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Development of a short-term chronic toxicity test with a tropical mysid

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ABSTRACT

There is an increasing need to develop reliable methodologies for chronic toxicity testing using tropical species. The present work aimed at developing a suitable short-term chronic toxicity test with *Mysidopsis juniae* using zinc (Zn) and nickel (Ni) as model chemicals and growth (length and dry weight), survival, and egg production (number of females with eggs) as endpoints after seven days of exposure. Survival and growth of newborn *M. juniae* were affected by chronic exposure to zinc, while nickel affected only survival. For zinc, dry weight was the most sensitive endpoint with significant effects even at the lowest tested concentration (75 μg Zn · L^{-1}), whereas for nickel, survival was the most sensitive parameter (LC_{20} of 26 μg Ni · L^{-1}). Egg production was not affected. *M. juniae* short-term chronic testing is a sensitive approach to evaluating metal toxicity; further studies are necessary to assess chronic toxicity for others contaminants in the proposed assay.

1. Introduction

Toxicity testing is widely used in water quality assessment since it integrates the response of biota to the bioavailable fraction or the mixture of contaminants present in the environment (Burton, 1999). Nonetheless, the establishment of reliable methodologies using tropical marine species is still limited, and most available data come from temperate species (Howe et al., 2014). There is increasing evidence regarding the differences between tropical and temperate ecosystems (Daam and Van den Brink, 2010). In Brazil, there are few standardized ecotoxicological assays for aquatic marine species, and the most commonly used are based on acute toxicity responses (Krull and Barros, 2012; Magalhães and Ferrão Filho, 2008; Weinstein and Birk, 1989). Despite the great value of acute toxicity tests in environmental toxicity assessment, they present several weaknesses, mostly related to the short exposure time and the limited ecological relevance (Emery et al., 1997). The USEPA (2002) has developed faster protocols (with durations of up to nine days) to assess chronic toxicity of effluents with five different species, *Cyoprinodon variegatus* (fish), *Menidia beryllina* (fish), *Arbacia punctulata* (sea urchin), *Champia parvula* (algae), and *Mysidopsis bahia* (mysid), to replace traditional long-term chronic toxicity tests.

Considering the gaps in tropical ecotoxicology, the aim of this study was to develop and assess a short-term chronic toxicity test with the tropical mysid *Mysidopsis juniae*. Two metals were chosen as model chemicals, zinc and nickel, and endpoints included were survival, body length, dry weight, and the number of fecund females following a seven-day exposure period. *M. juniae* are maintained in laboratory cultures and have a high sensitivity to chemicals (ABNT, 2011). Mysids inhabit pelagic and demersal environments, varying from 2 mm to 8 cm in length and feeding on suspended solids (algae and zooplankton) and are therefore considered omnivorous. They have a developed carapace covering the thorax, and pereopods are biramous and used to swim while pleopods are often reduced; in males, pleopods are modified for reproduction (Brusca and Brusca, 2003). Their reproduction occurs by copulation and the eggs are fertilized immediately after being expelled from the oviducts to the marsupium (Mauchline, 1980). According to Prósperi (1998), the sexual maturity of *M. juniae* occurs around the 15th day of life of organism. Eggs are visible inside the marsupium between 18 and 20 days, and hatching occurs around day 22. The mean number of offspring per female per week is usually around eight (ABNT, 2011).

2. Materials and methods

2.1. Chemicals

Zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$) and nickel chloride hexahydrate II ($NiCl_2 \cdot 6H_2O$) were purchased from Sigma-Aldrich. The stock

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solutions were prepared in distilled water with 1% HNO_3 (65%, Dinâmica Química Contemporânea LTDA), at a concentration of 10 g · L^{-1} for both metals. Stock solutions of 0.5 and 1 mg · L^{-1} for zinc and 1 and 2 mg · L^{-1} for nickel were prepared in naturally filtered (0.8 μm) seawater (salinity of 35 ppt), and the total metal concentrations were analyzed by Flame atomic absorption spectrometry using the Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 2005). As suggested by Rauf and Hanan (2009), calibration curves for standard calibration solution were also carried out in salt water in order to attain accurate results during the analysis. The coefficients of correlation were 0.9933 for zinc and 0.9993 for nickel curves. It is important to mention also the detection and quantitation limits for each metal: 0.12 and 0.40 mg · L^{-1} for zinc and 0.24 and 0.80 mg · L^{-1} for nickel, respectively. Test solutions were then prepared by dilution in naturally filtered (0.8 μm) seawater (salinity of 35 ppt) to obtain the nominal concentrations of 19, 37.5, 75, 150, 300, and 600 μg · L^{-1} for zinc and 3.8, 7.5, 15, 30, 60, 120 μg · L^{-1} for nickel.

2.2. Test organisms

M. juniae (Fig. 1) were obtained from a well-established culture at the Laboratory of Marine Ecotoxicology, Fortaleza, Brazil. Cultures were maintained in natural sea water collected offshore at Mucuripe beach (03°40′20.9856″S, 038°30′10.8288″W), Fortaleza, Brazil, and filtered (0.8 μm filter); 10 males and 30 females (1:3 ratio) were kept per glass aquaria (12 L volume capacity). All aquaria were continuously aerated and covered with a plastic film to avoid water evaporation and the water was renewed weekly. Organisms were kept at 24 ± 2 °C, salinity of 35, and a 12 h photoperiod (12 h light: 12 h dark); they were fed daily *ad libitum* with 48 h-old *Artemia* sp. nauplii. Dissolved oxygen, salinity, and pH were measured weekly and all the procedures followed the recommendations of the Brazilian Association of Standards Techniques NBR 15.308 (ABNT, 2011).

2.3. Chronic toxicity testing

The experimental procedures were divided into two different protocols to evaluate lethality, body length, and dry weight (protocol I) and fecundity (protocol II) after 7 days of exposure. All experiments followed the method described by USEPA (2002) for *M. bahia* with some modifications for the native tropical species.

In protocol I, five neonates (age ≤ 24 h) were exposed to a range of zinc (37.5, 75, 150, 300, and 600 μg · L^{-1}) and nickel (7.5, 15, 30, 60, and 120 μg · L^{-1}) concentrations for seven days in acid-washed glass beakers in quadruplicates (final volume 250 mL), without media renewal or aeration. Parameters for water pH, dissolved oxygen (DO) content, and salinity were measured in each beaker at the beginning and end of the experiment, or when there was 100% mortality in a replicate. Experiments were maintained in a temperature controlled room (24 ± 2 °C) with a photoperiod of 12 h light to 12 h dark, and fed daily with *Artemia* sp. nauplii (48 h old) at a rate of 20 nauplii per mysid during the first 48 h, and 40 nauplii per mysid until the end of the experiment at seven days. Daily, the number of live mysids was counted and excess food and dead animals removed. At the end of the test, surviving organisms were counted, washed in distilled water, and measured individually to determine total body length from the head to the end of the last segment using a stereoscopic microscope (Fig. 1A). Due to their small size, mysids were then pooled by concentration, dried in aluminum bags at 60 °C for 24 h in an oven, and finally weighted in an analytical balance (accuracy 0.0001 g). Tests were repeated four times.

For protocol II, twenty neonate mysids (age ≤ 24 h) were maintained over 10 days in an aquarium at a culture density of one mysid per 50 mL and fed daily with 48 h old *Artemia* sp. nauplii at a rate of 60 nauplii per mysid. These juveniles (10 d old) were then exposed to four concentrations of zinc (19, 37.5, 75, and 150 μg · L^{-1}) and nickel (3.7, 7.5, 15, and 30 μg · L^{-1}) for seven days. All other conditions were identical to protocol I. After exposure, gender was determined and the number of males, fecund females, and immature females was recorded (Fig. 1B–E). Males were identified by the presence of a pair of penes located at the bases of eighth thoracic appendages (Fig. 1B). To evaluate fecundity, females were observed for the presence or absence of eggs in the oviduct (Fig. 1D) and marsupium (Fig. 1E). Females were considered immature when the marsupium was in an early stage of development (Fig. 1C). Adults that died during the test were excluded from the calculations. Concentrations in which there were no significant exposure effects on adult survival were considered for the evaluation of the fecund females' endpoint. Tests were repeated three times.

The acceptability criteria for chronic short-term tests with *M. juniae* was based on the criteria established by USEPA (2002) for *M. bahia*. Control survival must be greater or equal to 80% at the end of exposure and fecundity can only be used as an evaluation criterion if 50% or more of the females in the control have eggs in the oviduct or in the pouch.

2.4. Statistical analysis

As suggested by USEPA (2002) for *M. bahia*, separate analyses of survival data were performed to estimate the median lethal concentration (LC_{50}) and the lethal concentration for 20% of tested organisms (LC_{20}) endpoints. Normal distribution of arc sine square root transformed

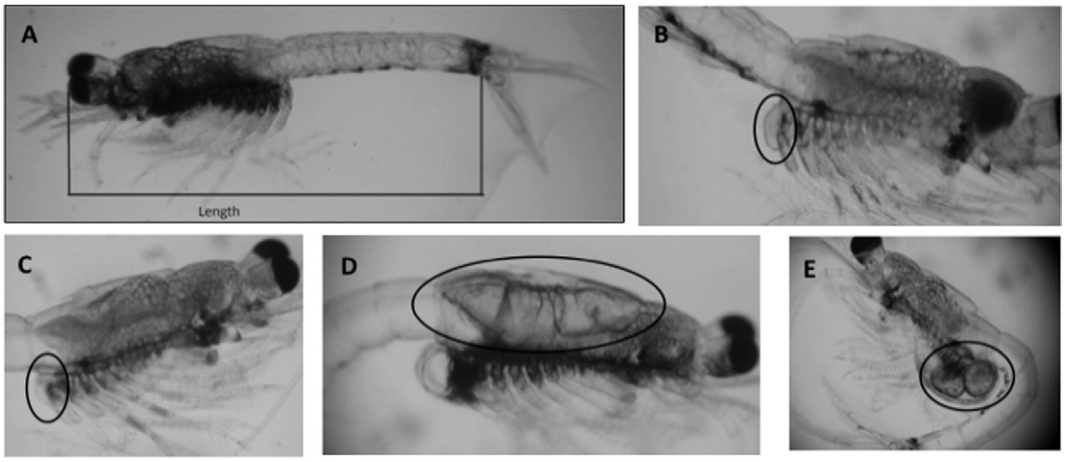

Fig. 1. Microphotographs of the tropical mysid *Mysidopsis juniae*. A — Immature individual, showing the length. B — Male, highlighting with a circle the male reproductive organ (pair of penes located medially at the bases of the eighth thoracic appendages). C — Immature female circling to indicate the absence of male pair penes, to differentiate from B. D — Female with eggs in the oviduct (circling to indicate). E — Female with eggs in the marsupium (circling to indicate). 10× magnification.