Ecotoxicology and Environmental Safety ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

The serpulid *Ficopomatus enigmaticus* (Fauvel, 1923) as candidate organisms for ecotoxicological assays in brackish and marine waters

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ARTICLE INFO

Article history:
Received 8 May 2015
Received in revised form
7 October 2015
Accepted 8 October 2015

Keywords:
Ficopomatus enigmaticus
Sperm toxicity
Larval development
Bioassays
Ecotoxicity

ABSTRACT

Ficopomatus enigmaticus is an ubiquitous fouling reef-forming species, easy to sample and recognize, diecious with gamete spawning along different seasons in different salinity conditions. Due to its characteristics it could become a good candidate for the monitoring of both marine and brackish waters. The suitability of *F. enigmaticus* as a promising model organism in ecotoxicological bioassays was evaluated by a sperm toxicity and a larval development assay. The fertilization rate in different salinity conditions (range 5–35‰) was first assessed in order to detect the salinity threshold within which profitably perform the assays. Afterward copper (Cu^{2+}), cadmium (Cd^{2+}), sodium dodecyl sulfate (SDS) and 4-*n*-nonylphenol (NP) were used as reference toxicants in exposure experiments with spermatozoids (sperm toxicity assay) and zygotes (larval development assay). A dose-response effect was obtained for all tested toxicants along all salinity conditions except for 5‰ salinity condition where a too low (< 30%) fertilization rate was observed. NP showed the highest degree of toxicity both in sperm toxicity and larval development assay. In some cases the results, expressed as EC_{50} values at 35‰ salinity condition, were similar to those observed in the literature for marine organisms such as the sea urchin (*Paracentrotus lividus*) and the marine serpulid *Hydroides elegans*, while the exposure of *F. enigmaticus* spermatozoids' to Cd^{2+} and NP resulted in toxicity effects several orders of magnitude higher than observed in *P. lividus*. Spermatozoids resulted to be slightly more sensitive then zygotes to all different toxicants.

1. Introduction

Human activities increase the background levels of different toxic contaminants such as heavy metals and organic compounds in natural waters. Chemical analyses allow a determination of the degree and nature of pollution, but they do not provide evidence regarding the biologic consequences (Chapman et al., 1987). While the majority of effluent licensing programs currently rely solely on chemical-based monitoring, the use of toxicity testing and other biological methods to assess effluent quality is gaining acceptance (Hickey, 1995). Bioassays allow the detection of these effects by measuring the biologic response of marine organisms. The test species must be sensitive enough to respond to low levels of contaminants and must be available for use from either laboratory cultures or from field collection throughout the year. Additionally, if biologic tests should be ecologically relevant, the species should be widespread and easily available (Richardson and Martin, 1994). The early developmental stages of marine invertebrates have repeatedly been found to be more sensitive to environmental pollutants than their adult counterparts (Connor, 1972; Rand et al., 1995; His et al., 1999) and different early life-stage toxicity testing protocols have been developed and effectively applied for the characterization of effluent toxicity using marine species such as bivalves and echinoderms in both fertilization and larval development assays (*Paracentrotus lividus* (Lamarck, 1816), *Mytilus edulis* (Linnaeus, 1758), *Crassostrea virginica* (Gmelin, 1791), and *C. gigas* (Thunberg, 1793)). The importance of invertebrates in the ecosystems has lead to an increase of interest in their use as model organisms in biological assays (e.g. *Artemia* sp. (Leach, 1819), *Corophium* sp. (Latreille, 1806), *Acartia tonsa*, (Dana, 1849) etc., in addition to the organisms cited before); currently, few invertebrate species have been used in toxicity studies, especially for brackish waters for which dedicated assays are really few (e.g. the copepod *Nitocra spinipes* (Boeck, 1865) used in ISO/DTS 18220 procedure).

Polychaetes bioassays, in particular, are generally focused on evaluation of bioaccumulation of pollutants (e.g. *Hediste*

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http://dx.doi.org/10.1016/j.ecoenv.2015.10.006
0147-6513/

diversicolor (Müller, 1776; ASTM, 1997)) or acute toxicity assay with mortality and/or growth endpoints (Díaz-Castañeda et al., 2009).

Although environmental levels of several pollutants has been largely documented both in marine and brackish ecosystems, data on their effects on marine/brackish organisms early life stages are scarce if compared with the source of data available for freshwater organisms such as of daphnids, ostracods and others (Hutchinson et al., 1998; Leung et al., 2001).

The sea urchin's sperm cells appears as the most widespread standardized bioassay in regulatory programs dealing with the evaluation of seawater quality and marine or estuarine sediments toxicity (United States Environmental Protection Agency, 1994). This toxicity tests rely on field-collected organisms and the main problems encountered in performing the assay are represented by the lack of gamete emission during certain periods of the year and the low adaptability to salinity condition different from 35‰. These two aspects represent a limit in performing the assay during all the year and in all salinity conditions.

The introduction of a new model organism needs the consideration of various aspects such as the conditions and reproducibility of the test that should be suitably standardized.

Since a few numbers of bioassays are standardized in brackish waters, the aim of this work was to evaluate the suitability of *Ficopomatus enigmaticus* as potential candidate for sperm toxicity and larval development assays for the monitoring of both marine and brackish waters. *F. enigmaticus* is a reef building serpulid polychaete widespread in all tropical and temperate areas (Fofonoff et al., 2003), even if of uncertain origin. The body bauplan consists of a cephalic area with breathing/feeding radioles and the characteristic operculum, a thorax and an abdomen with reduced appendices. The presence of a typical operculum morphology, together with a easily distinguishable tube, is an important taxonomic characteristic that permits to easily discriminate *F. enigmaticus* not only from other widespread reef-building serpulids such as *Hydroides elegans* (Haswell, 1883) and *H. dianthus* (Verrill, 1873), but even from other genera of the family (ten Hove and Weerdenburg, 1978; ten Hove and Kupriyanova, 2009). It is an invasive alien species and its spread is probably due to passive transport attached on ships' hulls (Çinar, 2013; Newman, 1995). It is an eurialine species that can survive in a wide range of salinity conditions (up to 55‰), pH (4–9) and temperature (0–35 °C); however the best reproduction conditions in natural environments are represented by salinity values comprised within the range 10–35‰ and a temperature not lower than 18 °C (Straughan, 1972; Dixon, 1981).

F. enigmaticus is a diecious species with external fertilization. Its choice as a good candidate for ecotoxicological testing derived from several favorable biological characteristics, such as the widespread distribution and the taxonomic characteristics, which permit the ease of sampling (it's a sedentary organism forming dense colonies), the ease in forcing gamete release, the feasibility of the assay during wide time windows, generally, for Northern Emisphere, from May–June to October–November, depending on temperature and geography (Dixon, 1981; Obenat and Pezzani, 1994) and the ease in laboratory maintaining. In this study, different bioassays (sperm toxicity and larval development) were adapted from methods previously used for other invertebrates. After gamete emission, which can be forced by tube breaking, sperm toxicity and larval development assays were performed at five different salinity conditions (5‰–10‰–15‰–30‰–35‰) with the following reference toxicants: copper (Cu^{2+}), cadmium (Cd^{2+}), sodium dodecyl sulfate (SDS) and 4-n-nonylphenol (NP).

Sperm toxicity test involved the initial exposure of sperm to different chemical concentrations, followed by the addition of eggs and the consecutive assessment of fertilization. On the other hand, larval development test consisted in the exposure of fertilized eggs to different reference toxicant dilutions for 24 h and the subsequent assessment of normal larval development.

2. Materials and methods

2.1. Artificial seawater (ASW)

ASW was prepared according to Peltier and Weber (1985). Starting from a 35‰ salinity ($S=35$‰) stock solution, other salinity conditions ($S=30$‰, $S=25$‰, $S=10$‰, $S=5$‰) were prepared by dilution of stock solution with bi-distilled water. All pH values were 8.1 ± 0.2, except for $S=5$‰ that measured 7.76 ± 0.1.

2.2. Chemicals

Selected reference toxicants were cadmium (Cd^{2+}), copper (Cu^{2+}), sodium dodecyl sulfate (SDS) and 4-nonylphenol (NP). Cd^{2+} and Cu^{2+} were standard solutions AAS grade from Fluka (Italy).

The organic compounds sodium dodecyl sulfate (SDS) and 4-nonylphenol (NP) were purchased from Sigma-Aldrich (Italy).

Six dilutions of each reference toxicant were freshly prepared for each experiment dissolving reference substances in ASW at $S=5$‰, $S=10$‰, $S=15$‰, $S=30$‰, $S=35$‰. Toxicant concentrations were: 2–1–0.5–0.25–0.125–0.062–0.031 mg/L for Cd^{2+}, 0.5–0.25–0.125–0.062–0.031–0.015 mg/L for Cu^{2+}, 10–5–2.5–1.25–0.625–0.312 mg/L for SDS and 12–6–3–1.5–0.75–0.375 μg/L for NP. NP is soluble in seawater at 3630 μg/L (U.S. EPA, 2005): for this reason no solvents were used.

2.3. Analytical chemistry

Concentrations of all reference toxicants were measured in experimental solutions at $S=10$‰, $S=25$‰ and $S=35$‰ salinity condition and three representative toxicant concentrations (highest, medium and lowest) were chosen for chemical analyses.

Concentrations of Cd^{2+} and Cu^{2+} were measured according to Toyota et al. (1982) via graphite furnace atomic absorption spectroscopy (220, Varian, Palo Alto, CA. USA). The detection limits for Cd^{2+} and Cu^{2+} 0.1 and 0.2 μg/L respectively. Quality control of metal analysis was performed using reference material (IAEA simulated freshwater W-4) obtaining values in good agreement with the certified values ($<10\%$ deviation).

Concentration of SDS was measured as methylene blue active substance (MBAS) as reported by George and White (1999). Samples were extracted with chloroform and the absorbance at 650 nm of complex chloroform–methylene blue was measured by the use of a Perkin Elmer Lambda 45 spectrophotometer. Calibration curves were performed in different salinity conditions in order to normalize salt interference. The assay showed linearity in the range 1–25 mg/l^{-1}.

Concentration of NP was measured via an HPLC-fluorescence detection method according to Cruceru et al. (2012). Samples were extracted with dichloromethane (pH 3.00–3.50) followed by HPLC analysis with a chromatographic system that consisted of a Jasco 880 pump and Jasco 821 fluorescence detector (Jasco, Tokyo, Japan). The excitation wavelength (λ_{ex}) and emission wavelength (λ_{em}) were set at 220 and 315 nm, respectively. The reversed-phase column was a 5-μm, 4.6×150 mm^2 C8 column (Zorbax ECLIPSE XDB, Agilent Technologies, USA) and the isocratic elution was performed with a mixed solvent acetonitrile/water 65:35 (flow rate 1 mL/min). The detection limit, calculated as signal-to-noise ratio 3:1, was 0.088 μg/L. JascoBorwin software was used for data processing.

Please cite this article as: Oliva, M., et al., The serpulid *Ficopomatus enigmaticus* (Fauvel, 1923) as candidate organisms for ecotoxicological assays in brackish and marine waters. Ecotoxicol. Environ. Saf. (2015), http://dx.doi.org/10.1016/j.ecoenv.2015.10.006