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Estuarine sediment acute toxicity testing with the European amphipod *Corophium multisetosum* Stock, 1952

Ana Ré, Rosa Freitas, Leandro Sampaio, Ana Maria Rodrigues, Victor Quintino *

CESAM (Centro de Estudos do Ambiente e Mar), Departmento de Biologia, Universidade de Aveiro, 3810 – 193 Aveiro, Portugal

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ABSTRACT

This study assessed the use of the European amphipod *Corophium multisetosum* Stock [Stock, J.H., 1952. Some notes on the taxonomy, the distribution and the ecology of four species of the genus *Corophium* (Crustacea, Malacostraca). Beaufortia 21, 1–10] in estuarine sediment acute toxicity testing. The sensitivity of adults to the reference toxicant $CdCl_2$ was determined in water-only 96 h exposures in salinity 2. LC_{50} values ranged from 0.33 $mgCd^{2+}\ L^{-1}$ at 22 °C to 0.57 $mgCd^{2+}\ L^{-1}$ at 15 °C. Adult survival was studied in control sediment with water salinity from 0 to 36 and with fine particles content (<63 μm) from 2% to 97% of total sediment, dry weight. Experiments were conducted at 15, 18 and 22 °C and the results indicate that the species can be used under the full salinity range although higher mortality was observed at the lower salinity in the higher water temperature, and at the higher salinity in the lower water temperature. The species also tolerated the studied range of sediment fines content and showed the highest sensitivity at intermediate values of fines, especially at the higher temperature, thus advising that tests which have to accommodate sediments with a wide range in fines content should preferably be conducted at 15 °C rather than at 22 °C. The response in natural sediments was studied in samples collected yearly from 1997 to 2006, at a site located off the Tagus Estuary, western Portugal. A major flood event in winter 2000–2001 induced detectable alterations in sediment baseline descriptors (grain-size, redox potential and total volatile solids), organic contaminants (PAHs, PCBs, DDT metabolites and γ-HCH) and the macrofauna benthic community. Mortality of the amphipod diminished significantly from the before to the after flood period, in close agreement with diminishing sediment contamination and increasing benthic fauna diversity, in the same time period. *C. multisetosum* is suitable to conduct acute sediment toxicity tests and presents good potential for the development of a full life-cycle sediment test, due to its amenability to laboratory culture and high survival in the control sediment.

1. Introduction

Sediment bioassays are a powerful tool for the study of sediment toxicity and are recommended along with others methodologies to classify and prioritise areas of contaminated sediments (Chapman and Long, 1983; Luoma and Carter, 1993; Riba et al., 2004; Kirkpatrick et al., 2006; Allen et al., 2007; Scarlett et al., 2007; Morales-Caselles et al., 2008). Amphipods are an abundant component of the marine and estuarine soft bottom and are amongst the principal prey of many fish, birds and larger invertebrate species. Many species are detritus feeders and ingest sediment, which may directly expose them to sediment bound contaminants. Amphipods have been shown to be sensitive to contaminated sediments and to disappear from benthic communities impacted by pollution and reappear under recovery conditions (Swartz et al., 1986). The negative effects of toxicants on amphipod populations may alter the structure and functioning of ecosystems by affecting the availability of food for higher trophic levels. This led to the development of benthic quality indices based upon the relationship between amphipods and other fauna, namely opportunistic polychaetes (Dauvin and Ruellet, 2007), and also to a worldwide development of sediment toxicity protocols using amphipods as test-organisms (Lamberson and Swartz, 1988, 1989; DeWitt et al., 1988, 1992; Lamberson et al., 1992; Bat and Raffaelli, 1998; Bat et al., 1998; Woodworth et al., 1999; Bat, 2005; ISO, 2005; Prato et al., 2006; McCready et al., 2006; Allen et al., 2007; ASTM, 2008; Picone et al., 2008; Prato et al., 2008).

Amphipods have been successfully used in sediment toxicity testing namely due to their sensitivity to a wide variety of contaminants, easy collection and handling in the laboratory, but also because their protection ensures the protection of the whole benthic community. Amphipod toxicity tests have been successfully used in the United States and Canada to assess coastal sediment toxicity

* Corresponding author. Address: CESAM (Centro de Estudos do Ambiente e do Mar), Departamento de Biologia, Universidade de Aveiro, Campus Universitario de Santiago, 3810-193 Aveiro, Portugal. Tel.: +351 234370769; fax: +351 234372587.
E-mail address: victor.quintino@ua.pt (V. Quintino).

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since the mid eighties (Chapman and Long, 1983; Swartz et al., 1985; DeWitt et al., 1992; USEPA, 1994; ASTM, 2008), and were later developed with European species (Ciarelli et al., 1997; Bat et al., 1998; Bat and Raffaelli, 1998; Costa et al., 1998; 2005; Brown et al., 1999; Casado-Martinez et al., 2007; Guerra et al., 2007; van den Heuvel-Greve et al., 2007; Prato et al., 2008). Also, most sediment assessment methods developed to date are primarily devoted to fresh or marine waters, whereas there is a relative paucity of applications in the estuarine environment (Chapman and Wang, 2001). *Corophium multisetosum* has been previously used as a test organism in sediment acute and chronic toxicity tests (Castro et al., 2006) as well as in whole effluent testing (Ré et al., 2007). Results indicated that this could be a suitable test species for Southern European transitional waters, where it is more abundant than the Northern counterpart *Corophium volutator*, also reported as a suitable test species (Ciarelli, 1994; Ciarelli et al., 1997; Peters and Ahlf, 2005; van den Heuvel-Greve et al., 2007). With a growing number of species reported as suitable for sediment toxicity testing, it is imperative to assure that the results obtained at the measured endpoint reflect a true negative effect due to contamination and not the sensitivity of the test species to natural factors, namely temperature, salinity, sediment grain size or organic matter, factors which may confound the toxicity evaluation of sediment samples is some test protocols (Benton et al., 1995; Quintino et al., 1995).

This work presents a study of baseline conditions for the development of an acute sediment toxicity test with the Southern European estuarine amphipod *C. multisetosum*. It includes the setting up of laboratory cultures and the experimental study of adult amphipods response when exposed to natural factors, namely a range of temperature, of interstitial and overlying water salinity and of sediment grain size, as well as to the reference toxicant cadmium chloride. Using the proposed test procedure, the species was also exposed to sediments collected yearly from 1997 to 2006, at a site located off the Tagus estuary, Western coast of Portugal. Within this period, a major flood event occurred in the winter 2000–2001, the effects of which were analysed in the benthic macrofauna community, on sediment baseline descriptors and organic compounds as well as on the survival of *C. multisetosum* in acute sediment toxicity exposures.

2. Materials and methods

2.1. Species characteristics and sampling site

Species of the genus *Corophium* Latreille are common in estuaries worldwide. The European species *C. multisetosum* has been found in the upper, less saline, parts of estuaries (Stock, 1952) and in Portugal is known from the Sado and Mondego estuaries (Marques and Bellan-Santini, 1985), Ria Formosa (Marques and Bellan-Santini, 1990) and Ria de Aveiro (Queiroga, 1992). In Ria de Aveiro, *C. multisetosum* is distributed over most of the length of the Mira Channel, in a soft-bottom and shallow water habitat, with densities up to hundreds of individuals per square meter. The species breeds throughout the year, but in May, July and August only a few incubating females are present in the population. An intense recruitment peak occurs in autumn and a smaller peak in spring (Cunha et al., 2000).

In Ria de Aveiro, *C. multisetosum* is mainly found on clean medium sand and tends to avoid sediments with high levels of grain size below 125 μm and rich in organic matter (Queiroga, 1992). In order to set up laboratory cultures, the species was sampled in a meso/oligohaline site in Ria de Aveiro where salinity remains below 5 during most of the year, the water temperature varied from 20–24 °C in summer to 7–15 °C in winter/spring, and the species can be extremely abundant (>80 000 ind m^{-2}) (Queiroga, 1992).

2.2. Laboratory cultures

Specimens of *C. multisetosum* were collected in an intertidal sand bank during low tide, separated from most of the sediment by gentle sieving in the field through 0.5 mm mesh screen and were transported to the laboratory in water collected in the sampling site. In the laboratory, they were kept in cultures in climatic chambers at 15, 18 and 22 °C. The cultures were set up in plastic containers (0.38 m length × 0.24 m width × 0.12 m height), holding about 6 L of filtered and UV sterilised water (6–7 cm) and an approximately 2 cm thick sediment layer from the field sampling location. The sediment layer needs to be adequate because the organisms check its depth by probing the substrate with their elongated second antennae. If the conditions are suitable they burrow but if not they move off to investigate other sites. Each culture container holds several hundred mature animals. The cultures were maintained with natural seawater diluted to salinity 18 in deionised water. The culture water was filtered, UV sterilized and constantly aerated. In the field, the water salinity in the amphipod sampling area ranges from 0, in winter and low tide to 22 in summer and high tide.

In the present work salinity was measured with a handheld refractometer and expressed using the Practical Salinity Scale that defines salinity as a pure ratio, with no dimensions. By decision of the Joint Panel of Oceanographic Tables and Standards, salinity should be reported as a number with no symbol, or indicator of proportion after it, such as ppt or ‰, and it is not correct to add the letters PSU, implying Practical Salinity Units, after the number.

The sediment used to set up the cultures was collected at the amphipods collecting site and was sieved through a 0.5 mm mesh screen to remove larger animals and debris. Unused sieved sediment was stored in the dark at 4 °C for later use. The culture containers were kept under constant aeration, in walk-in climatic chambers, with a photoperiod of 14 h light: 10 h dark. About 30% of the culture water was renewed three times a week. At this time water added to the culture was enriched with algal food (10^6 cells per mL – Coast Seafood Algae, Diet C). Feeding was also combined with water changes, consisting of dry food components (i.e. 48% Tetramin®, 24% dried alfalfa, 24% dried wheat leaves and 4% Neo-Novum® (DeWitt et al., 1992)), combined and ground to a fine powder and sprinkled on the water surface.

2.3. Test design and procedure: negative control and response to a reference toxicant

The acute sediment toxicity test used adult specimens only, isolated from the cultures. The static exposures were conducted in 1000 mL beakers containing 200 mL of sediment and 800 mL of overlying water. The exposure beakers, each containing 20 adult amphipods, were placed in walk-in chambers with controlled temperature and photoperiod, 14 h light: 10 h dark. Up to five replicate samples per treatment were considered in the experiments. The exposure containers were permanent aerated, without disturbing the sediment, and monitored daily for temperature and aeration. Acute sediment toxicity duration was set to 10 d, following the standard procedure with other species (USEPA, 1994; Bat and Raffaelli, 1998; Bat et al., 1998; ISO, 2005; Casado-Martinez et al., 2007; Guerra et al., 2007; ASTM, 2008; Prato et al., 2008). During the 10 d period, the amphipods were not feed and the water was not renewed. At the end of the exposure, the sediment from each beaker was sieved through a 0.5 mm mesh screen to collect and count the surviving amphipods.

The response criterion in the acute test is survival. Dead animals are recognized by their discoloration, absence of pleopod movements or lack of response to an external mechanical stimulation. Missing animals are assumed to have died during the exposure.