Marine Pollution Bulletin 91 (2015) 82–86

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

A macroalgal germling bioassay to assess biocide concentrations in marine waters

J.A. Girling [a], K.V. Thomas [c], S.J. Brooks [c], D.J. Smith [a], E. Shahsavari [b], A.S. Ball [a,b,*]

[a] School of Biological Sciences, University of Essex, Colchester CO43SQ, Essex, UK
[b] School of Applied Sciences, RMIT University, Bundoora, Victoria 3083, Australia
[c] Norwegian Institute for Water Research (NIVA), Oslo, Norway

ARTICLE INFO

Article history:
Available online 31 December 2014

Keywords:
Macroalgae germlings
Bioassay
Biomonitoring
Antifouling paint
Biocides
Toxicity

ABSTRACT

A bioassay method using the early life stages (germlings) of macroalgae was developed to detect toxicity of anti-fouling paint biocides. A laboratory based bioassay using *Ulva intestinalis* and *Fucus spiralis* germlings was performed with 4 common anti-fouling biocides (tributyltin (TBT), Irgarol 1051, Diuron and zinc sulphate), over a range of environmentally relevant concentrations (0.0033–10 μg l^{-1}). Comparison between the two species showed that germlings of *U. intestinalis* were better adapted for *in-situ* monitoring, as germlings of *F. spiralis* appeared to be too robust to display sufficient growth differences. The response of *U. intestinalis* germling growth appeared to reflect environmental biocide concentrations. Overall the developed method showed potential for the assessment of the sub-lethal effects of anti-fouling biocides on the early developmental stages of *U. intestinalis*.

1. Introduction

In recent years, there has been growing concern over the range and quantity of chemical pollutants that are discharged into marine and coastal environments from anthropogenic inputs (Gelcich et al., 2014). One such form of aquatic chemical pollution, especially in coastal zones, has been attributed to the leaching of biocide compounds from anti-fouling paints, into the surrounding water column (Boxall et al., 2000; Thomas and Brooks, 2010). Anti-fouling paints are applied to the immersed exteriors of ships, boats and marine structures to prevent fouling of marine organisms, such as algae and molluscs, attaching to their surfaces.

Owing to the total ban on tributyltin (TBT)-based anti-fouling paints, the industry has been forced to find alternatives to biocides (Chen et al., 2014; Dafforn et al., 2011). As a result an increasing number of alternatives such as copper or zinc based anti-fouling paints are now being used. However, since copper and zinc are not as effective in preventing algal fouling as organotins are (Thomas et al., 2001), "booster" biocide additives were developed and added to the anti-fouling paint formulations (Dafforn et al., 2011; Thomas, 2001). The "booster" biocides are usually herbicidal compounds (Irgarol 1051 & Diuron) that target copper resistant species, such as macroalgae (Bao et al., 2011). The deleterious environmental effects of the common biocide TBT are well known (Alzieu, 2000; Harino et al., 2012; Kim et al., 2014; Mercier et al., 1997; Ruiz et al., 1996; Thomas and Brooks, 2010). However, the impact that the "new" booster biocides have on "non-target" marine organisms, especially macroalgal communities is relatively unknown.

Macroalgae are important organisms in coastal zones, where they serve as primary producers and sheltered rocky shore habitat modifiers (Stagnol et al., 2013). Using the developmental stages (germlings) of macroalgae to determine harmful concentrations of aquatic pollutants could be an extremely useful tool for coastal monitoring. The current literature on the use of macroalgal germlings as biological indicators of pollution is limited. Most studies using *in-situ* macroalgae as test subjects used adult specimens (Fong et al., 1998), although several studies have reported the use of macroalgal germlings in laboratory toxicity tests (e.g. Brooks et al. (2008)). To date there are no studies using the germlings of *Ulva intestinalis* as an *in situ* monitoring species. In a review of macroalgae toxicity tests, (Eklund and Kautsky, 2003) recommended the use of *Fucus vesiculosus* in future toxicity tests and for the development of a standardised test method. *F. vesiculosus* was found to be sensitive to environmental concentrations of copper in controlled laboratory exposures (Brooks et al., 2008). However, at present no international standardised test method exists for germlings of any macroalgal species.

* Corresponding author at: School of Applied Sciences, RMIT University, Bundoora, Victoria 3083, Australia. Tel.: +61 3 9925 6594; fax: +61 3 9925 7110.
E-mail address: andy.ball@rmit.edu.au (A.S. Ball).

http://dx.doi.org/10.1016/j.marpolbul.2014.12.025
0025-326X/

In this study, germlings were used from two common species of macroalgae found in the UK, *U. intestinalis* and *F. spiralis*. *U. intestinalis* germlings were chosen as they are relatively simple to sporulate and are considered to be a common fouling organism (Callow and Callow, 2002; Tolhurst et al., 2007). Two species of macroalgae were chosen as the use of multiple indicator species would provide a better understanding of the pollution of a marine environment, especially if the species respond to the different portions of the total pollutant load on the ecosystem. In addition, the sensitivity of germling growth between the two algal species following environmental exposure could be compared and assessed for their suitability as a biomonitoring organism. The overall aim was to develop a standardised test method for using macroalgal germlings as potential indicators of pollution, with a focus towards antifouling biocides.

2. Materials and methods

2.1. Macroalgae spawning

Zoospores and embryos were extracted from two common species of macroalgae that are commonly found within the intertidal zone of the United Kingdom coastline. The species used were the common *Chlorophyceae* macroalgae, *U. intestinalis* and the *Phaeophyceae* macroalgae, *F. spiralis*. Adult samples of *U. intestinalis* were collected at low tide from St Lawrence Bay, Essex, UK (51°43.010N, 00°50.110E). The individual specimens were washed with 0.1 μm filtered seawater from the Crouch estuary and carefully wiped with a damp cloth to remove any detritus. The algae were then placed onto a damp paper towel, transferred into a covered container and left for a period of 24 h at 18 °C. The algae were then removed and divided into ten beakers, each containing 100 ml of filtered seawater at ambient temperature (18 °C) and a magnetic stirrer bar. The ten beakers were placed onto a magnetic stirring plate and left to stir for a period of 2 h. The rewetting of the fronds after desiccation subjects the algae to hydrolytic shock, which initiates the algae to release motile zoospores into the surrounding water (Reed and Russell, 1979). After 2 h of stirring the algae, the seawater was filtered through a 20 μm mesh. The filtrate was collected and examined through a microscope at ×80 magnification for the presence of motile zoospores (Fletcher, 1989). The concentration of the zoospores was calculated and adjusted by pipetting the motile zoospores out at the water–air interface and adding the zoospores to a smaller volume of seawater. This was achieved by placing the beaker of zoospore filtrate onto a light box, this made the zoospores congregate at the water–air interface as they exhibit negative phototaxis, moving away from the light; they are also held at the interface by the surface tension of the water (Callow and Callow, 2002). This adjustment gave a final zoospore concentration of approximately 75 per 50 μl.

Adult samples of *F. spiralis* with swollen receptacles were collected at low tide from Burnham-on-Crouch, Essex, UK (51°37.544N, 00°48.243E). The receptacles were removed from the stipe and washed under 0.1 μm filtered seawater to remove any detritus. The receptacles were mildly desiccated by wrapping them loosely in a damp paper towel and refrigerated at 4 °C for a period of 24 h. The reproductive receptacles were then transferred to beakers of 100 ml filtered seawater (0.1 μm) at ambient temperature (18 °C). The reproductive receptacles were left in the filtered seawater for a period of 1 h, after which time the receptacles and seawater were washed through a 100 μm mesh to trap detritus; below this a 20 μm mesh collected the embryos. A period of 1 h was found to be the optimum time for egg and sperm release (data not shown). The collected embryos were re-suspended in 50 ml of filtered seawater. The density of the embryo suspension was calculated using a Sedgewick rafter cell and a binocular microscope (×15 magnification). The density of the embryos was adjusted with the addition of filtered seawater to give a final concentration of approximately 20 embryos per 200 μl.

2.2. Laboratory toxicity tests

Laboratory based toxicity tests were conducted (in triplicate) using 3 anti-fouling paint biocides, Irgarol 1051, Diuron and TBT, and also a reference compound, zinc sulphate. Nominal test concentrations ranged from 0.01 to 10 μg l^{-1} for the anti-fouling compounds and 0.03 to 3.3 mg l^{-1} for zinc sulphate.

For toxicity tests, filtered seawater from the Crouch estuary was used, which was the seawater supply at the CEFAS marine laboratory, Burnham on Crouch, Essex, UK. For exposure experiments, only nominal concentrations of the herbicides were used. The filtered seawater supply was considered suitable as seawater control/reference water and was widely used in toxicity studies performed at the laboratory. No effects on germling growth were observed in the controls, which indicates no or low concentrations of herbicides or other contaminants present.

The collected zoospore suspensions were placed in the wells of clear plastic 12 well plates containing 5 ml volumes of the appropriate test solutions. For *U. intestinalis*, coverslips (Ø 16 mm) were placed into each well prior to the addition of the zoospore suspension (50 μl, giving approximately 33 zoospores per well). The cover slips provided a surface for the adherence of the zoospores. For *F. spiralis*, test solutions aliquots (5 ml) were placed in the wells and the zoospore suspension (20 μl) was added to each well, giving 50 zoospores per well. Plates were incubated at 19 °C for 21 d under 16 h light, 8 h dark (dark-light cycle). Germling measurements were recorded at 4 d intervals throughout the incubation using an image analysis system composed of an Olympus IX 70 inverted microscope equipped with an Hitachi camera and image capture facility. For *U. intestinalis* total shoot length was measured (Scarlett et al., 1997); for *F. spiralis*, development of the rhizome was followed (Scanlan and Wilkinson, 1987). All data were recorded using Image-Pro plus v5.1 imaging software (Datacell®). Growth rates and the toxicity end points EC_{50} (median effective concentration, the effective concentration that produces a response in 50% of the organisms tested) and NEC (no effect concentration, the highest concentration that produces no statistically significant response) were determined as previously described (Walker et al., 1996).

2.3. Statistical analyses

Regression models were produced for comparisons between the macroalgal species. The coefficient of determination (R^2) and the growth rate in μm day^{-1} were calculated using regression. To provide statistical comparisons of the data, an analysis of variance (ANOVA) test was conducted. The tests were performed on all of the experimental data, between the 2 species of macroalgae. ANOVA results were produced using the statistical software package, Minitab®.

3. Results and discussion

The impact of the four test compounds on the germling growth rates of both *U. intestinalis* and *F. spiralis* was examined in a laboratory controlled system. The environmental conditions were monitored throughout the 21 d experiments (Table 1). The temperature remained relatively constant, with a mean value of 19.5 (±0.6) °C throughout the experiment. Dissolved oxygen, 7.50 (±0.96) mg l^{-1}, salinity, 32.7 (±0.32)‰, and pH, 7.95 (±0.26) all remained relatively