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Prior exposure to Cu contamination influences the outcome of toxicological testing of *Fucus serratus* embryos

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Abstract

Brown seaweeds are often employed in single species toxicity testing to study the association between the pollutant and the biota in contaminated marine habitats. We have used *Fucus serratus* (Phaeophyta) from one Cu resistant and one non-resistant population to evaluate the effect of prior exposure to metal pollution on toxicological endpoints. Analysis of comparative toxicity was conducted for embryo rhizoid elongation and adult relative growth rate (RGR). Algae that had previously been exposed to Cu expressed consistently lower levels of sensitivity to Cu than those that had no history of exposure to the pollutant. For both non-resistant and resistant populations rhizoid length was a more sensitive endpoint than adult RGR. While early life history stages of brown algae are generally regarded as being pollution-sensitive and inhibition of spore and embryo rhizoid elongation is frequently used as endpoints in bioassays, the test results may be affected by prior exposure of the parent algae to the pollutant. We conclude that the effect of prior exposure should be considered when comparing endpoints between studies and when selecting material for future testing.

Keywords: Toxicity test; Macroalgae; *Fucus*; Heavy metal; Copper; Resistance

1. Introduction

Heavy metal ions derived from anthropogenic activities are significant pollutants of coastal and estuarine waters, worldwide (Bryan and Langston, 1992; Castilla, 1995; Grout and Levings, 2001; Eriksen et al., 2001). Brown macroalgae often dominate the vegetation of these shallow pollution-vulnerable marine areas in the temperate latitudes of the northern and southern hemispheres. Here they are principle primary producers and provide shelter and substrata for a variety of marine animals and epiphytic algae (Ramus, 1992).

The impacts of sewage effluents and heavy metals on brown seaweeds are frequently studied by application of single species toxicity testing (Burridge and Bidwell, 2002). This approach exploits the concentration dependent inhibitory effects of heavy metal ions (e.g. Cu) on spore and embryo germination and rhizoid elongation that are known for a variety of both fucoid (Andersson and Kautsky, 1996; Bond et al., 1999; Nielsen et al., 2003a) and laminarian species (Chung and Brinkhuis, 1986; Anderson et al., 1990; Bidwell et al., 1998). The inhibition of spore and embryo germination and rhizoid elongation occurs at ecologically relevant metal ion concentrations. Therefore, the juvenile stages of brown algae offer suitable endpoints for toxicological testing (Bidwell et al., 1998). The abundance and year-round reproduction of brown algae as well as the ease with

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which spores and zygotes are obtained and cultured in large numbers, provides a good model system for the study of species-specific association between the algae and the pollutant. Such information compliments chemical testing as well as biomonitoring and is critical for predicting stress responses in biota in marine areas. It is generally assumed that the microscopic stages of brown algae are more sensitive to environmental stress than the adults (Coelho et al., 2000) and the development of tests has focused on identification of suitable species and test parameters (Burridge and Bidwell, 2002). Little attention has been paid to inter-population differences (Anderson et al., 1990; Bidwell et al., 1998) and the implications of using material with increased resistance to the pollutant on the interpretation of the test results.

Brown seaweeds can develop resistance during prolonged exposure to pollutants such as heavy metals (Nielsen et al., 2003b), and lush populations may be found in contaminated sites such as Restronguet Creek in the South West of England, an area with a contamination history dating to the 19th century (Bryan and Gibbs, 1983). The water in the creek is contaminated with up to 0.5–1.5 μM total copper (Cu) (peak concentrations) whereas natural background estuarine and coastal concentrations of Cu_T are about 20 nM (The Environment Agency, Cornwall and Devon, UK, 2000). The increased Cu resistance of *Fucus serratus* (L.) growing in Restronguet Creek compared to those from clean sites is an inherited character (Nielsen et al., 2003b). As a consequence, the impact of heavy metal ions on toxicological endpoints in brown seaweeds may not only depend on the concentration to which the microscopic life cycle stages are exposed but also on the exposure history of the algal material from which they are derived.

Using *F. serratus* as a representative brown macroalgae and Cu as a model heavy metal, this paper will evaluate the effect of natural prior exposure of algae to heavy metal ions on toxicological endpoints in embryos and adults. This is achieved by comparing the toxicological endpoints expressed by of embryos (rhizoid elongation) and adults (daily relative growth rate (RGR)) of material from the long-term contaminated Restronguet Creek with those expressed by material from an un-contaminated site.

2. Materials and methods

Individual *Fucus serratus* plants bearing vegetative fronds and mature receptacles were collected in November 2001 at low tide from a Cu resistant population at Restronguet Creek (50° 11′ N, 5° 50′ W), and from a Cu non-tolerant populations at Wembury Beach (50° 18′ N, 4° 05′ W) in South West England (Nielsen et al., 2003b). The algae were transported to the laboratory in sealed plastic bags at ambient temperature (<20 °C) within 2 h. Mature receptacles were cut from separate male and female fronds, blotted dry and stored in the dark wrapped in paper towels at 3–5 °C for up to 10 days. Vegetative tips (3–4 cm in length) were cut from the fronds and allowed to recover in filtered (0.45 μm cellulose nitrate membrane) natural seawater (FSW) for 8 days during which period FSW was changed every 1–2 days. Frond tips were maintained at 15 °C under 250 μmol m^{-2} s^{-1} photosynthetic active radiation (PAR) provided by white fluorescent lamps on a 16/8 h light/dark cycle. Test solutions were based on an artificial seawater (ASW) culture medium (Morel et al., 1979) enriched with $CuSO_4 \cdot H_2O$ to yield total copper concentrations ([Cu]) ranging between 0 and 127 μg l^{-1}.

Gamete release was stimulated by thoroughly rinsing receptacles in tap water followed by slight desiccation during exposure to natural sunlight for approximately 20 min. Subsequently receptacles were transferred to FSW that induced gamete release within 1 h. Mixing sperm and eggs under fluorescent white light at 16 °C for 30–45 min induced fertilization. The resulting zygotes were filtered through a 100 μm nylon mesh into FSW. Tests on embryos were conducted in 2.5 cm diameter Petri dishes fitted with cover slip bases onto which zygotes were sown at a density of approximately 100 per dish. After zygote settlement 1–2 h after fertilization (AF), the incubation dishes were filled with 5 ml Cu-free ASW and the zygotes could be observed on an inverted microscope whilst retained in the incubation medium.

To measure rhizoid elongation, zygotes were allowed to develop in Cu-free ASW in unidirectional white light at 60 μmol m^{-2} s^{-1} PAR until 18 h AF at which time germination was initiated. Subsequently the embryos were transferred to ASW containing 0, 6, 32 or 127 μg Cu l^{-1} and grown for a total of 10 days. To compensate for ligand release during Cu exposure (Gledhill et al., 1999), ASW was changed daily. Bright field images of 25 embryos in each dish (3 replicate dishes for each of the 4 Cu treatments) were recorded digitally. Rhizoid length defined as the distance of the thallus/rhizoid dividing cell wall to the rhizoid tip was measured using analytical software (LUCIDA, Kinetic Imaging, Liverpool, UK).

To measure adult RGR, vegetative frond tips were transferred to individual beakers containing 100 ml ASW and 0, 6, 32, 64 or 127 μg Cu l^{-1} under the temperature and light regime described above for 23 days during which ASW was changed every two days. Five replicates of each population were exposed to the 5 treatments. Calculation of RGR was based on fresh weight measurements. Frond tips were removed from the beakers, blotted dry and weighed at 0.1 mg accuracy.