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Testing lagoonal sediments with early life stages of the copepod *Acartia tonsa* (Dana): An approach to assess sediment toxicity in the Venice Lagoon

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ABSTRACT

The early-life stages of development of the calanoid copepod *Acartia tonsa* from egg to copepodite I is proposed as an endpoint for assessing sediment toxicity by exposing newly released eggs directly onto the sediment-water interface.

A preliminary study of 5 sediment samples collected in the lagoon of Venice highlighted that the larval development rate (LDR) and the early-life stages (ELS) mortality endpoints with *A. tonsa* are more sensitive than the standard amphipod mortality test; moreover LDR resulted in a more reliable endpoint than ELS mortality, due to the interference of the sediment with the recovery of unhatched eggs and dead larvae. The LDR data collected in a definitive study of 48 sediment samples from the Venice Lagoon has been analysed together with the preliminary data to evaluate the statistical performances of the bioassay (among replicate variance and minimum significant difference between samples and control) and to investigate the possible correlation with sediment chemistry and physical properties.

The results showed that statistical performances of the LDR test with *A. tonsa* correspond with the outcomes of other tests applied to the sediment-water interface (*Strongylocentrotus purpuratus* embryotoxicity test), sediments (*Neanthes arenaceodentata* survival and growth test) and porewater (*S. purpuratus*); the LDR endpoint did, however, show a slightly higher variance as compared with other tests used in the Lagoon of Venice, such as 10-d amphipod lethality test and larval development with sea urchin and bivalves embryos. Sediment toxicity data highlighted the high sensitivity and the clear ability of the larval development to discriminate among sediments characterized by different levels of contamination. The data of the definitive study evidenced that inhibition of the larval development was not affected by grain-size and the organic carbon content of the sediment; in contrast, a strong correlation between inhibition of the larval development and the sediment concentrations of some metals (Cu, Hg, Pb, Zn), acid-volatile sulphides (AVS), polychlorinated biphenyls (PCBs) and polynuclear aromatic hydrocarbons (PAHs) was found. No correlation was found with DDTs, hexachlorobenzene and organotin compounds.

1. Introduction

Planktonic copepods represent the major component of the marine ecosystem. They feed on phytoplankton and protozoans and serve as food reservoir for the planktonic larvae of fishes (Turner, 2004). As such they play a key role in the food webs of the marine and oceanic environments. A number of factors make the copepods a useful bioindicator for the assessment of the adverse effects of chemicals and effluents in surface waters after short-term exposure, including their worldwide distribution, ecological relevance, short generation times and easy culturing (US EPA, 1978). Indeed copepods have been used for this purpose since the late seventies (USEPA, 1978; Bengtsson, 1978); in fact, copepod mortality is a methodologically easy endpoint to evaluate. An international standard is also available for the acute lethality test with the calanoid *Acartia tonsa* Dana, the harpacticoids *Nitocra spinipes* Boeck and *Tisbe battagliai* Volkmann-Rocco (ISO, 1999).

Nevertheless, several studies performed over recent years have evidenced the ability of endpoints such as egg-production, hatching success and early life stage development to indicate significant impairments at concentration levels several times lower than those

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affecting survival (Hutchinson et al., 1994; Kusk and Petersen, 1997; Lotufo, 1997; Andersen et al., 1999; Breitholtz and Bengtsson, 2001; Christoffersen et al., 2003; Gorbi et al., 2012; Zhou et al., 2016). In addition, the endocrine regulation of critical processes including molting, sexual differentiation and growth, makes the copepods particularly well suited for detecting the effects of chemicals that affect or interfere with neuro-endocrine signalling (the synthesis of hormones, binding to receptors or simulating hormone functions). The suitability of the copepods and the growing interest for endocrine disrupters testing has led to the recent development of several methods for detecting sub-lethal and chronic effects towards the calanoid *A. tonsa* (Andersen et al., 2001) and the harpacticoids *N. spinipes* (Breitholtz et al., 2003), *T. battagliai* (Hutchinson et al., 1999a, 1999b), *Tigriopus japonicus* Mori (Marcial et al., 2003; Kyun Woo et al., 2013), *Amphiascus tenuiremis* Brady and Robertson (ASTM, 2004a) and *Eurytemora affinis* Pope (Forget-Leray et al., 2005; Lesuer et al., 2013). An OECD guideline for detecting reproductive and developmental effects of endocrine disrupters with *A. tonsa*, *N. spinipes*, *T. battagliai* and *A. tenuiremis* has been under validation (Kusk and Wollenberger, 2007; OECD, 2007) and a ISO Standard Method (ISO 16778) for assessing water quality with the early life stages of *A. tonsa* has been recently published (ISO, 2015).

Although several studies have been dedicated to testing chemicals, the use of sub-lethal endpoints and chronic tests for assessing environmental matrices is not yet widespread; in fact, only very few references are available in the literature on elutriate (Williams, 1992) and sediment testing (Kovatch et al., 1999; Wollenberger and Kusk, 2006). This is surprising given that a number of factors make copepods particularly useful for elutriate, pore-water and sediment testing including their sensitivity to toxic substances, easy laboratory cultivation, the widespread need for small volume samples and the availability of several sub-lethal endpoints.

Sediment toxicity is most often assessed using adult amphipods (or less frequently with polychaetes) following their acute exposure to the whole sediment (Kennedy et al., 2009); nevertheless, although these methods have proved to be a valuable tool for the detection of acute effects in hot-spots of contamination, their ability to highlight impairments in sediment with low to moderate contamination is uncertain (USEPA, 2001; Picone et al., 2016). Sub-lethal and chronic effects are seldom investigated in whole-sediment testing, since the procedures are often expensive and time-consuming, both for amphipods and polychaetes, requiring greater scientific and technological effort as compared to acute tests (Costa et al., 2005). In contrast, early-life stages of benthic and planktonic invertebrates offer the possibility of assessing the sub-lethal effects exerted by sediment-bound contaminants on the sediment-water interface (SWI) (Anderson et al., 1996, 2001). SWI testing with early-life stages is ecologically relevant for a number of reasons: firstly, early-life stages are more sensitive than adult stages (Ringwood, 1992; Hutchinson et al., 1998) and the sub-lethal effects that can be measured on early-life stages may have greater ecological relevance than lethality to identify possible impairments due to the exposure to the contaminants.

Secondly, the SWI plays a crucial role in the marine environment since gametes and larval stages of many marine species are negatively buoyant and spend most of their life span at this interface, where mineralization of detritus, deposition from water columns and release from the sediments may contribute to the accumulation of toxicants (Anderson et al., 2001, and citation herein).

The larval development test (LDR test) proposed by Wollenberger and Kusk (2006) allows the determination of the effects due to the fraction of soluble contaminants released from the sediment into the overlying water during the test. The method is based on the exposure of the copepods at early stages of development to the sediments, starting with the egg-stage. The test assesses the ratio of surviving animals that passed through the nauplii stages and reached a copepodite stage after 5–6 days of exposure (Andersen et al., 2001). Within this period about the 50% of control animals should have metamorphosed into at least copepodite stage I. The 50% ratio was selected as it represents the optimal development point to enable the observation of potential inhibitory or stimulatory effects (Kusk and Wollenberger, 2007). Furthermore, nauplii and copepodites are morphologically clearly distinct, so that the transition from the sixth naupliar stage to the first copepodite stage is quite easy to observe under a dissecting microscope. Moreover, the establishment of a laboratory culture of *A. tonsa* enables the recruitment of organisms of good quality and sensitivity throughout the year, avoiding the disadvantages (limited in-field availability and seasonal changes of health and sensitivity) related to the use of wild populations of other crustaceans, e.g. amphipods.

Taking the Lagoon of Venice as the selected area of investigation, the present study focuses on the evaluation of the sensitivity of the larval development test with *A. tonsa* as an applicable method for the routine testing of sediment toxicity. A first, preliminary application of the LDR test on a restricted number of frozen samples (n = 5) has been performed to compare the sensitivity of the early life stages toxicity test with *A. tonsa* with the standard 10-d lethality test with the amphipod *Corophium orientale* (both performed on whole sediment, but *C. orientale* on freshly collected, not frozen sediment). The promising results obtained in the preliminary study suggested the need to perform further assessment on LDR test performance in a definitive study, by testing a larger number of freshly collected sediments, characterized by different grain size, total organic carbon (TOC) content and occurrence of contaminants which may cause risk or adverse effects to the biota (Contaminants of Potential Concern - COPC). The definitive study was then performed on 48 sediment samples collected in the framework of the HICSED. This whole-lagoon scale project is promoted and funded by the Ministero delle Infrastrutture e dei Trasporti – Magistrato alle Acque di Venezia through its concessionaire Consorzio Venezia Nuova, with the aim of quantifying the hazard due to sediment contamination, using ecotoxicological criteria.

The data collected during both the preliminary and definitive study have been analysed with the following specific goals: 1) the assessment of the statistical performances of the bioassay (especially as concern the LDR endpoint), through analysis of the variance and subsequent calculation of a toxicity threshold (TT) using the minimum significance difference criterion (Thursby et al., 1997); 2) the evaluation of the potential role of grain-size, organic carbon and acid volatile sulphides as confounding factors; 3) a verification of the ability of the test to discriminate among sediments of different degrees of contamination; 4) an identification of possible correlations between LDR and contaminant's concentrations, also taking into account their bioavailability and mobility, i.e. Simultaneously Extracted Metals (SEM) and Acid Volatile Sulphides (AVS).

2. Materials and methods

2.1. Study area

The Venice lagoon is a coastal wetland with a surface of about 549 km^2 located in North-Eastern Italy, with an average mean depth referred to the mean sea level of about 1 m (Sfriso and Marcomini, 1996). The lagoon is connected with the Adriatic Sea by 3 sea inlets that allow for the exchange of about 60% of the water in any 12-h cycle (Sfriso and Marcomini, 1996). It includes estuarine and marine environments, pristine salt marshes and shallows, as well as reconstructed marshes, reclaimed land and human environments such as the city of Venice and the industrial district. Point and nonpoint sources of pollution flowing into the lagoon include: industrial waste from the area of Porto Marghera, treated and untreated municipal wastewaters from the cities of Venice and Mestre, streams, agricultural runoff, boat traffic and atmospheric deposition (Volpi Ghirardini et al., 2005).