rate of detoxification and elimination (Rainbow and Luoma, 2011a; Simpson and King, 2005). This study was designed to gain more information and understanding about the different effects of continuous and intermittent exposures to metal contaminants and their implications on subcellular metal distribution. Thus, the aim of this work was to investigate the links between the kinetics of subcellular copper bioaccumulation and the growth response of *Ruditapes philippinarum* exposed to increasing, continuous and pulsed exposure to copper-spiked sediments and during a recovery period. This bivalve is an infaunal suspension feeder and was selected as test organism according to earlier studies (Chong and Wang, 2000; Fan and Wang, 2001) that have demonstrated that sediments often constitutes an important food source for this clam due to its resuspension.

2. Materials and methods

2.1. Test organisms

Ruditapes philippinarum (Adams and Reeve, 1850) is a native bivalve from the Indo-Pacific region introduced in Europe in the 1970s for commercial purposes and appreciated for human consumption (Delgado and Pérez-Camacho, 2007). This bivalve is an infaunal suspension feeder and earlier studies (Chong and Wang, 2000; Fan and Wang, 2001) have demonstrated that sediment often constitutes an important food source for this clam due to its resuspension. Juveniles of *R. philippinarum* (8–10 mm shell length) were purchased from an aquaculture farm (Amalthea, Cadiz), held in polypropylene tanks in a flow-through system and acclimated 7 days in a temperature controlled room (dissolved oxygen 10.1 ± 4.1 mg L^{-1}, pH_{water} 7.4 ± 0.1, temperature 19.0 ± 0.6 °C, salinity 34.6 ± 0.5; means ± SD). Clams were fed twice a week ad libitum with Sera Micron powdered fish food (Fishtamins).

2.2. Sediment collection and spiking

Estuarine sediment was collected from a pristine site in the Bay of Cadiz (SW Iberian Peninsula; 36°23′31.80″N, 6°12′24.01″W). At the time of collection sediment was press-sieved through a 1.0-mm mesh to remove large debris and indigenous macrofauna and stored at 4 °C in the dark for up two weeks until spiking. Sediment at this location has been previously characterized and found to have low metal contamination (Campana et al., 2013a). After thorough homogenization, a subsample was used for the geochemical characterization including the determination of the particle size content (25% particles <63 μm), particulate organic carbon content (2% OC) and bulk sediment copper concentration (28 μg Cu g^{-1}). Because it has been demonstrated (Campana et al., 2013b; Campana et al., 2012; Strom et al., 2011) that the geochemical properties of the sediment affect copper bioavailability for deposit feeder species, these values were taken into account to yield OC-normalized copper spiking concentrations suitable to induce a sublethal effect without affecting the survival rate based on an previous, not published (O. Campana personal communication) study with *R. philippinarum*.

Sediments were spiked following the procedure described by Simpson et al. (2004). Briefly, copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) was prepared as an aqueous stock solution and added on a per dry weight basis to the sediment in order to yield three nominal copper concentration of 10 (low), 15 (medium) and 25 (high) mg Cu <63 μm g^{-1} OC taking into account spike and background. Required amounts of stock solution were added on a sediment to water ratio 4:1 (v/v). Twenty-four hours after spiking, pH was measured (7.7 ± 0.1; mean ± SD) and pH neutralization was considered unnecessary. Sediments were thoroughly mixed using a plastic spatula several times during the first week and then allowed to equilibrate for 30 days, at 4 °C.

2.3. Experimental design

In order to study the effects of the different types of exposure, sublethal whole-sediment toxicity tests were conducted exposing clams to spiked sediments into 8 L polycarbonate tanks in a flow-through system, at flow rate of 130 mL/min, during 40 days and sampled at different times (T0 = initial time, T1 = 10 days, T2 = 20 days, T3 = 30 days and T4 = 40 days). Sampling periods of 10 days were selected to obtain a sensitive measure of the juveniles' growth variation. At T0, 60 juvenile clams were separated from the batch, weighed and put into each tank. The average wet weight (tissue + shell) of the bivalves at T0 was recorded for each tank and used to calculate growth rate. Four treatments, each one tested in triplicate, were set up simultaneously with: (1) *control*, a group kept in unspiked sediment; (2) *increasing exposure*, where bivalves were subsequently exposed to increasing copper concentrations during 10 days each; (3) *continuous exposure*, where bivalves were maintained at the highest concentration for 30 days; and (4) *pulsed exposure*, where organisms were exposed to pulsed events by alternating the highest copper concentration and the control every 10 days. All trials ended after a recovery period (T4) of 10 days during which bivalves were exposed to the unspiked original sediment. Each 10 days, 10 clams were sampled from each replicate, allowed to depurate 24 h in natural clean seawater, weighed for growth analysis and soft tissues were pooled and frozen at −80 °C until metal analysis. Throughout the experiments subsamples of sediment, pore and overlying water were collected to determine copper concentrations and physico-chemical variables were recorded. Following the experimental design, tanks were set up (including sediment, water and aeration) two days before the shift of the bivalves for the next exposure to allow the equilibration of the physico-chemical conditions into the tank. The diet of the bivalves was supplemented with 1 mg per tank of Sera Micron powdered fish food (Fishtamins) on alternate days.

2.4. Sediment and water analyses

All plastic and glassware were pre-cleaned by soaking in 10% HNO_3 (AR grade, Merk, Germany) for 24 h and rinsed with higher grade deionized water (Milli-Q, 18 MΩ cm, Millipore). Sediment particle size was determined by wet-sieving through a 63-μm sieve using a minimum amount of deionized water followed by gravimetry. Particulate organic carbon (OC) content was determined by wet oxidation followed by titration using 888 Titrando analyzer (Metrohm, USA). Copper concentrations were analyzed in bulk sediment, in the <63 μm fraction and pore and overlying water. Sediment samples were oven dried at 60 °C for 24 h and digested according to the following procedure. Briefly,