Mar. Freshwater Res., 1996, **47**, 1009–14

Effect of Sewage Effluents on Germination of Three Marine Brown Algal Macrophytes

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Abstract. Inhibition of germination of zygotes of the fucoid macroalgae *Hormosira banksii* and *Phyllospora comosa* and zoospores of the laminarian *Macrocystis angustifolia* was used as an end-point to assess the toxicity of three sewage effluents of differing quality. For each species, between-assay variation was low and results of tests with the reference toxicant 2,4-dichlorophenoxyacetic acid suggested that results are reproducible, especially in *P. comosa*. Each species showed a greater sensitivity to primary-treated effluent than to secondary-treated effluent, and higher variability in response to the primary effluent. High variation in response for each species when exposed to the primary effluent (compared with that for the secondary effluent) is presumably indicative of variation in quality of the primary effluent. The capacity to reproduce these assays, the sensitivity of species employed, and the ecological relevance of germination as a toxicological end-point suggest that germination tests of this nature may be useful in biological testing of effluent quality at discharge sites in south-eastern Australia.

Introduction

Toxicity testing is a critical tool for both predicting and monitoring the effects of marine effluent discharges. In Australia there is a need for an increased focus on toxicity test development and the use of local organisms in monitoring effluent quality (Richardson and Martin 1994; Martin and Richardson 1995). Few marine toxicity tests available in Australia can be readily applied to coastal discharges, and those that have been developed have focused on marine invertebrates (e.g. Ahsanullah *et al.* 1981). There are few reports on the effects of toxicants on Australian marine macrophytes (Burridge *et al.* 1995*a*, 1995*b*; Gunthorpe *et al.* 1995), and information on the sub-lethal, toxicological effects of sewage effluent on Australian marine macroalgae is scant.

Inhibition of germination offers a toxicological end-point of substantial ecological significance. Benthic organisms including algal macrophytes are particularly susceptible to chronic effluent discharges and macrophyte community structure may be modified in areas adjacent to sewage effluent discharge (e.g. Borowitzka 1972; Murray and Littler 1978; May 1985; Tewari and Joshi 1988; Brown *et al.* 1990). Adhesion and early development constitutes one of the most important phases of sessile development of benthic macrophytes and can be quite sensitive to physical and biological stress (Deysher and Dean 1986; Reed 1990; Brawley and Johnson 1991). Inhibition of germination may lead to diminished recruitment, population decline, and changes in community structure. This might also occur at effluent concentrations that have no apparent effect on larger life stages (Burridge *et al.* 1995*a*, 1995*b*).

This laboratory study assesses the effects of three different sewage effluents (primary, low-secondary and secondary treated) and a reference toxicant on germination of unicellular life phases of the three phaeophyte marine macroalgae *Hormosira banksii* (Turner) Decaisne, *Phyllospora comosa* C. Agardh and *Macrocystis angustifolia* Bory. All are large species of brown algae and occur widely in south-eastern Australia. *H. banksii* grows in profuse mats on intertidal rock platforms and *P. comosa* forms dense forests in the upper sublittoral zone, extending down to approximately 15 m. Both are members of the Fucales and are reproductive all year. Reproduction is oogamous involving eggs (approximately 40 μm and 145 μm in diameter for *H. banksii* and *P. comosa* respectively) and sperm; germination results in polarization and development of an attachment rhizoid from a unicellular, apolar zygote (Forbes and Hallam 1978; Burridge *et al.* 1993). *M. angustifolia*, in the Laminariales, also forms dense forests in the sublittoral zone. *Macrocystis* species exhibit a hetero-morphic alternation of generations and the large sporophyte plants produce small zoospores (approximately 6·0 μm in diameter) that adhere, germinate and develop into micro-scopic gametophytes (Fritsch 1945; Anderson and Hunt 1988).

Materials and Methods

Fertile plants of each species were collected at Barwon Heads, Victoria (144°30′E,38°17′S), and were maintained on ice or refrigerated until used. Gamete release for *H. banksii* followed the methodology of Forbes and Hallam (1978). Thallus material was rinsed with fresh water and laid on trays to dry at room temperature (approximately 20°C); exudation of gametes usually occurred about 1 h later. Five male and five female plants,

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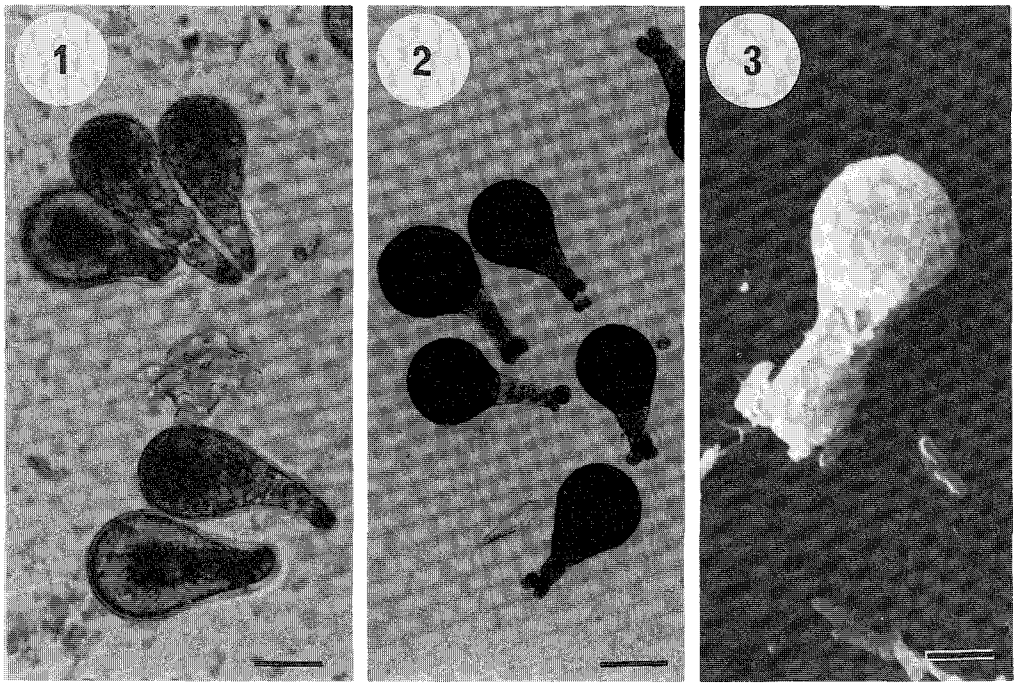
each about 12 cm in length, were washed separately into 400 mL of filtered sea water (membrane pore size 0·2 μm). Gamete suspensions were diluted to achieve sperm and egg densities of 10^6 and 150 cells mL^{-1} respectively, and 10 mL of each gamete preparation were added to 40-mL glass beakers containing a microscope cover slip. After 12 h, the sea-water medium was decanted and sewage solutions were added to four replicates of each treatment. After a further 24 h, the microscope slide and attached zygotes were placed on a compound microscope and the first 40 zygotes identified were scored for the presence or absence of a germination rhizoid (Fig. 1).

Collection and gamete release for *P. comosa* followed the methodology of Burridge *et al.* (1993), and the toxicity testing procedure was a modification of Burridge *et al.* (1995*a*). Sexed, fertile receptacles were placed in 50-mL polyethylene culture vials filled with sea water. In the laboratory the vials were placed on ice under fluorescent light of 150 μmol $m^{-2} s^{-1}$; release of gametes usually occurred after approximately 4 h. Sperm are released directly into the water, whereas eggs remain attached by stalks to the receptacle surface until fertilized. Following gamete release, a 2-cm-long piece of receptacle with attached eggs and 10 mL of a sperm solution (10^6 sperm mL^{-1}) were added to 20 mL of filtered sea water in 40-mL glass beakers containing a filter-paper substratum. After 12 h and following zygote release from the stalks, the receptacle tissue was removed and each beaker was slightly agitated. After another 12 h, the sea-water medium was decanted and sewage/toxicant solutions were added to four replicates of each treatment. After a further 24 h, germination was scored by viewing the filter paper with a dissecting microscope and examining the first 40 zygotes/embryos identified for the presence of an attachment rhizoid (Fig. 2). This method produced a zygote density of approximately 60 cells cm^{-2}. By allowing the zygotes to settle and adhere without disruption, this method avoids placing handling stress on the zygotes as may occur with the methods of Burridge *et al.* (1995*a*). Any unattached, non-viable zygotes are removed 24 h after gamete mixing when the sea-water medium is decanted and the sewage/toxicant treatment added.

Collection and spore release for *M. angustifolia* was a modification of procedures employed in North America for toxicity testing with *Macrocystis pyrifera* (Anderson and Hunt 1988; Anderson *et al.* 1990). Sporophylls were washed in filtered sea water, blotted dry, and allowed to dry further at room temperature for about 60 min. Twenty-five sporophylls were then immersed in 400 mL of filtered 15°C sea water to initiate zoospore release. Spore solutions were diluted to 10^6 cells mL^{-1}, and 2 mL of the spore suspension were added to 40-mL beakers containing 20 mL of filtered sea water and a microscope cover slip. After 1 h, the sea water was decanted and sewage/toxicant solutions were added to four replicates of each treatment. After a further 24 h, the cover slip was examined under a compound microscope and the first 40 spores/gametophytes identified were scored for the presence or absence of a germination rhizoid (Fig. 3). For the three species, all beakers were covered with paraffin wax and stored in an incubation cabinet at 15°C at a light intensity of 120 μmol $m^{-2} s^{-1}$ under a 12:12 h light:dark regime.

Sewage effluents were obtained from treatment authorities and stored in plastic polyethylene containers at 4°C until used. The primary effluent was a low-quality effluent subjected to fine-screening (mesh size 500 μm) and grit removal. The low-secondary effluent was of intermediate quality and had undergone anaerobic digestion and sludge removal. The secondary effluent was of high quality and had been subjected to pondage treatment, anaerobic digestion and overland filtration. Before testing, all treatment solutions were adjusted for salinity, pH and dissolved oxygen to approximate the values found in sea water (temperature 15°C, pH 8 ± 1, dissolved oxygen 7 ± 1 mg L^{-1}, salinity 34 ± 1); salinity was adjusted with marine aquarium sea salt ('Marinemix', Marine Enterprises Inc., Baltimore, USA). Artificial sea-water controls in which (distilled) water chemistry had been similarly modified with the aquarium sea salt were undertaken with each test. Tests were also conducted with 2,4-dichlorophenoxyacetic acid (2,4-D; 98% pure, Aldrich D7,072-4) as a reference toxicant to provide data on background variability in germination response for each species.



Figs 1–3. **(1)** Light micrograph of *Hormosira banksii* germlings 36 h after mixing of sperm and eggs, showing rhizoidal development from the previously apolar egg. Scale bar, 40 μm. **(2)** Light micrograph of *Phyllospora comosa* germlings 48 h after mixing of sperm and eggs, showing rhizoidal development from the previously apolar egg. Scale bar, 115 μm. **(3)** Scanning electron photomicrograph of a germinated *Macrocystis angustifolia* spore 24 h after release from the adult sporophyte. Scale bar, 3.0 μm.

Percentage germination data were arcsine-transformed and subjected to single-factor analysis of variance (ANOVA). Pairwise comparisons of treatments and controls were undertaken with the aid of Dunnet's multiple comparison test (Zar 1984) to provide lowest observable effect concentration (LOEC) and no observable effect concentration (NOEC). Median-effect concentration (24-h EC_{50}) was derived by the trimmed Spearman–Karber method (Hamilton *et al.* 1978), and coefficients of variation and statistical power were determined according to Zar (1984). All effluent concentrations are presented in percentage (v/v) form and the results refer to nominal concentrations of effluent or 2,4-D in initial treatments.

For scanning electron microscopy, spores were fixed in 10% glutaraldehyde in sea water with pH adjusted to 8·0 and salinity to 34 mg L^{-1}. Spores were allowed to adhere to cover slips and germinate prior to removal of sea water and addition of fixative. Spores were fixed for 1 h at 15°C, followed by three 15-min washes in sea water. A 10% stepped ethanol series was used to dehydrate specimens, which were then critical-point-dried, sputter-coated, and examined in a Hitachi 570 scanning electron microscope at 10 kV accelerating voltage.

Results

Each species showed a high degree of consistency in germination success of controls, and the results of 15 separate tests conducted over a five-month period from June through October 1994 are outlined in Table 1. There was also no significant difference ($P < 0·05$) between natural and artificial sea-water controls for each species. Tests using 2,4-D produced 24-h EC_{50} values between 2 and 6 mg L^{-1} for all three species and no significant difference in EC_{50} values between species ($n = 4$; $P < 0·05$). Dose response curves for 2,4-D are shown in Fig. 4. Individual tests for each species with all effluent/toxicant forms including sewage produced very high statistical power (power >0.99).

Response to each effluent differed among species and also varied over time. Table 2 summarizes a single assay series in which each species has been tested against each of the three effluent forms. The EC_{50} values enable comparison of the response by each species to the same effluent form, and threshold concentrations lie between the LOEC and NOEC values. Where a discrete NOEC was not obtained, threshold concentrations are lower than the LOEC value.

The germination success for *H. banksii* when tested against different effluent samples is shown in Fig. 5. For *H. banksii*, EC_{50} values were between 10% and 25% for the primary effluent, between 9% and 24% for the low-secondary effluent, and between 30% and 40% for the secondary effluent. Fig. 6 shows the germination success for *P. comosa* when exposed to the three effluent types. Although the primary and low-secondary effluents both produced variable results, the secondary effluent resulted in much more uniform responses. *P. comosa* generally appeared to be more sensitive than *H. banksii*, with EC_{50} values under 10% for the primary effluent, between 10% and 20% for the low-secondary effluent, and between 21% and 30% for the secondary effluent. *M. angustifolia* responded to the effluent treatments in a manner similar to that of the two fucoid algae, with variable response to the primary and low-secondary effluents and more uniform results for the higher quality secondary effluent (Fig. 7). EC_{50} values were between 1% and 8% for the primary effluent, between 12% and 45% for the low-secondary effluent, and between 32% and 47% for the secondary effluent.

Table 1. Summary of mean (sea-water) control germination responses ($n = 15$) and mean 2,4-D 24-h EC_{50} concentrations ($n = 4$), each with associated coefficient of variation (CV)

EC_{50} values refer to initial (nominal) concentrations of 2,4-D in sea water

Species	Mean control germination (%)	CV (%)	Mean 2,4-D 24-h EC_{50} (mg L^{-1})	CV (%)
H. banksii	89	4·8	3·6	37
P. comosa	91	4·7	2·6	14
M. angustifolia	89	1·6	3·8	45

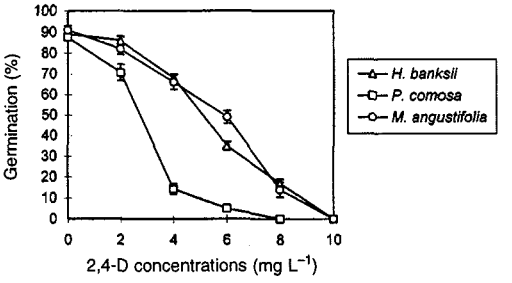


Fig. 4. Dose response curves for each of the three macrophytes when assayed with 2,4-dichlorophenoxyacetic acid (2,4-D). Each data point represents the mean of four replicates with associated error (s.e.m.) bars. Concentrations refer to initial (nominal) concentrations of 2,4-D in sea water.

Table 2. Summary of a single germination test series for each species when assayed against each of the effluent forms: primary treatment (P), low-secondary treatment (LS) and secondary treatment (S)

True threshold concentrations are lower than the LOEC values. Numbers are percentages (v/v, nominal). NOEC, no observable effect concentration; LOEC, lowest observable effect concentration

	NOEC	LOEC	24-h EC_{50}	95% CI
H. banksii				
P	<5	5	14.6	13.5–15.8
LS	<5	5	10.8	9.7–12.0
S	10	20	35.1	32.5–38.0
P. comosa				
P	<2	2	4.4	4.1–4.8
LS	<5	5	6.5	5.6–7.5
S	<10	10	23.7	21.6–26.0
M. angustifolia				
P	<1	1	2.0	1.8–2.3
LS	<5	5	19.1	17.9–20.2
S	<10	10	38.7	32.6–45.9

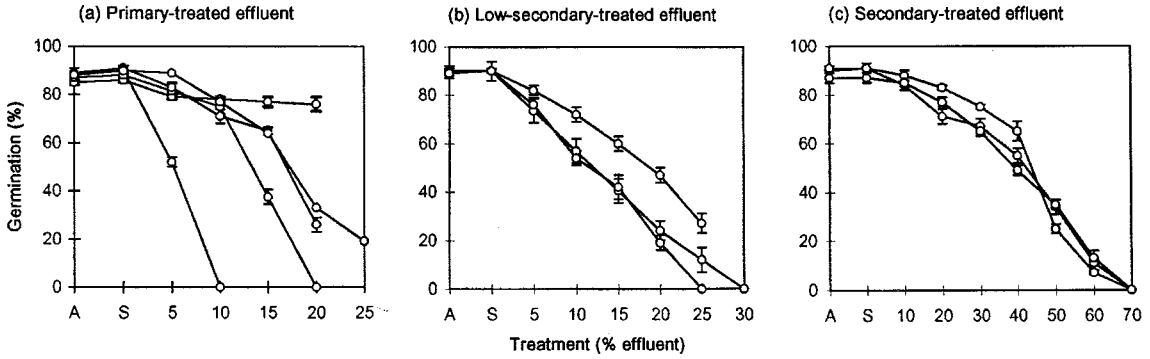


Fig. 5. Dose response curves for *Hormosira banksii* zygotes when assayed against each of the sewage effluent forms. Each curve represents a single assay series. Note the high variability in response for the primary-treated effluent and the more uniform results for the low-secondary-treated and secondary-treated effluents. Each data point represents the mean of four replicates with associated error (s.e.m.) bars. A, artificial sea-water control; S, natural sea-water control. Concentrations are nominal and refer to initial dilutions of sewage effluent in sea water.

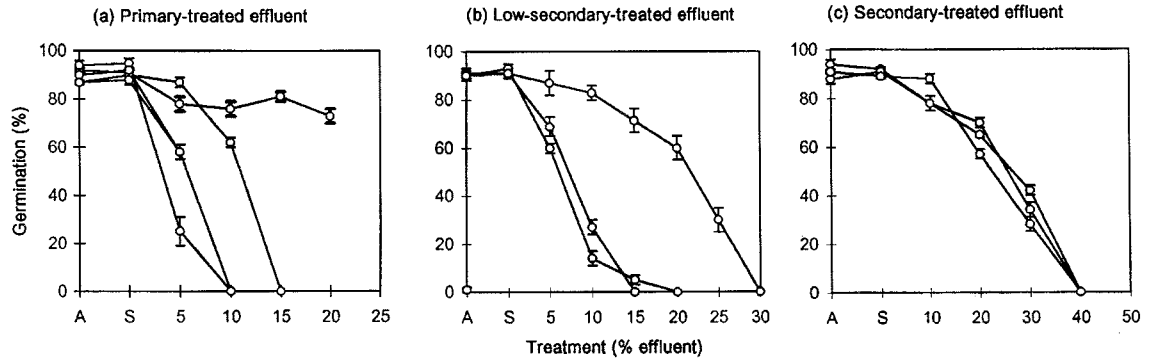


Fig. 6. Dose response curves for *Phyllospora comosa* zygotes when assayed against each of the sewage effluent forms. Each curve represents a single assay series. Note the greater variability in response for the primary-treated and low-secondary-treated effluents compared with that for the secondary-treated effluent. Each data point represents the mean of four replicates with associated error (s.e.m.) bars. A, artificial sea-water control; S, natural sea-water control. Concentrations are nominal and refer to initial dilutions of sewage effluent in sea water.

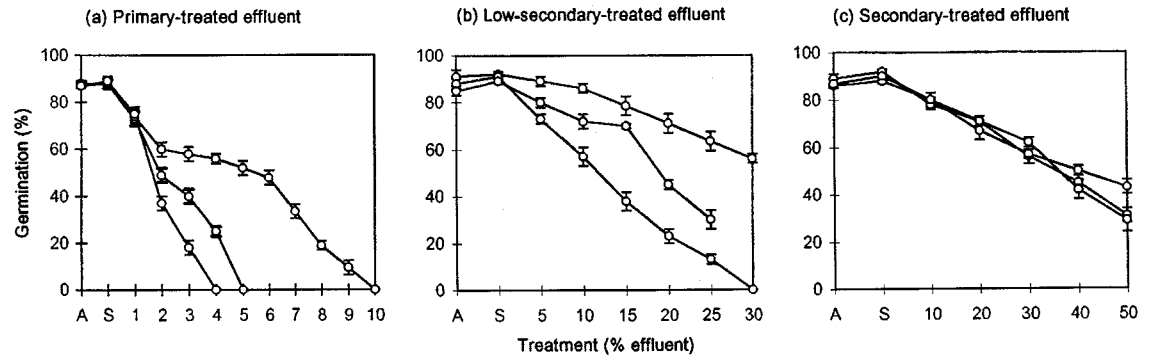


Fig. 7. Dose response curves for *Macrocystis angustifolia* zoospores when assayed against each of the effluent forms. Each curve represents a single assay series. Note the greater variability in response for the primary-treated and low-secondary-treated effluents compared with that for the secondary-treated effluent. Each data point represents the mean of four replicates with associated error (s.e.m.) bars. A, artificial sea-water control; S, natural sea-water control. Concentrations are nominal and refer to initial dilutions of sewage effluent in sea water.

Discussion

The results indicate that each of the algal species tested possesses substantial sensitivity to different types of effluent and also to variation in quality of particular effluent forms. Variation in germination response for the primary and low-secondary effluents contrasts with the more uniform responses for exposure to different samples of the secondary effluent and to the reference toxicant 2,4-D. This is presumably an indication of greater variability in constituents in the less-treated primary and low-secondary effluents. There is also a trend for lower inhibition of germination in the low secondary and secondary effluents than in the primary effluent. This is presumably an indication of a reduction in concentration of potential toxicants as influent is subjected to a greater degree of treatment.

The threshold concentration for germination inhibition of *M. angustifolia* spores exposed to the primary effluent is consistent with reported effects of primary-treated sewage on germination of *M. pyrifera* zoospores (Anderson and Hunt 1988). For the latter species, significant inhibition of zoospore germination was also reported at concentrations of primary-treated sewage effluent greater than 1%. The greater sensitivity for *M. angustifolia* (compared with that for the two fucoid species) when exposed to the primary effluent contrasts with the response to the low-secondary effluent, to which *M. angustifolia* appeared to be less sensitive. Smaller life stages of marine macroalgae are particularly sensitive to exogenous compounds (Thursby *et al.* 1985; Anderson *et al.* 1990; Burridge *et al.* 1995*a*, 1995*b*), and the smaller size of the *M. angustifolia* zoospore suggests a possible greater sensitivity than that of the larger zygotes of the two fucoid species when exposed to the same effluent. Although interspecific variation in response may be related to size, the present results indicate that variation may also be attributable to different physiological responses from each species.

The actual expression of toxicity by components of the sewage effluent(s) is unknown. Although the effects of exogenous compounds on macroalgal life stages have been recorded (Steele and Thursby 1983; Thursby *et al.* 1985; Anderson and Hunt 1988; Anderson *et al.* 1990; Burridge *et al.* 1995*a*, 1995*b*; Gunthorpe *et al.* 1995), the effects of components of a complex effluent such as sewage are difficult to quantify given the potential for synergism and antagonism between constituents. Anderson and Hunt (1988) discussed the effects of a primary sewage effluent on germination of *M. pyrifera* zoospores and indicated that the contribution to toxicity by individual components of the effluent was unclear. A possible mechanism to quantify component effects may be to correlate routine toxicity screening for several species with data derived from detailed analytical analyses of the same effluent. Potential toxicants identified in this process could then be assayed individually against the same species.

The 24-h EC_{50} values obtained here for 2,4-D suggest that algal macrophytes may be less sensitive than animal species to 2,4-D. In the estuarine crab *Chasmagnathus granulata*, Rodriguez and Amin (1991) reported 24-h median-lethal concentrations (LC_{50}) of 4·5–13·0 mg L^{-1} for zoea larvae and >6·4 mg L^{-1} for juveniles. For adult crabs, 72-h LC_{50} values have been reported as 6·7 mg L^{-1} for *C. granulata* and 213 mg L^{-1} for *Uca uruguayensis* (Rodriguez and Lombardo 1991). Wan *et al.* (1991) have reported 96-h LC_{50} values for Pacific salmonids (*Oncorhynchus* sp.) of 10 mg L^{-1} and 500 mg L^{-1} when assayed with formulated products of 2,4-D. Burridge *et al.* (1995*b*) have reported LOEC values for mortality of 1- and 7-day-old *P. comosa* germlings exposed to 2,4-D at 100 mg L^{-1} and 1000 mg L^{-1} respectively. Burridge *et al.* (1995*a*, 1995*b*) have also shown that germination of *P. comosa* is inhibited by formaldehyde at a concentration an order of magnitude less than that causing mortality of 7-day-old plants. For the algal species used in the present study, mortality of juvenile plants is likely to occur at 2,4-D concentrations substantially higher than those for the animal taxa previously assayed.

The lack of significant difference between 2,4-D median-effect concentrations for each species, and the similarity in dose response characteristics, suggest that the mechanism of toxic action of 2,4-D may be similar for each. The physiological effect of 2,4-D on algal germination is unknown, although inhibition of ATPase activity by chlorophenol compounds (Arguello *et al.* 1990) could reduce rates of photophosphorylation and oxidative phosphorylation and thereby lead to delayed germination. Inhibition of germination could also be related to inhibition of polar expression in the spore or zygote. Arguello *et al.* (1990) have reported 2,4-D-induced alteration in uptake kinetics of Ca^{2+} in avian skeletal muscle and have suggested that alterations in permeability of membranes to Ca^{2+} may be responsible for myopathic actions of 2,4-D. Other studies (e.g. Robinson and Jaffe 1975; Jaffe *et al.* 1976) have proposed that cation (Ca^{2+}) movement across the cell membrane in the basal pole of *Fucus* (Fucales) zygotes is a precursor to zygote germination. Movement of Ca^{2+} into the base of the zygote has been described as generating a transcellular electrical field that initiates the movement of negatively charged vesicles into the basal pole, leading to adhesion and ultimately rhizoid formation. Exposure to 2,4-D and alteration of Ca^{2+} membrane permeability could inhibit this mechanism of cellular polarization and delay germination of fucoid zygotes. The same process may apply to *M. angustifolia* zoospores; Anderson and Hunt (1988) have proposed a similar mechanism for germination inhibition of *M. pyrifera* when exposed to high Zn^{2+} concentrations. Burridge *et al.* (1995*a*) have previously indicated that inability of *P. comosa* zygotes to germinate in the presence of tributyltin may result from inhibition of development of the apico-basal axis associated with normal development.

The methods outlined here offer simple, easy and ecologically relevant toxicological tests that can be used by regulatory authorities in southern Australia for routine effluent screening. The tests are very reproducible, especially for *P. comosa*, for which initial work suggests a coefficient of variability under 20%, and they offer exceptionally high statistical power. The year-round fertility and availability of gametes and zoospores also provides the basis for uninterrupted monitoring of effluent quality. Further work to validate these tests could involve use of reference toxicants to develop spatial and temporal data sets to quantify background variation in control germination and toxic responses.

Acknowledgments

We thank Barwon Water and Melbourne Water for support with this study and David Greenwood for assistance with figures and review of the manuscript.

References

Ahsanullah, M., Negilski, D. S., and Mobley, M. C. (1981). Toxicity of zinc, cadmium and copper to the shrimp *Callianassa australiensis*. I. Effects of individual metals. *Marine Biology (Berlin)* **64**, 299–304.

Anderson, B. S., and Hunt, J. W. (1988). Bioassay method for evaluating the toxicity of heavy metals, biocides and sewage effluent using microscopic stages of the giant kelp *Macrocystis pyrifera* (Agardh): a preliminary report. *Marine Environmental Research* **31**, 113–34.

Anderson, B. S., Hunt, J. W., Turpen, S. L., Coulon, A. R., and Martin, M. (1990). Copper toxicity to microscopic stages of giant kelp *Macrocystis pyrifera*: interpopulation comparisons and temporal variability. *Marine Ecology Progress Series* **68**, 147–56.

Arguello, J. M., Evangelista, A. M., and Duffard, R. O. (1990). Ca^{2+} homeostasis alterations induced by 2,4-dichlorophenoxyacetic butyl ester and 2,4-dichlorophenoxyacetic acid on avian skeletal muscle. *Biochemical Pharmacology* **40**, 2441–8.

Borowitzka, M. A. (1972). Intertidal algal species diversity and the effect of pollution. *Australian Journal of Marine and Freshwater Research* **23**, 73–84.

Brawley, S. H., and Johnson, L. E. (1991). Survival of fucoid embryos in the intertidal zone depends upon developmental stage and microhabitat. *Journal of Phycology* **27**, 179–86.

Brown, V. B., Davies, S. A., and Synnot, R. N. (1990). Long-term monitoring of the effects of treated sewage effluent on the intertidal macroalgal community near Cape Schanck, Victoria, Australia. *Botanica Marina* **33**, 85–98.

Burridge, T. R., Clayton, M. N., and Hallam, N. D. (1993). Oogenesis and stalk mediated fertilisation in *Phyllospora comosa* (Labillardière) C. Agardh (Seirococcaceae, Phaeophyta). *Botanica Marina* **36**, 223–32.

Burridge, T. R., Lavery, T., and Lam, P. K. S. (1995*a*). Effects of tributyltin and formaldehyde on the germination and growth of *Phyllospora comosa* (Labillardière) C. Agardh (Phaeophyta:Fucales). *Bulletin of Environmental Contamination and Toxicology* **55**, 525–32.

Burridge, T. R., Lavery, T., and Lam, P. K. S. (1995*b*). Acute toxicity tests using *Phyllospora comosa* (Labillardière) C. Agardh (Phaeophyta:Fucales) and *Allorchestes compressa* Dana (Crustacea:Amphipoda). *Bulletin of Environmental Contamination and Toxicology* **55**, 621–8.

Deysher, L. E., and Dean, T. A. (1986). *In situ* recruitment of sporophytes of the giant kelp, *Macrocystis pyrifera* (L.) C. A. Agardh: effects of physical factors. *Journal of Experimental Marine Biology and Ecology* **103**, 41–63.

Forbes, M. A., and Hallam, N. D. (1978). Gamete structure and fertilisation in the brown alga *Hormosira banksii* (Turner) Decaisne. *British Phycological Journal* **13**, 299–310.

Fritsch, F. E. (1945). 'The Structure and Reproduction of the Algae.' Vol. II. (Cambridge University Press: Cambridge.)

Gunthorpe, L., Nottage, M., Palmer, D., and Wu, R. (1995). The development of a fertilisation inhibition assay using gametes of the brown alga *Hormosira banksii*. *Australasian Journal of Ecotoxicology* **1**, 25–31.

Hamilton, M. A., Russo, R. C., and Thurston, R. V. (1978). Trimmed Spearman–Karber method for estimating lethal concentrations in toxicity bioassays. *Environmental Science and Technology* **11**, 714–18.

Jaffe, L. F., Robinson, K. R., and Nuccitelli, R. (1976). Local cation entry and self electrophoresis as an intracellular localisation mechanism. *Annals of the New York Academy of Sciences* **238**, 372–89.

Martin, M., and Richardson, B. J. (1995). A paradigm for integrated marine toxicity research? Further views from the Pacific rim. *Marine Pollution Bulletin* **30**, 8–13.

May, V. (1985). Observations on algal floras close to two sewerage outlets. *Cunninghamia* **1**, 385–94.

Murray, S. N., and Littler, M. M. (1978). Patterns of algal succession in a perturbated marine intertidal community. *Journal of Phycology* **14**, 506–12.

Reed, D. C. (1990). The effects of variable settlement and early competition on patterns of kelp recruitment. *Ecology* **71**, 776–87.

Richardson, B. J., and Martin, M. (1994). Marine and estuarine toxicity testing: a way to go? Additional sitings from Northern and Southern Hemisphere perspectives. *Marine Pollution Bulletin* **28**, 138–42.

Robinson, K. R., and Jaffe, L. F. (1975). Polarizing fucoid eggs drive a calcium current through themselves. *Science (Washington, DC)* **187**, 70–2.

Rodriguez, E. M., and Amin, O. A. (1991). Acute toxicity of Parathion and 2,4-D to larval and juvenile stages of *Chasmagnathus granulata* (Decapoda, Brachyura). *Bulletin of Environmental Contamination and Toxicology* **47**, 634–40.

Rodriguez, E. M., and Lombardo, R. J. (1991). Acute toxicity of Parathion and 2,4-D to estuarine adult crabs. *Bulletin of Environmental Contamination and Toxicology* **46**, 576–82.

Steele, R. L., and Thursby, G. B. (1983). A toxicity test using life stages of *Champia parvula* (Rhodophyta). In 'Aquatic Toxicology and Hazard Assessment'. Sixth Symposium, ASTM STP 802. (Eds W. E. Bishop, R. D. Cardwell and B. B. Heidolph.) pp. 73–89. (American Society for Testing and Materials: Philadelphia.)

Tewari, A., and Joshi, H. V. (1988). Effect of domestic sewage and industrial effluents on biomass and species diversity of seaweeds. *Botanica Marina* **31**, 389–97.

Thursby, G. B., Steele, R. L., and Kane, M. E. (1985). Effects of organic chemicals on growth and reproduction in the marine red alga, *Champia parvula*. *Environmental Toxicology and Chemistry* **4**, 797–805.

Wan, M. T., Watts, R. G., and Moul, D. J. (1991). Acute toxicity to juvenile Pacific Northwest salmonids of basacid blue NB755 and its mixture with formulated products of 2,4-D, glyphosate and triclopyr. *Bulletin of Environmental Contamination and Toxicology* **47**, 471–8.

Zar, J. H. (1984). 'Biostatistical Analysis.' (Prentice-Hall: Englewood Cliffs, NJ.)

Manuscript received 1 November 1995; revised and accepted 28 June 1996