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Acartia tonsa eggs as a biomonitor to evaluate bioavailability/toxicity of persistent contaminants in anoxic/sulfidic conditions: The case of cadmium and nickel

Sandra Sei [a], Marion Invidia [a], Marco Giannetto [b], Gessica Gorbi [a,*]

[a] Department of Life Sciences – University of Parma, Parco Area delle Scienze 11/A, 43124 Parma, Italy
[b] Department of Chemistry – University of Parma, Parco Area delle Scienze 17/A, 43124 Parma, Italy

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ABSTRACT

The evaluation of toxicity due to persistent pollutants in anoxic aquatic environments has met with various problems, as most test organisms can not withstand oxygen lack and exposure to free sulfide. We evaluated the suitability of the eggs of the brackish copepod *Acartia tonsa* for bioassays in anoxic/sulfidic conditions: when exposed to deep hypoxia and free sulfide, the eggs become quiescent and are able to resume hatching after restoring normoxic conditions. Tests with cadmium and nickel were performed in normoxic and deeply hypoxic conditions and in anoxic water containing H_2S or $H_2S+FeSO_4$ on an equimolar basis. Active and quiescent eggs showed equivalent sensitivity to the metals, both suffering significant reductions in hatching success at 89 μM Cd and 17 μM Ni. As expected on the basis of the SEM/AVS model, Cd toxicity was almost completely suppressed in presence of sulfides. Dissolved Cd concentration drastically dropped and hatching success was generally $>80\%$, as against values $<6\%$ observed in sulfide-free water, indicating that the applied experimental procedure can simulate metal-sulfide interaction. Ni toxicity was only slightly reduced by the presence of sulfides. High dissolved Ni concentrations were detected and mean hatching percentages were $\leq 32\%$, suggesting that Ni bioavailability/toxicity was only partially controlled by excess reactive sulfides. The results suggest that *A. tonsa* eggs could be a useful biomonitor to evaluate toxicity due persistent contaminants in anoxic conditions and the role of sulfides in reducing metal bioavailability/toxicity.

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1. Introduction

The evaluation of toxicity due to persistent pollutants in anoxic aquatic environments has met with various problems. Oxygen lack is often, if not always, coupled with the presence of free sulfides and both can contribute to negatively affect aquatic organisms, so that the cause of toxicity could be not properly identified. While sulfide-contaminated sediments can be rapidly rendered harmless by re-oxygenation (Wang and Chapman, 1999), sediments polluted by persistent contaminants require more complex remedial approaches. Heavy metal pollution is one of the critical issues since metal bioavailability strongly depends on partitioning processes, among which formation of poor soluble metal sulfides appears to have a key role. Di Toro et al. (1990, 1992) evidenced the role of the reactive pool of solid-phase sulfides, termed Acid Volatile Sulfides (AVS), in controlling metal bioavailability, and hence toxicity, in marine and freshwater sediments. AVS, operationally defined as the fraction of sulfides that releases H_2S when treated with cold acid, are thought to be primarily constituted by iron and manganese monosulfide and are available to bind with metals: Fe and Mn can be displaced by other more toxic metals (such as Cd and Ni) which form less soluble sulfides. Cationic metals, extracted under the same condition as AVS, are operationally defined as Simultaneously Extracted Metals (SEM). According to Di Toro and colleagues, when the molar concentration of AVS is higher than that of SEM (SEM/AVS <1 or SEM - AVS <0), no metal toxicity is expected. On this basis, they proposed the SEM/AVS approach to predict sediment toxicity. It is well known that, in addition to AVS, metal bioavailability can be influenced by other binding phases such as organic matter. More recently, Di Toro et al. (2005) proposed an extension of the SEM/AVS method that also takes into account metal partitioning to sediment particulate organic carbon.

Numerous studies focussed on verifying the accordance between toxicity predicted on the basis of the SEM/AVS model and toxicity determined by bioassays. Ankley et al. (1996) summarized results from laboratory and field bioassays with marine and freshwater sediments and several species. They suggested that "an absence (but not necessarily a presence) of metal toxicity can be

* Corresponding author.
E-mail address: gessica.gorbi@unipr.it (G. Gorbi).

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reliably predicted based upon metal-sulfide relationships and/or pore-water metal concentrations". Berry et al. (1996) observed little or no mortality in a tube-dwelling amphipod exposed to sediments with SEM/AVS >1 and reported literature data indicating that mortality of various test species increased only in sediments with SEM/AVS >5.9. They hypothesized that, along with additional binding phases, organism behaviour during toxicity tests, such as avoidance of contaminated sediments and lack of burrowing, could have controlled exposure thus limiting metal impact. In in situ experiments on benthic organisms exposed to Cd-spiked freshwater sediments, Warren et al. (1998) observed that, while cadmium activities in pore waters were in agreement with SEM/AVS model, Cd accumulation by organisms did not and most animals bioaccumulated Cd almost exclusively from the overlying water. Vandegehuchte et al. (2007) observed a significant toxic effect, due to nickel, in the benthic oligochaete *Lumbriculus variegatus* exposed to bulk (whole) sediment cores, although no toxicity was expected at [SEM-AVS] <0. According to the authors, toxicity was due to oxidation of AVS in the top layer at the sediment-overlying oxidized water surface.

These studies underline two relevant aspects of metal bioavailability/toxicity assessment in anoxic/sulfidic conditions: i) organism behaviour (such as bioturbation, burrow depth, change in frequency and/or irrigation rate, feeding particle source) can influence their relative exposure to metals (Simpson and Batley, 2007); ii) although sulfide concentration and metal speciation can be maintained by testing under nitrogen atmosphere, most test organisms can not withstand anoxia and require oxygen-rich waters that can modify sediment and pore water chemistry (Wang and Chapman, 1999).

Our previous work (Invidia et al., 2004) demonstrated that the subitaneous eggs of the planktonic copepod *Acartia tonsa* Dana (Crustacea, Copepoda) become quiescent when exposed to severe hypoxia (dissolved oxygen concentration $<8\times10^{-3}$ mmol L^{-1}) or to dissolved sulfides (1 mM). The eggs are able to resume hatching when normoxic conditions are restored (see also Marcus and Lutz (1994) and Nielsen et al. (2006)) and, when exposure to hypoxia or sulfide lasts a time period ≤ 96 h, they show a hatching success not significantly different from that of the eggs maintained in normoxic conditions. These findings have suggested that *Acartia* eggs could allow to overcome most of the constraints encountered in tests with other species in anoxic/sulfidic conditions: i) being able to withstand deep hypoxia, they do not require oxidized water so that the risk of modifying chemical speciation is ruled out; ii) being able to withstand free sulfides (≤ 1 mM), they can allow to exclude this additional confounding cause of toxicity in anoxic water when toxicity due to contaminants other than sulfide has to be detected. Furthermore, the eggs of *Acartia* are sensitive to environmental pollutants (Barata et al., 2002; Lindley et al., 1998; Lindley et al., 1999) and, as they tend to sink and can not actuate avoidance behaviours, exposure is certain. On these bases, we hypothesized that *A. tonsa* eggs could constitute a suitable tool to evaluate bioavailability/toxicity of metals and the protective role of different binding substances in anoxic/sulfidic water. A procedure for conducting bioassays with *A. tonsa* eggs in anoxic/sulfidic conditions was set up by testing cadmium and nickel toxicity. The suitable conditions for the evaluation of Cd and Ni toxicity in presence of sulfides (both free sulfides and iron sulfides) were recreated in laboratory taking into account the fast precipitation reaction between aqueous iron (II) and dissolved sulfide (Rickard, 1995) and the competition of divalent cationic metals such as Cd and Ni with Fe(II) for binding sites on AVS (Di Toro, 1992; Nieboer and Richardson, 1980). Cd and Ni are among the most used metals in studies focussed on SEM/AVS model. Their toxicity is well documented both in wildlife and humans (Eisler, 1998; UNEP, 2010; Sarkar et al., 2013): both metal are able to interfere with various biochemical and physiological pathways/processes, are regarded as inducers of oxidative stress and cancer and are among hazardous contaminants of aquatic ecosystems.

As quiescence is a depressed metabolic state that allows organisms to limit the accumulation of toxic end-products of metabolism (Clegg, 1997; Nielsen et al., 2006), test with metals in deeply hypoxic conditions were performed on both active and quiescent eggs to check if in this metabolic state sensitivity to toxicants may be altered.

2. Materials and methods

2.1. Sampling and maintenance

Acartia tonsa was originally collected in the Venice lagoon (North Adriatic Sea, Italy) and successively reared in laboratory in aquaria containing natural seawater (NSW) (about 8 l; S $=30\pm2$‰; pH $=8.2\pm0.1$) filtered on 0.45 μm mixed cellulose ester filter. The aquaria were maintained in a constant temperature room at 20 ± 1 °C and a 14 h light: 10 h dark cycle and continuously aerated. The cultures were fed to excess a mixture of three algal species: *Isochrysis galbana* (CCAP 927/1), *Tetraselmis suecica* (CCAP 66/22A) and *Rhinomonas reticulata* (CCAP 995/2). Algal species were cultured at the same conditions as described by Invidia et al. (2004).

Information about biology/physiology of *A. tonsa* is available in the Additional Supporting information of the article by Gorbi et al. (2012).

2.2. Experimental test procedures

The eggs for the experiments were obtained from adults isolated from laboratory-reared populations. About 48 h before the beginning of the experiments, samples of the stock cultures were siphoned out of the aquaria and poured into crystallising dishes. Afterward, adult females and males were sorted from the samples of the stock cultures (the gender of each individual was rapidly checked under a dissection microscope), transferred into a crystallising dish containing 700 ml of NSW and fed with *Isochrysis galbana*, *Tetraselmis suecica*, and *Rhinomonas reticulata.* Approximately fifteen hours before the beginning of the experiments, the sorted organisms were randomly distributed into smaller crystallising dishes (10–12 organisms per crystallising dish) containing 200 ml NSW and fed. The eggs spawned during the subsequent 15 h were collected into Petri capsules and used for the bioassays. At the above-described culture conditions in normoxic water, most *A. tonsa* eggs hatch within 24 h after spawning. Hatching success was used as endpoint.

Adult sorting, egg collection and transferring as well as hatching and nauplius viability check were done under a dissection microscope. To guarantee the constant composition of the medium, all the bioassays were carried out in artificial seawater (ASW, pH 8.2 ± 0.1), prepared according to APHA, AWWA and WPCF (1989) and modified in the content of NaCl to obtain a salinity of 30 ± 1‰.

This method has been proven to guarantee a good repeatability and reproducibility of toxicity tests with the egg/nauplius stages of *A. tonsa* (Gorbi et al., 2012).

2.2.1. Test with metals in hypoxic conditions

Pilot experiments in deeply hypoxic water were performed to evaluate if quiescence may alter egg sensitivity to metals. To this aim, i) active eggs were directly transferred to hypoxic metal solutions: in this case the eggs become quiescent during exposure to the metal; ii) active eggs were incubated for 24 h in metal-free

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