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SWAN RIVER TRUST

**A trial to determine the
effect of the herbicide
fluazifop-butyl on flora and
fauna of the Swan River
system**



Swan River Trust
Report No 12
1993



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A TRIAL TO DETERMINE THE EFFECT OF THE HERBICIDE FLUAZIFOP- BUTYL ON FLORA AND FAUNA OF THE SWAN RIVER SYSTEM

Report to the Swan River Trust
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FOREWORD

The infestation of exotic perennial plants into areas of native wetland is of considerable concern in terms of long term management and productivity of wetland environments. Control of these plants is both labour intensive and difficult.

A trial was conducted to determine the effectiveness of the herbicide fluazifop-butyl to eliminate monocotyledons from amongst native vegetation.

Trial results indicate that the herbicide fluazifop-butyl may be considered effective in eliminating introduced monocotyledons from a riverine wetland environment and no visually apparent effects upon the native vegetation were observed.

In addition the application of the herbicide to the *Juncus* complex had little effect on the terrestrial invertebrates inhabiting the *Juncus* beds or on the aquatic invertebrates inhabiting the adjacent shallow inshore river region.

Recommendations for the use of fluazifop-butyl are contained within the report.

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1. INTRODUCTION

The peripheral vegetation of estuarine systems contributes to the ecological health and aesthetics of waterways. It does so by trapping nutrients, providing food and creating habitats for a diverse range of vertebrate and invertebrate fauna (Pen 1983). Considerable clearing of the peripheral estuarine vegetation has occurred since European colonisation and today much of the remnant vegetation around the waterways of the south-west are inundated with weeds associated with grassland and human accessways.

The infestation of exotic perennial plants into areas of native wetland vegetation is of considerable concern in terms of the long term management and productivity of wetland environments. Introduced species, particularly monocotyledons such as kikuyu, watercouch, couch, buffalo and wild/bearded oats, compete with the native species for water, nutrients, light and space and often are more successful due to their colonising life strategies. In addition attempts to control exotic grasses by mowing may lead to the destruction of rush beds. Control of these species is both labour intensive and difficult.

Agencies responsible for management of foreshore areas are expected by the public to provide grassed areas for recreation. In doing so they are often encouraging the growth of these species. Management agencies are therefore faced with the dilemma of providing grassed recreation areas as well as controlling the spread of these plants into native foreshore vegetation. Moreover these agencies are expected to be environmentally responsible and financially accountable for their actions.

Possible techniques to remove introduced species include:

- chemical application,
- physical or mechanical removal,
- biological control, and;
- ecological control (Klemm, Siemon and Ruiz-Avila 1993).

Physical or mechanical removal involves the use of machinery or people to remove the weed species. One of the disadvantages of mechanical removal is that it disturbs the soil and the native vegetation further promoting weed growth. The area and accessibility of the infestation often also make it unfeasible. In addition it is likely that only part of the plant is removed leaving roots to regenerate. It is also labour intensive and consequently financially prohibitive. It is however possible to use physical removal in conjunction with other techniques.

Biological control involves the study and use of parasites, predators, and pathogens for the regulation of host population densities. The object of this method of control is not to eradicate the weed but to reduce the level of infestation to a point where the plant is no longer perceived to be a problem. Although this technique may have the lowest long-term environmental impact, the chances of discovering a suitable biological control agent is remote.

Ecological control involves modifying the environment to affect the growth of an introduced weed. Modifications may include flooding, nutrient reduction, salt water inundation, and light reduction. Monocotyledons are hardy plants and none of these strategies are considered feasible.

The advantages of chemical application are that it is labour and cost effective. However, potential impacts of using herbicides include toxic effects on fish and aquatic invertebrates and indirectly, birds through a reduction of food source. In addition, herbicide residues may impact on water users. Although it is possible to minimise the potential for herbicides to get into water, concern often remains and any ill health of foreshore plants or animals in the area is rightly or wrongly blamed on the herbicides. Consequently proposals to use such herbicides need to undergo environmental assessment.

One option for control considered worthy of environmental investigation is the use of the selective post-emergent herbicide, fluazifop-butyl (Fusilade®) which has been specifically formulated for the control of a broad range of annual and perennial grasses in non-gramineous crops (Finney and Sutton 1980). Such a study also requires an assessment of the potential harmful effects on the terrestrial and aquatic invertebrate fauna associated with the remnant native foreshore vegetation.

1.1 Aim

To determine if fluazifop-butyl is a safe, cost effective, but environmentally acceptable herbicide which can be used to eliminate monocotyledons from amongst native foreshore vegetation.

1.2 Choice of herbicide

Herbicides kill plants either through the inhibition or disruption of vital biochemical processes such as photosynthesis, respiration and protein synthesis. The effect is caused by either direct contact (cell desiccation) or systemic (translocative) action. Contact herbicides kill only the plant part to which the chemical is applied whereas systemic herbicides are absorbed by the plant parts and are then translocated throughout the plant system.

The advantage of translocated herbicides is that they are taken to the site of metabolic action of the plant, and prevent regeneration. Contact herbicides only kill the parts of the plant in contact with it. One of the problems associated with contact herbicides is the variation in selectivity and resultant damage to non-target plant species.

The herbicide fluazifop-butyl (Fusilade®) is a selective, post emergent, translocated herbicide used for the control of certain grass species. It was chosen first, due to its comparatively low toxicity and second, its specific nature to monocotyledons (Young et al 1993) (see Appendix 1). Trials by the Kings Park Board (n d) also indicated its value for control of Veldt grass (*Ehrharta calycina*).

1.3 How the herbicide works

The selectivity of this herbicide is due to a number of factors including the uptake of the compound by the plant, spray retention, absorption and translocation, site of action and metabolism (Derr et al. 1985). As a foliar spray, fluazifop-butyl is readily absorbed through the leaf surface of grasses and de-esterified to fluazifop. The parent acid is then translocated through the plant to the rhizome nodes and meristematic tissue via the phloem and xylem (Coupland 1989). Within 48 hrs of the herbicide application a cessation of growth occurs. However, existing leaves may be slow to die, often taking 3 to 4 weeks depending upon the application rate (Chandrasena and Sagar 1984). In time, the meristematic tissue in the nodes and buds becomes necrotic and young leaves show signs of chlorosis then necrosis, while older leaves show senescent pigment changes (Plowman et al. 1980).

Fluazifop-butyl is reported to be effective regardless of growth stage (Plowman et al. 1980) although this would be dependent upon rates of application which usually range between 0.25 and 2 kg active ingredient (a. i.) / ha. Kings Park Board (n d) reports that fluazifop-butyl must be applied when the grass is actively growing, not when under drought stress, and should not be used when rain is expected, though 60% of the chemical is absorbed into the leaf within 1 hour of application. It is important to use a wetting agent or spreader eg. Agral 60, as this can improve the effectiveness of the herbicide by up to 40%.

1.4 Safe use of fluazifop-butyl

This chemical is fairly safe to use having an LD50 >4000 mg/kg of body weight oral and >2000 mg/kg dermal acute, which compares favourably with other chemicals in common use, especially when diluted for field use (Kings Park Board n d).

The concentrate should be handled with care, not least because it is highly flammable. As with all herbicides/insecticides protective clothing should be worn when using them, eg PVC gloves, respirator etc. More specific instructions can be found on the commercial packaging.

1.5 Method

There are many issues involved when herbicides are used on or adjacent to waterways and these need to be carefully considered. The use of herbicides may represent the cheapest and potentially most efficient form of removing a weed species from adjacent to a river system, however their use must be evaluated carefully in terms of environmental impacts and benefits. The foremost consideration in dealing with herbicides adjacent to a river system is their impact on non-target native vegetation and animal life, and on the environment generally.

Based on the experience of the Kings Park Board (n d) the trial was designed to establish:

- whether monocotyledons could be eliminated from amongst native foreshore vegetation,
- whether the native flora and fauna of the river environment was adversely effected,
- best time to spray,
- concentration required for effective control,
- numbers of applications required, and;
- the best conditions for effective control.

In order to achieve this the study was undertaken in two parts. Section 2.0 outlines the effectiveness of the herbicide fluazifop-butyl on monocotyledons and on native foreshore vegetation and Section 3.0 determines the effect on fauna of the river environment.

1.5.1 Study site

The following criteria were used to determine the study area:

- (a) must be a wetland environment,
- (b) contain both native and introduced plant species,
- (c) consist of a relatively homogeneous mixture of species over a 50 metre distance parallel to the shoreline, and
- (d) accessible to the spraying contractor.

The area chosen was the Canning River foreshore, 120 metres south of the Cloisters Avenue boat ramp, Como, adjacent to the Kwinana Freeway (see Figure. 1).

The area selected has a 6–7 m band of *J. kraussii* stretching over approximately 500 m. The *Juncus* is interspersed with *Sarcocornia* spp. and bounded by an open woodland consisting of *Casuarina obesa* and *Melaleuca raphiophylla*. At high tide, water and wind induced waves reach the *Juncus* complex which buffers the other vegetation from the direct affects of river water inundation.

1.5.2 Pesticide application

A transect was established parallel to the shoreline for a distance of 50 metres, taken from high water mark.

Four quadrats (5 m x 5 m) were created at 5 metre intervals with a 10 metre gap between the second and third quadrats (see Figure. 2.).

Fluazifop-butyl was applied by a spraying contractor to the designated experimental plots between 7.30 am and 10.30 am the morning of December 13, 1991. The herbicide was applied using an hand-sprayer and at a rate of 1 kg a. i./ha. At the time of application the wind was blowing from an south-westerly direction at 1-2 km/hr and the tide was low (46 cm above the Fremantle low water datum).

Quadrats 2 and 4 were sprayed and treated as trial plots. Quadrats 1 and 3 remained untouched and served as the control plots. A red dye was incorporated into the spray to aid visibility and ensure that there was no overspray onto the control plots.

Water samples were taken following the herbicide application to be analysed for fluazifop-butyl by the Chemistry Centre of WA. Unfortunately these samples were lost during the analyses.

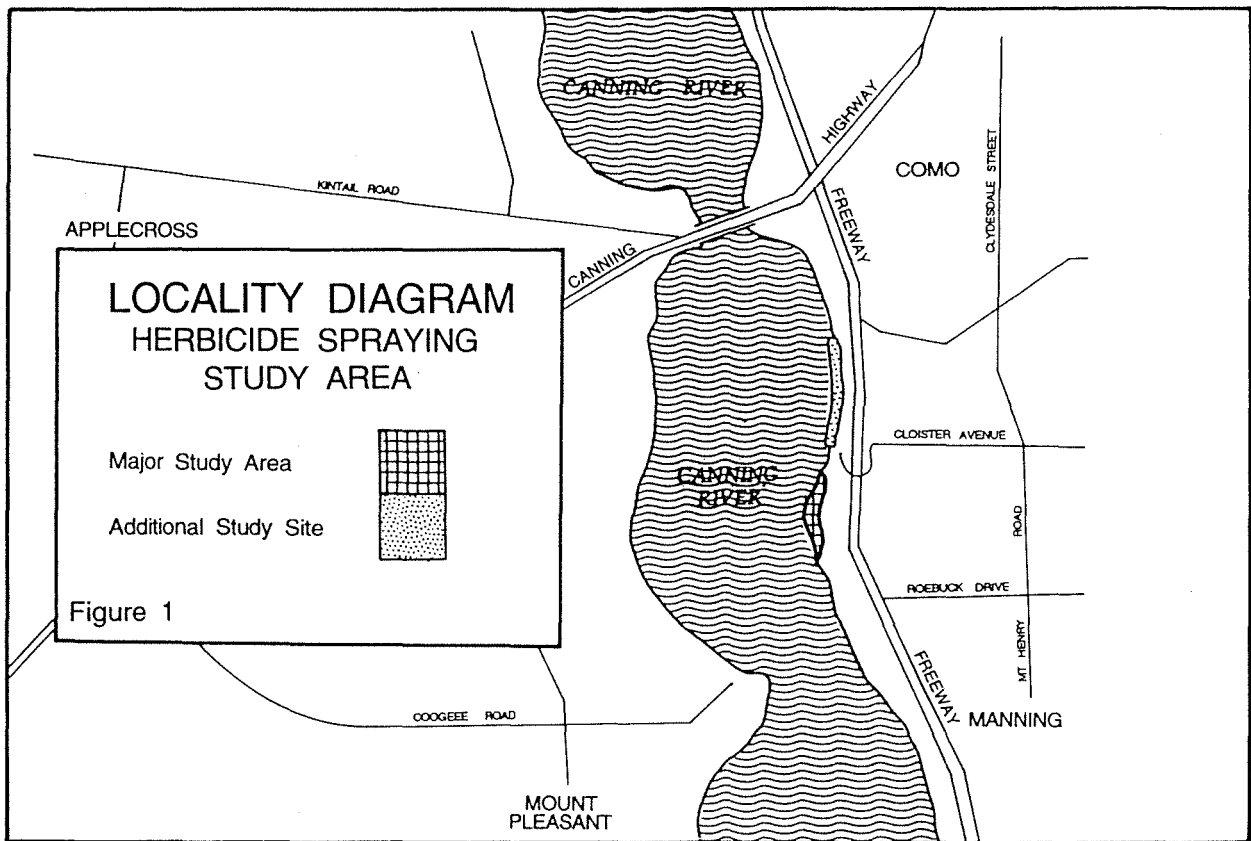


Figure 1: Locality diagram - herbicide spraying study area

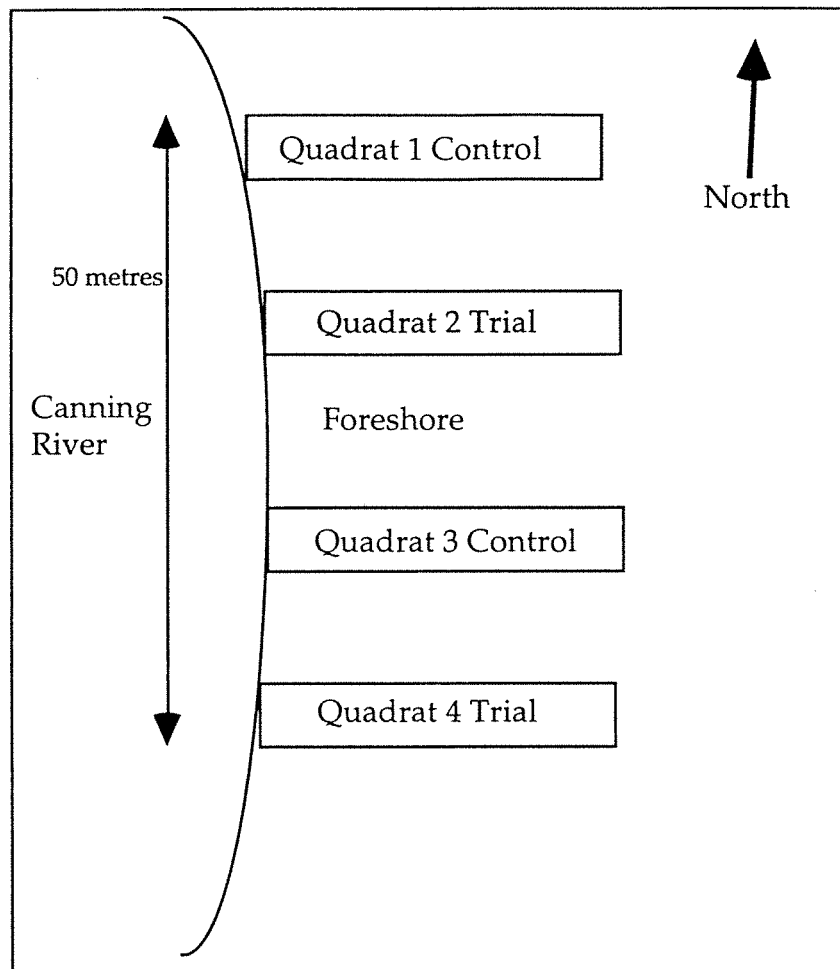


Figure 2: Transect area

2. EFFECT OF FLUAZIFOP-BUTYL ON MONOCOTYLEDONS AND NATIVE FORESHORE VEGETATION

This study aimed to investigate the effect of fluzifop-butyl on the vegetation of a riverine wetland environment consisting of both native and exotic species.

2.1 Aim

The aim of the first part of the study was to:

- determine the effectiveness of the herbicide fluzifop-butyl in eliminating introduced monocotyledons from a wetland environment.
- examine the visual effects of fluzifop-butyl application on native vegetation.

2.2 Methods and materials

2.2.1 Experimental design

Vegetation surveys of the four quadrats were undertaken before and after spraying (December 12, 1991 and March 10, 1992 respectively) to :

- (a) identify the plant species present,
- (b) quantify the extent of each species within the quadrat as a percentage of the total area before spraying using a visual estimation,

- (c) quantify the extent of introduced monocotyledon deaths as a percentage of the total number of weeds present prior to spraying using a visual estimation,
- (d) subjectively determine the visual effect of the herbicide upon the native vegetation after spraying.

In addition, photographs were taken as a visual record on December 17 1992 and March 10 1992.

An advertisement was placed in the state newspaper advising of the experiment. Trust staff were also on site during the spraying directing the public away from the area. Permission was also obtained from the Department of Health to conduct the spraying trial.

2.3 Results

The initial vegetation survey revealed that a high proportion of the native riverine vegetation chosen consisted of *Juncus kraussii* (Shore Rush) followed by *Sarcocornia quinqueflora* (Samphire). Other species found in relative abundance included *Isolepis nodosa* (Knotted Club Rush) and *Suaeda australis* (Seablite) (see Table 1).

The introduced monocotyledons were primarily represented by *Pennisetum clandestinum* (Kikuyu), *Stenotaphrum secundatum* (Buffalo) and *Cynodon dactylon* (Couch). Other plants identified included the herbs *Aster subulatus* (Wild aster) and *Rumex crispus* (Dock).

The vegetation survey undertaken on March 10, 1992 after the application of the herbicide, revealed a high weed mortality rate in the trial quadrats. Only 5% and 3% of the original Kikuyu remained in Quadrats 2 and 4 respectively. Similar results were obtained with the other weeds in the trial quadrats (see Table 2). However, some reinfestation of Kikuyu encroaching from the surrounding untreated area into Quadrat 4 was observed in the second vegetation survey.

In the control quadrats the weed population remained quite constant with some growth of Kikuyu recorded in Quadrat 3.

Visual examination of the native and introduced dicotyledon vegetation did not reveal any adverse effects from the spraying. Some reed deaths were observed in all quadrats.

The red dye showed that the fluzifop-butyl application was restricted to the trial quadrats and that there was no overspray into the control quadrats.

2.4 Discussion

The results suggest that the herbicide fluzifop-butyl has a significant effect upon introduced monocotyledon species, resulting in the eventual death of a majority of the population sprayed. Some reinfestation was noted but the incidence was probably the result of vegetative propagation from plants outside the trial quadrat, colonising the area.

TABLE 1: SPECIES LIST

PLANT SPECIES		% SPECIES OF TOTAL AREA BEFORE SPRAYING			
Scientific name	Common name	Quadrat 1 Control	Quadrat 2 Trial	Quadrat 3 Control	Quadrat 4 Trial
<i>Juncus kraussii</i>	Shore rush	30	70	50	60
<i>Isolepis nodosa</i>	Knotted club rush	5	6	4	0
<i>Suaeda australis</i>	Seablite	5	2	7	0
<i>Sarcocornia quinqueflora</i>	Samphire	50	5	15	4
<i>Atriplex hastata</i> *	Marsh saltbush	0	0	3	0
<i>Rumex crispus</i> *	Dock	0	0	4	0
<i>Lactuca serriola</i> *	Prickly lettuce	0	0	0	4
<i>Aster subulatus</i> *	Wild aster	0	7	2	0
<i>Paspalum distichum</i> *	Watercouch	0	0	0	2
<i>Pennistum clandestinum</i> *	Kikuyu	6	10	8	28
<i>Cynodon dactylon</i> *	Couch	4	0	0	0
<i>Stenotaphrum secundatum</i> *	Buffalo	0	0	7	0
<i>Avena barbata</i> *	Wild/bearded oats	0	0	0	2
	Total	100	100	100	100

TABLE 2: WEED DEATHS

PLANT SPECIES		MONOCOTYLEDON DEATHS % WEEDS REMAINING AFTER SPRAYING			
Scientific name	Common name	Quadrat 1 Control	Quadrat 2 Trial	Quadrat 3 Control	Quadrat 4 Trial
<i>Paspalum distichum</i> *	Watercouch	0	0	0	4
<i>Pennistum clandestinum</i> *	Kikuyu	98	5	102	3
<i>Cynodon dactylon</i> *	Couch	0	0	100	0
<i>Stenotaphrum secundatum</i> *	Buffalo	100	0	0	0
<i>Avena barbata</i> *	Wild/bearded oats	0	0	0	1

Drought stress and trampling by the invertebrate team contracted to study the effects of the herbicide on the fauna population may explain the few observed reed deaths.

Given that the first set of results were considered favourable for controlling introduced monocotyledons in an riverine wetland environment, an area north of the transect was identified for a full scale application. A three square metre section around a drain outfall, a 50 x 2 metre section north of the boat ramp and another 50 x 2 metre section 20 metres north of the previous site were applied with fluazifop-butyl by a licensed contractor. The herbicide was applied at the rate used in the previous trials.

The area was inspected one month after application and it was noted that a very high percentage of the weeds were dead with no visual effects upon the native vegetation being observed.

Observations after 12 months, however, indicated that regrowth of all weed species was occurring. Further investigation indicated that spraying at 1 kg a. i./ha was insufficient to kill the stolons. This indicated that regrowth at this rate would be an ongoing problem.

2.5 Conclusion

In conclusion, the herbicide fluazifop-butyl may be considered effective in eliminating introduced monocotyledons from a riverine wetland environment and no visually apparent effects upon the native vegetation were observed. However spot spraying may be required about one month after the initial treatment to eliminate regenerating weeds.

3. EFFECT OF FLUAZIFOP-BUTYL ON FAUNA OF THE RIVER ENVIRONMENT

Information relating to the toxicity of fluazifop-butyl to fauna is limited. It is reported to have low toxicity to mammals (acute oral LD50 to rats and guinea pigs being 3300 and 2660 mg/kg, respectively) and birds (LD50 17000 mg/kg in Mallard ducks) (Pesticide Manual 1987). The compound is thought to be moderately toxic to fish; LC50 96 hr for rainbow trout (*Salmo gairdneri*) is 1.6 mg/L (Plowman et al. 1980). Tests performed on invertebrates have shown fluazifop-butyl to have no visible affect on bees when applied orally and by contact (rates of 240 and 120 mg a. i./bee, respectively). Earthworms were also unaffected one and six months after an application to field plots at 5 kg a. i./ha (Plowman et al. 1980). In addition, the median effective concentrations (EC50, 24 and 48 hr) of fluazifop-butyl to the cladoceran *Daphnia magna*, an aquatic crustacean, were greater than 10 mg a. i./L (Plowman et al. 1980). Some conflicting information relating to the toxicity of fluazifop-butyl arises from ICI, the manufacturers. An ICI product bulletin (ICI 1987) suggests that under practical conditions of use, dilute concentrations of fluazifop-butyl reaching waterways, as a result of spray drift, would not adversely affect fish or daphnia. However, a safety data sheet (ICI 1988) suggests that Fusilade® is highly toxic to aquatic life and that users should avoid contaminating waterways.

3.1 Aim

This study has been designed to assess the potentially harmful effects of fluazifop-butyl on the terrestrial and aquatic invertebrate fauna associated with the remnant *Juncus kraussii* communities.

3.2 Materials and methods

3.2.1 Experimental design

A BACI (Before After Control Impact) design was set up to assess the acute effects of fluazifop-butyl on invertebrates over a one week period. Four experimental plots (5 m x 5 m) were sectioned off within the *J. kraussii* complex with a 5 m buffer zone separating each plot (Figure 1). Two plots were allocated as control and two to be applied with the herbicide. In addition, four experimental plots (5 m x 5 m) in the shallow water areas adjacent the vegetation

plots were also selected. Although no pesticide was directly applied to the aquatic plots, those buffering the treated or control sites within the vegetation were considered to be treated or control sites respectively (Figure 3). The herbicide would only be present in the 'treated' aquatic plots if there was contamination of these sites via drifting spray or runoff from the treated vegetation plots.

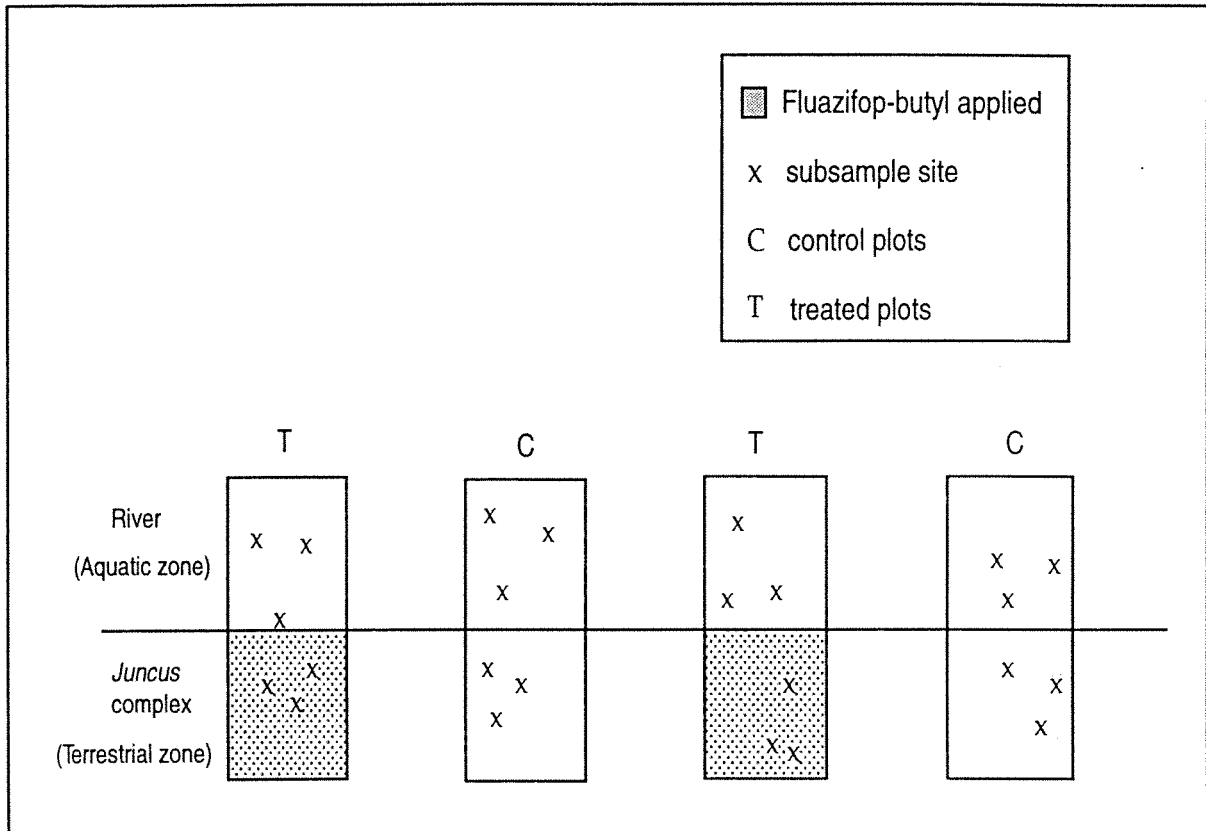


Figure 3 Invertebrate sampling site

3.2.2 Invertebrate sampling

Terrestrial and aquatic invertebrates within the experimental plots were sampled on December 12, 1991, one day prior to the herbicide application and then on two further occasions two and six days following the application (December 15 and 19, 1991). On each sampling occasion the fauna were collected according to the following method:

- 1 Terrestrial invertebrates were removed by taking three cores from each experimental plot.
- 2 Each sample was treated in the following manner.
 - A quadrat (30 x 30 cm) was randomly placed within the plot and all vegetative material within that area was removed using secateurs and bagged.
 - A spade was then used to remove the substrate beneath the quadrat to a depth of 15 cm and this was added to the bagged vegetative material.
 - Both vegetation and substrate were then preserved with 70% ethanol.
 - The aquatic invertebrates were sampled by randomly taking three cores from each experimental plot using an 11 cm diameter corer (ca. surface area = 0.01 m²) driven approximately 15 cm deep into the substrate.

- Cores were bagged and preserved with 10% formalin.
- All samples were rinsed and sorted on a 1 mm screen and animals retained were stored in 70% ethanol before being counted and identified.

3.2.3 Analyses

The abundance of the invertebrate taxa from the two habitat types were expressed as number of individuals per m². The top ten taxa from each habitat were ranked according to their contribution to the total number of organisms. Taxa contributing more than 10% of the total numbers were included in the statistical analyses.

Repeated measures analyses of variance (ANOVA) were used to test whether the abundance of the selected aquatic and terrestrial taxa were affected by the application of the herbicide. This statistical method was chosen because it accounts for the lack of independence in the data and separates the between and within subjects effects (Potvin et al. 1990). ANOVA enables orthogonal contrasts of interest to be examined. In this case, the contrast of most relevance was the difference between the densities of the animals in each treatment type obtained from pre- and post-treatment sampling occasions. A significant interactive effect would be expected to occur if the herbicide had a detrimental effect on the abundance of the animals in the treated enclosures.

All dependent variables were tested for heteroscedasticity by using Cochran's C-test and those variables showing significant ($P < 0.05$) heterogeneity were log transformed [$\log_{10}(n+1)$]. All of the analyses were run using the SPSS-X statistical package (SPSS Inc 1988) and differences were accepted as significant at $P < 0.05$ except where Cochran's C test showed that the variance was still heterogeneous after transformation. Where the data were still heterogeneous, results of the ANOVA were only considered to be significant at the 0.01 level of probability (Underwood 1981). Prior to the examination of the ANOVA results, the assumption of compound symmetry of the variance-covariance matrix was tested using Mauchly's criterion and where this was rejected, the results were corrected using the Huynh-Feldt value for epsilon (Potvin et al. 1990).

The Bray-Curtis index was used to compare the degree of similarity between the invertebrate communities of the treated and control enclosures in terms of species richness and abundance. For each sampling occasion, the index was calculated according to the following formula:

$$D = \sum (x_{1j} - x_{2j}) / \sum (x_{1j} + x_{2j})$$

where x_{1j} = abundance of species in the control enclosures and x_{2j} = abundance of species in the treated enclosures. In the strict sense, this index is actually an index of dissimilarity. The values generated by the index range from 0 (identical samples) to 1.0 (no species in common).

3.3 Results

3.3.1 Invertebrate abundance

3.3.1.1 Terrestrial

A total of 45 invertebrate taxa were collected from the experimental sites located in the *J. kraussii* complex (Table 3). These comprised four taxonomic groupings including crustaceans (5 spp.), arachnids (9 spp.), insects (30 spp.) and oligochaetes. Although many species were collected, the majority of these occurred infrequently and in low numbers during the study period (Appendix 2). Only two species contributed more than 10% to the total abundance of invertebrates collected, the oniscid isopod sp1. (54.89%) and the eusirid amphipod, *Telorchestes* sp. (25.24%) (Table 3). The abundance of these species within the treated and

control experimental plots during the study are shown in Figure 4. Whilst there was no clear detrimental effect of the herbicide application on the abundance of the amphipod, the isopod showed a slight decline in abundance. However, ANOVA showed that there was no significant ($P>0.05$) treatment by time interactive effect associated with the application of the herbicide for either species (Table 5).

3.3.1.2 Aquatic

A total of 20 invertebrate species were collected from the aquatic sites during the experimental period (Table 4). These comprised four taxonomic groupings including molluscs (4 spp.), polychaetes (6 spp.), crustaceans (9 spp.) and insect (1 spp.) Three species contributed more than 10% to the total abundance of invertebrates within this region, the spined worm *Boccardiella limnicola* (41.07%), the nereid, *Ceratonereis aequisetis* (30.02%) and the amphipod *Erichthonius* spp. (14.86%). The abundance of these species within the treated and control sites were plotted over time (Figure 5) and showed no detrimental effect of the herbicide treatment. This was supported by the ANOVA results which showed that there was no significant ($P>0.01$) effect of the herbicide on the abundance of these species (Table 5).

Many of the aquatic invertebrates collected were common throughout the experiment (Appendix 3). However, they contributed less than 10% to the total abundance of individuals (Table 4). These species were grouped into their respective taxa, along with those species which did contribute more than 10%, and the abundance of these taxa in the treated and control sites was plotted over time (Figure 6). Once again, there was no apparent effect of the herbicide on the abundance of the taxonomic groups and this was supported by the ANOVA results (Table 5).

3.3.2 Change in community structure

The Bray - Curtis index provides a measure of the similarity of the communities within the treated and control plots. For both habitat types, the change in the index during the study period is shown in Figure 7. The degree of similarity between the invertebrates within the control and treated plots of the terrestrial zone was low (0.65) prior to the herbicide being added. Following the application the two plots converged slightly but were highly dissimilar (0.89) one week after the application. Conversely, the degree of similarity between the treated and control plots of the aquatic zone was high (0.2) prior to the application of the herbicide. These became increasingly similar on subsequent sampling occasions (Figure 7).

Table 3: Mean density (No.per sq.m \pm 1s.e.) of invertebrate taxa collected from the terrestrial zone

TAXA	DENSITY	%	RANK
CRUSTACEA			
Isopoda			
Oniscidae sp. 1	129.6 \pm 42.2	54.89	1
Oniscidae sp. 2	3.9 \pm 2.0	1.65	5
Sphaeromatidae sp.	0.6 \pm 0.4	0.25	24"
Amphipoda			
Eusiridae			
<i>Telorchestes sp.</i>	59.6 \pm 45.8	25.24	2
Decapoda			
Sundathelphusidae			
<i>Holthuisana sp.</i>	2.4 \pm 1.1	1.02	9"
ARACHNIDA			
Chelonethi	0.3 \pm 0.3	0.13	33"
Araneida			
sp. 1	2.5 \pm 1.0	1.06	7"
sp. 2	2.4 \pm 0.9	1.02	9"
sp. 3	0.6 \pm 0.6	0.25	24"
sp. 4	0.6 \pm 0.4	0.25	24"
sp. 5	0.4 \pm 0.3	0.17	30"
sp. 6	0.7 \pm 0.4	0.30	18"
sp. 7	0.4 \pm 0.3	0.17	30"
<i>Gasteracantha minax</i>	0.3 \pm 0.3	0.13	33"
OLIGOCHAETA	1.5 \pm 0.9	0.63	12"
INSECTA			
Hemiptera			
Henicocorinae sp.	0.7 \pm 0.4	0.30	18"
Cicadidae sp.	2.5 \pm 1.2	1.06	7"
Ecinetidae sp.	0.4 \pm 0.3	0.17	30"
Meenopilidae sp.	1.0 \pm 0.5	0.42	15"
Bryocorinae sp.	0.3 \pm 0.3	0.13	33"
Derbidae sp.	0.6 \pm 0.4	0.25	24"
Rhyparochrominae sp.	0.3 \pm 0.3	0.13	33"

Table 3 Cont.

TAXA	DENSITY	%	RANK
INSECTA cont.			
Diptera			
larvae sp. 1	0.7 ± 0.4	0.30	18"
larvae sp. 2	0.3 ± 0.3	0.13	33"
larvae sp. 3	0.3 ± 0.3	0.13	33"
Chironomidae			
Orthocladinae sp.	4.4 ± 1.9	1.86	4
<i>Chironomus alternans</i>	0.3 ± 0.3	0.13	33"
Blattodea			
sp. 1	5.9 ± 2.4	2.50	3
sp. 2	0.3 ± 0.3	0.13	33"
Strepsiptera	3.4 ± 1.3	1.44	6
Coleoptera			
larvae sp. 1	1.5 ± 1.0	0.63	12"
larvae sp. 2	0.9 ± 0.5	0.38	17"
Curculionoidae	0.6 ± 0.4	0.25	24"
Oomatidae	1.8 ± 1.0	0.76	11
Eucinetidae	0.3 ± 0.3	0.13	33"
Orthoptera			
Dentridactylinae	0.7 ± 0.4	0.29	18"
Biroellinae			
sp. 1	1.0 ± 0.5	0.42	15"
sp. 2	0.7 ± 0.4	0.29	18"
Hymenoptera			
Symphyta	0.3 ± 0.3	0.13	33"
Tethredinidae	0.3 ± 0.3	0.13	33"
Apocrita			
sp. 1	0.6 ± 0.4	0.25	24"
sp. 2	1.5 ± 0.8	0.63	12"
Lepidoptera	0.7 ± 0.4	0.30	18"

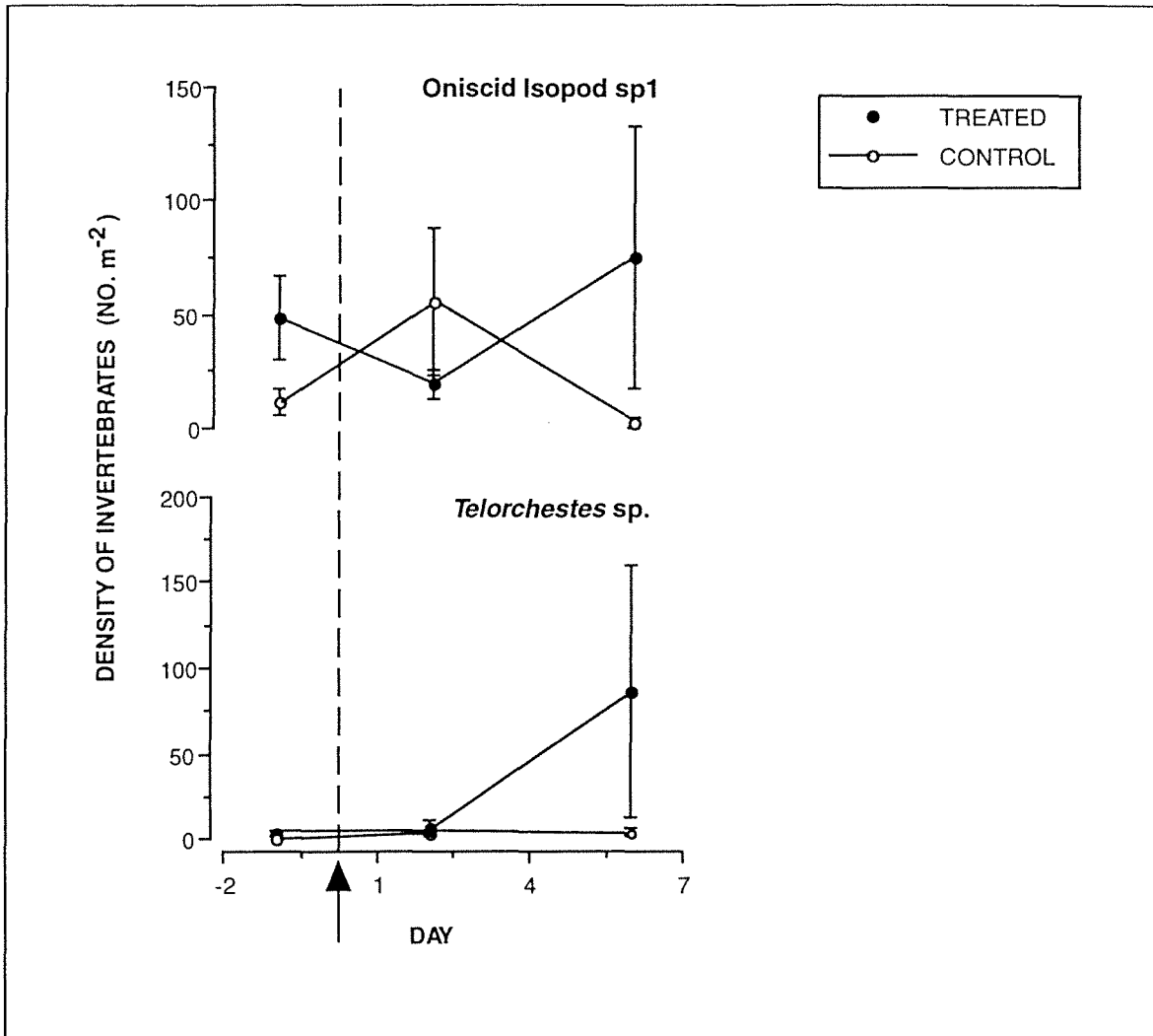


Figure 4: Mean densities (± 1 s.e.) of the two most common invertebrates occurring in the control and treated plots within the *Juncus* complex.

NB The arrow indicates the day on which the herbicide was applied.

Table 4: Mean density (No.per sq.m \pm 1s.e.) of benthic invertebrate taxa collected from the aquatic zone.

TAXA	DENSITY	%	RANK
MOLLUSCA			
Mytilidae			
<i>Xenostrobus securis</i>	258.2 \pm 34.4	2.21	5
<i>Musculista senhousia</i>	122.2 \pm 29.6	1.05	9
Trapeziidae			
<i>Fluviolanatus subtorta</i>	247.2 \pm 37.9	2.12	6"
Sanguinolariidae			
<i>Sanguinolaria biradiata</i>	2.8 \pm 2.8	0.02	15"
POLYCHAETA			
Spionidae			
<i>Boccardiella limnicola</i>	4792.2 \pm 455	41.07	1
Orbiniidae			
<i>Leitoscoloplos normalis</i>	377.8 \pm 58.2	3.24	4
Capitellidae			
<i>Capitella capitata</i>	41.7 \pm 16.1	0.36	11
Nereidae			
<i>Ceratonereis aequisetus</i>	3502.8 \pm 246.2	30.02	2
Serpulidae			
<i>Ficopomatus enigmaticus</i>	33.3 \pm 22.5	0.29	12"
Eunicidae			
<i>Marphysa sanguinea</i>	5.6 \pm 3.8	0.05	14
CRUSTACEA			
Amphipoda			
Gammaridae			
<i>Melita matilda</i>	205.6 \pm 64.5	1.76	8
Ischyroceridae			
<i>Erichthonius sp.</i>	1733.3 \pm 244.6	14.86	3
Corophiidae			
<i>Corophium minor</i>	63.9 \pm 31.2	0.55	10
<i>Paracorophium excavatum</i>	2.8 \pm 2.8	0.02	15"
Isopoda			
Sphaeromatidae			
<i>Syncassidina aestuaria</i>	247.2 \pm 35.1	2.12	6"
sp. 1	19.4 \pm 6.7	0.17	13"
sp. 2	2.8 \pm 2.8	0.02	15"
Decapoda			
Palaemonidae			
<i>Palaemonetes australis</i>	2.8 \pm 2.8	0.02	15"
Hymenosomatidae			
<i>Haliscarcinus bedfordi</i>	2.8 \pm 2.8	0.02	15"
INSECTA			
Neuroptera			
Sisyridae	2.8 \pm 2.8	0.02	15"

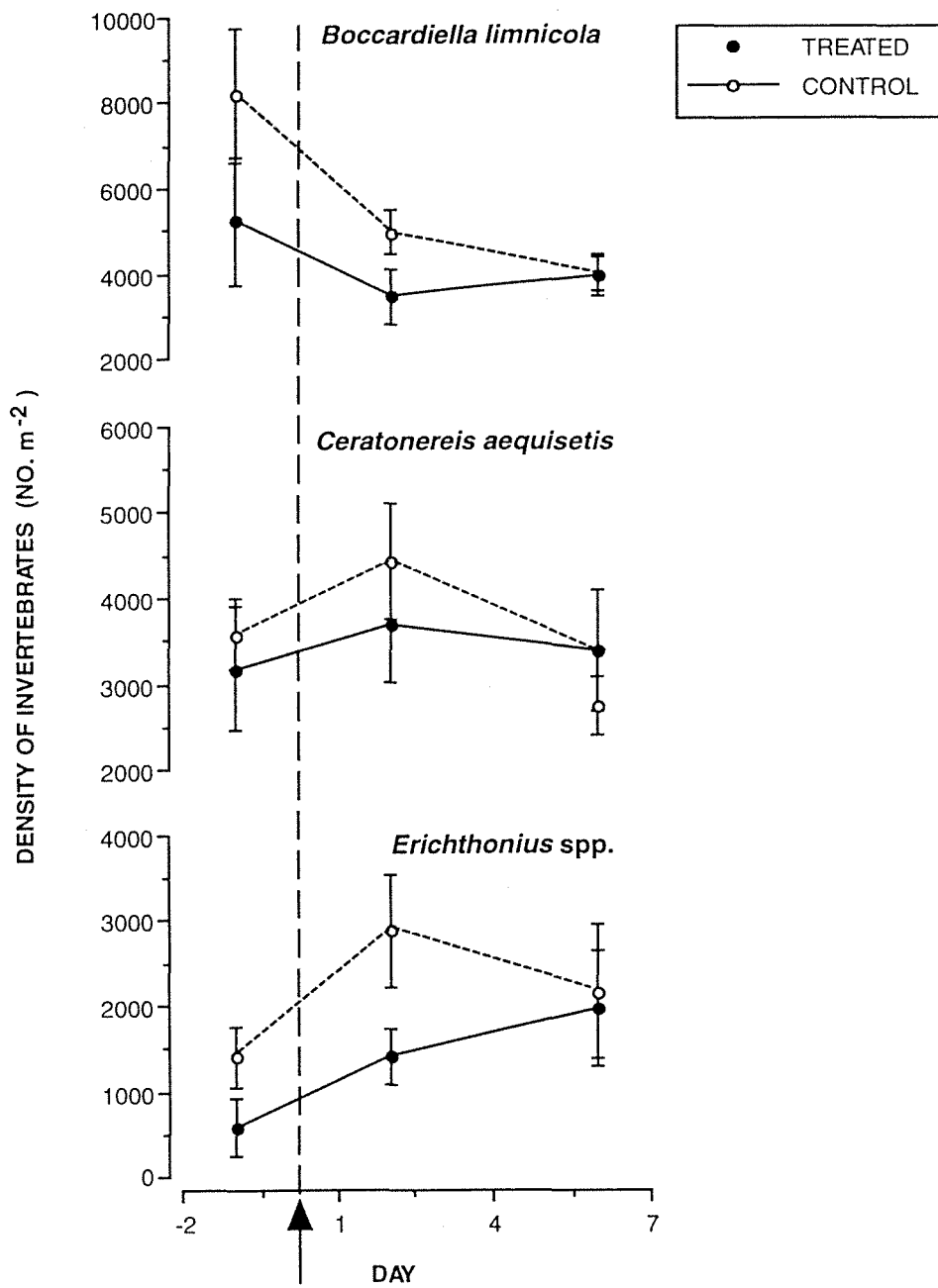


Figure 5: Mean densities (± 1 s.e.) of the three most common invertebrates occurring in the control and treated ponds within the aquatic zone.

NB: The arrow indicates the day on which the herbicide was applied to the *Juncus* complex.

Table 5: Summary of repeated measures analyses of variance for the contrast between pre and post-treatment macroinvertebrate densities.

NB: The contrast is between measures in the time factor and thus mean squares and F values for the contrast do not exist for the treatment factor. n.s. = not significant

Taxa	<u>Source of variation</u>										residual Mean squares	d.f.
	Mean squares	time d.f.	F	P	treatment x time Mean squares	d.f.	F	P				
Terrestrial												
<i>Telorchestes sp.</i>	0.39	2	0.37	0.69	n.s.	0.48	2	0.46	0.64	n.s.	1.03	20
Oniscid isopod sp1	0.52	2	0.84	0.45	n.s.	0.76	2	1.23	0.31	n.s.	0.61	20
Aquatic												
<i>Boccardiella limnicola</i>	0.03	2	0.31	0.74	n.s.	0.12	2	1.30	0.29	n.s.	0.09	20
<i>Ceratonereis aequisetis</i>	0.06	2	1.62	0.22	n.s.	0.01	2	0.37	0.69	n.s.	0.04	20
<i>Erichthonius sp.</i>	1.27	2	4.90	0.02	n.s.*	0.46	2	1.76	0.20	n.s.	0.26	20
Mollusca	0.28	2	0.98	0.39	n.s.	0.15	2	0.52	0.60	n.s.	0.29	20
Polychaeta	0.03	2	1.08	0.36	n.s.	0.04	2	1.51	0.24	n.s.	0.03	20
Amphipoda	0.42	2	2.51	0.11	n.s.	0.28	2	1.63	0.22	n.s.	0.17	20
Isopoda	0.89	2	1.86	0.18	n.s.	0.27	2	0.56	0.58	n.s.	0.48	20

* note: data was heterogenous after transformation therefore this result was not considered significant (P>0.01).

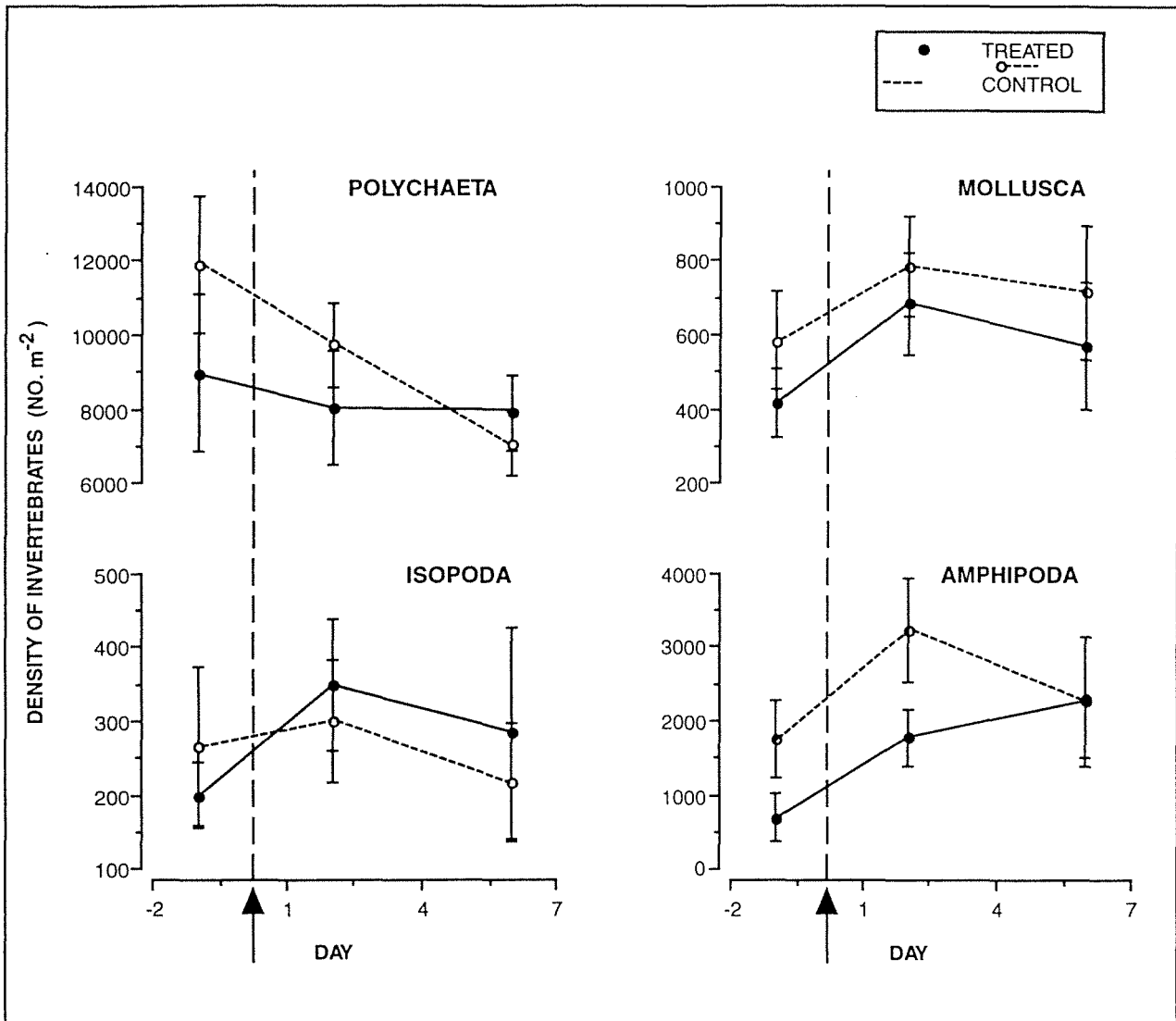


Figure 6: Mean densities (± 1 s.e.) of invertebrate taxa occurring in the control and treated plots within the aquatic zone.

NB: The arrows indicate the day on which the Herbicide was applied to the *Juncus* complex.

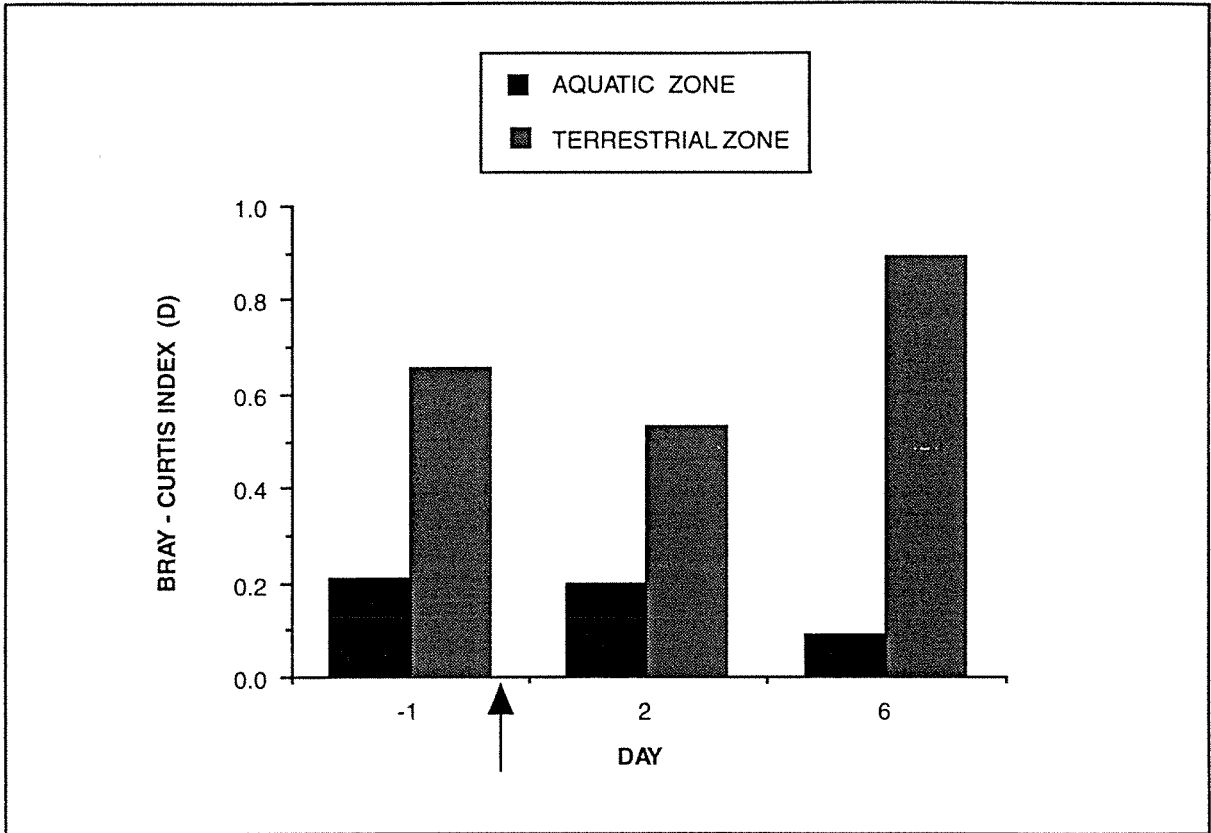


Figure 7: Similarity between control and treated plots in the terrestrial and aquatic zones.

NB: 0= identical samples; 1= no species in common. Arrow indicates the timing of the herbicide application.

3.4 Discussion

The invertebrates collected from the experimental plots located within the *J. kraussii* complex were highly diverse but most taxa present were low in abundance with many species occurring only once or twice during the study. The diversity of fauna present may have been a function of the many types of microhabitats present within both the *Juncus* complex and the experimental plots. These included above ground plant architectures, sand, loamy sand and decomposing mats of algae and detritus. The subsamples taken from the experimental sites were collected at random and hence no particular microhabitat was targeted. As a result, there was considerable variation in species abundance and richness within the terrestrial experimental plots and this accounts for the low levels of similarity between the treated and control plots prior to and following the application of the herbicide. The increasing dissimilarity between the treated and control plots following the application may have been a result of the herbicide, however the sampling procedure itself may have contributed to this.

Low abundance of individuals within a diverse array of species and taxa indicates that the composition of the invertebrate community within the *Juncus* complex may be extremely sensitive to disturbance. Environmental disturbances which occur frequently reduce diversity and result in communities dominated by opportunistic species with a reduction in the mean size of the dominating species (Gray 1989). This type of change in community structure within the peripheral vegetation may have a deleterious effect on trophic pathways.

Taxa collected during this study were non-sedentary, highly mobile fauna capable of recolonising depleted areas. The two most commonly collected invertebrate species, *Telorchestes* sp. and the oniscid isopod sp1 are important components of the detrital food chain within the *Juncus* complex. The application of fluzifop-butyl did not significantly decrease the abundance of these species despite a decrease in densities immediately following application. However, densities increased six days after application which suggests that the herbicide increased the amount of detritus available as food. In the long term an increase in these species might be expected to occur following an application of the herbicide. This could result from an increase in the amount of decaying vegetative material on which these invertebrates feed. However, the time over which this study was conducted was not sufficient to allow such changes to be observed or statistically proven.

The experimental sites within the aquatic zone were characterised by a continuous and homogeneous sandy substrate. Most of the invertebrates collected were common to the treated and control plots and hence the degree of similarity between the plots prior to the herbicide application was high. Following treatment this remained high indicating that the community integrity was retained. Neither the abundance of the common taxa (*Boccardiella limnicola*, *Ceratonereis aequisetis* and *Erichthonius* spp.) nor the common taxonomic groups (molluscs, polychaetes, crustaceans and insects) appeared to be affected by the herbicide application. However, since the concentration of fluzifop-butyl in the water column was not determined, it cannot be concluded that the aquatic invertebrate community was tolerant of the herbicide. The waterway may not have been contaminated when the herbicide was applied.

It is important to note that the abundance of invertebrates in both the vegetation and aquatic sites showed considerable variation around the means which indicates that perhaps not enough samples were taken during the study (Figure 4 - 6). This variation may have obscured any subtle, deleterious effects of the herbicide.

3.5 Conclusions

Under the conditions of application in this study fluzifop-butyl did not appear to adversely affect the common terrestrial invertebrates present. However the diverse composition of fauna within the *Juncus* complex is fragile and may be affected by the herbicide application. In order to reduce the effects of fluzifop-butyl upon the terrestrial invertebrate community it is recommended that, as part of a spraying regime, some areas within the peripheral vegetation do not have the herbicide applied to them. From these 'protected' areas, mobile invertebrate fauna could recolonise areas in which the fauna had become depleted due to a spraying programme.

In this study the application of the herbicide to the *Juncus* complex had no effect on the aquatic invertebrates inhabiting the adjacent shallow inshore river region. In order to avoid contamination of waterways the herbicide should always be applied when there is little or no wind or when it is blowing in a direction away from the water body. Applications should take place at low tide and preferably during a period in which rain is not expected during the next week so that runoff is minimised.

Fluazifop-butyl should be applied at a time at which it can be expected to have maximum efficacy against the weed species. Since its effectiveness is reduced by a soil moisture deficit (Coupland 1986,1989), it should be applied under conditions of adequate soil moisture. Fluazifop-butyl is also most effective under conditions which are warm (25-30°), humid and bright (ie. during spring).

It is highly recommended that a follow-up vegetation survey is carried out within 3 to 4 weeks after the application of fluazifop-butyl. This should determine whether the herbicide has been effective in the control of the perennial grasses and that there are no adverse effects on the peripheral vegetation. It would also allow field staff to observe if there have been undue and severe mortalities of invertebrates and other animal life.

This study investigated the acute effects of fluazifop-butyl on invertebrates when applied once at the rate of 1 kg a. i./ha. Further experimental investigation would be required to determine the exact effects of persistent usage over time or higher rates of application.

4. MANAGEMENT RECOMMENDATIONS

4.1 Weed regrowth

Two options are considered environmentally acceptable to address the problem of regrowth of weed species after 12 months:

- Application at 1.0 kg a.i./ha with spot spraying after 3-4 weeks if necessary.
- An increased rate of application upto 1.5 kg a.i./ha. Data analysis and literature investigation indicated that this increased rate of application is still environmentally acceptable. The following recommendations acknowledge this change in application rate and provision is made for ongoing monitoring.

4.2 Recommendations for use of fluazifop-butyl

- 1 The herbicide should only be used in accordance with manufacturers instructions.
- 2 Herbicide should be applied at a rate of no greater than 1.5 kg a. i./ha.
- 3 A follow-up survey should be conducted 3-4 weeks after application to determine:
 - effective control of perennial grasses,
 - the need for follow-up spot spraying
 - adverse effect on native vegetation, and
 - undue and severe mortalities of invertebrates and other animal life.
- 4 Any adverse effects on flora and fauna should be reported to the Swan River Trust.
- 5 A spraying regime should be established that includes areas within the foreshore area which do not have herbicide applied to them. This will allow mobile invertebrate fauna to recolonise areas which may have been depleted as a result of spraying.
- 6 The herbicide should be applied when there is little or no wind or when it is blowing in a direction away from the waterbody.

- 7 Applications should take place at low tide and preferably during a period in which rain is not expected during the next week so that runoff minimised.
- 8 Fluazifop-butyl should be applied under warm 25-30 °C humid and bright conditions (ie during spring).
- 9 An officer should be on-site to supervise spraying activity and restrict public access to the site.
- 10 The SRT will undertake further experimental investigation to determine the effects of persistent usage over time or at higher rates of application.

5. REFERENCES

- Anon (1991) The Agrochemical Handbook . Royal Society of Chemistry 3rd Edition UK
- Anon (1991a) The Pesticide Manual. British Crop Protection 9th Edition UK
- Brock MA and Pen LJ (1984) Ecological Studies of the Canning River Wetland. City of Canning Aust.
- Chandrasena, J.P.N.R. and Sagar, F.R. (1984) Effects of fluazifop-butyl on shoot growth and rhizome buds of *Elymus repens* (L.) Gould. Weed Research 24: 297 - 303.
- Coupland, D. (1986) The effects of environmental factors on the performance of fluazifop-butyl against *Elymus repens*. Annals of Applied Biology 108: 353 - 363.
- Coupland, D. (1989) Pre-treatment environmental effects on the uptake, translocation metabolism and performance of fluazifop-butyl in *Elymus repens*. Weed Research 29: 289 - 297.
- Derr, J.F., Monaco, T.J. and Sheets, T.J. (1985) Uptake and translocation of Fluazifop by three annual grasses. Weed Science 33: 612 - 617.
- Finney, J.R. and Sutton, P.B. (1980) Planned grass weed control with fluazifop-butyl in broad leaved crops. Proceedings British Crop Protection Council - Weeds pp. 427 - 436.
- Gray, J.S. (1989) Effects of environmental stress on species rich assemblages. Biological Journal of the Linnean Society 37: 19 - 32.
- ICI (1987) 'Fluazifop - butyl: its safety to human health and the environment'. (ICI Agrochemicals, England.)
- ICI (1988) 'Fusilade 212 - Safety Data Sheet'. (ICI Operations, Australia.)
- ICI (nd) ICI Technical Information - Fusilade. ICI Crop Care Melbourne Aust.
- Kings Park Board (n.d) Notes from *Ehrharta calycina* eradication trials.
- Klemm, V V, Siemon, N L and Ruiz-Avila, R J (1993) *Hydrocotyle ranunculoides* : A control strategy for the Canning River Regional Park. Swan River Trust Report No 6.
- Plowman, R.E. Stonebridge, W.C. and Hawtree, J.N. (1980) Fluazifop-butyl - a new selective herbicide for the control of annual and perennial grass weeds. Proceedings British Crop Protection Council - Weeds. pp 29 - 37.
- Potvin, C., Lechowicz, M.J. and Tardif, S. (1990) The statistical analysis of ecophysiological response curves obtained from experiments involving repeated measures. Ecology 7: 1389 - 1400.
- SPSS Inc. (1988) 'SPSS-X Users Guide, 3rd ed. (SPSS Inc: Chicago.)
- The Pesticide Manual - A World Compendium 8th Ed. 1987
- Thomson WT (1986) Agricultural Chemicals Book 2. Thomson Publications Fresno California USA
- Underwood, A.J. (1981) Techniques of analysis of variance in experimental marine biology and ecology. Oceanography and Marine Biology: An Annual Review 19: 513 - 605.
- Young L M, Klemm V V and Deeley D M (1993) Pesticide use adjacent to the Swan-Canning Estuary. Report to the Swan River Trust. Swan River Trust Report No 11.

Appendix 1: Characteristics of fluazifop-butyl

(From Young et al. 1993)

Characteristic

Chemical

Trade name

fluazifop-butyl

Fusilade

Registered uses in WA

Selective. Controls grass weeds in broad leaf crops

Restrictions on use, safety warnings

Use in Swan/Canning

5 authorities. 29.04 kg

Acute toxicity ratings and health effects

Mammals

Low

Birds

Low

Fish and aquatic life

Medium

Stability, persistence

In soil: persistence 3 - 20 weeks. Rapidly absorbed by vegetation. Further data unavailable.

Solubility

(@20⁰C) 1 mg/L

Sources

Anon (1991)

Anon (1991a)

Appendix 2: Mean density (No. per sq.m \pm 1 s.e.) of invertebrate taxa collected from the treated and control enclosures in the terrestrial zone on separate sampling occasions

TAXA	TREATED			CONTROLS		
	Day -1	Day 2	Day 6	Day -1	Day 2	Day 6
CRUSTACEA						
Isopoda						
Oniscidae sp. 1	177.8 \pm 67.8	72 \pm 23	275 \pm 210	42 \pm 23	202 \pm 119	9.2 \pm 7.2
Oniscidae sp. 2	3.7 \pm 2.3	1.8 \pm 1.8	3.7 \pm 2.3	0 \pm 0	15 \pm 11	0 \pm 0
Sphaeromatidae sp.	0 \pm 0	1.8 \pm 1.8	0 \pm 0	0 \pm 0	1.8 \pm 1.8	0 \pm 0
Amphipoda						
Eusiridae						
<i>Telorchestes</i> sp.	9.2 \pm 6.0	0 \pm 0	0 \pm 0	0 \pm 0	9.2 \pm 5.3	13 \pm 8.2
Decapoda						
Sundathelphusidae						
<i>Holthuisana</i> sp.	1.8 \pm 1.8	0 \pm 0	1.8 \pm 1.8	7.3 \pm 7.3	1.8 \pm 1.8	0 \pm 0
ARACHNIDA						
Chelonethi						
	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	1.8 \pm 1.8	0 \pm 0
Araneida						
sp. 1	3.7 \pm 3.7	1.8 \pm 1.8	0 \pm 0	2.4 \pm 1.8	5.5 \pm 3.8	1.8 \pm 1.8
sp. 2	0 \pm 0	7.3 \pm 2.3	5.5 \pm 3.8	1.8 \pm 1.8	0 \pm 0	0 \pm 0
sp. 3	0 \pm 0	0 \pm 0	1.8 \pm 1.8	0 \pm 0	0 \pm 0	1.8 \pm 1.8
sp. 4	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	3.7 \pm 3.7	0 \pm 0
sp. 5	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	2.4 \pm 1.8	0 \pm 0
sp. 6	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	2.4 \pm 1.8	0 \pm 0
sp. 7	0 \pm 0	1.8 \pm 1.8	0 \pm 0	0 \pm 0	2.4 \pm 1.8	0 \pm 0
<i>Gasteracantha</i> mino	0 \pm 0	0 \pm 0	0 \pm 0	1.8 \pm 1.8	0 \pm 0	0 \pm 0
OLIGOCHAETA						
	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	1.8 \pm 1.8
INSECTA						
Hemiptera						
Henicocorinae sp.	1.8 \pm 1.8	24 \pm 16	303 \pm 271	0 \pm 0	2.4 \pm 1.8	0 \pm 0
Cicadidae sp.	1.8 \pm 1.8	0 \pm 0	0 \pm 0	0 \pm 0	5.5 \pm 5.5	6.1 \pm 3.6
Ecinetidae sp.	0 \pm 0	1.8 \pm 1.8	0 \pm 0	2.4 \pm 1.8	0 \pm 0	0 \pm 0
Meenopilidae sp.	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	2.4 \pm 1.8	1.8 \pm 1.8
Bryocorinae sp.	0 \pm 0	0 \pm 0	1.8 \pm 1.8	0 \pm 0	0 \pm 0	0 \pm 0
Derbidae sp.	1.8 \pm 1.8	0 \pm 0	1.8 \pm 1.8	0 \pm 0	0 \pm 0	0 \pm 0
Rhyparochrominae sp.	0 \pm 0	0 \pm 0	1.8 \pm 1.8	0 \pm 0	0 \pm 0	0 \pm 0

TAXA	TREATED			CONTROLS		
	Day -1	Day 2	Day 6	Day -1	Day 2	Day 6
Diptera						
larvae sp. 1	0 ± 0	0 ± 0	0 ± 0	2.4 ± 2.4	1.8 ± 1.8	0 ± 0
larvae sp. 2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
larvae sp. 3	0 ± 0	1.8 ± 1.8	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Chironomidae						
Orthocladinae sp.	1.8 ± 1.8	1.8 ± 1.8	1.8 ± 1.8	1.8 ± 1.8	6.1 ± 3.6	0 ± 0
<i>Chironomus alternans</i>	0 ± 0	9.2 ± 9.2	7.3 ± 5.4	0 ± 0	1.8 ± 1.8	0 ± 0
Blattodea						
sp.1	0 ± 0	1.8 ± 1.8	1.8 ± 1.8	0 ± 0	17 ± 11	9.8 ± 7.3
sp.2	0 ± 0	1.8 ± 1.8	7.3 ± 4.6	0 ± 0	0 ± 0	0 ± 0
Strepsiptera	0 ± 0	3.7 ± 3.7	0 ± 0	1.8 ± 1.8	5.5 ± 3.8	9.2 ± 6
Coleoptera						
larvae sp. 1	1.8 ± 1.8	1.8 ± 1.8	0 ± 0	0 ± 0	5.5 ± 5.5	0 ± 0
larvae sp. 2	1.8 ± 1.8	1.8 ± 1.8	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Curculionoidae	0 ± 0	0 ± 0	1.8 ± 1.8	0 ± 0	0 ± 0	0 ± 0
Oomatidae	0 ± 0	1.8 ± 1.8	1.8 ± 1.8	0 ± 0	3.7 ± 2.3	0 ± 0
Eucinetidae	0 ± 0	1.8 ± 1.8	5.5 ± 5.5	1.8 ± 1.8	0 ± 0	0 ± 0
Orthoptera						
Dentridactylinae	0 ± 0	0 ± 0	0 ± 0	0 ± 0	4.3 ± 2.2	0 ± 0
Biroellinae						
sp.1	0 ± 0	0 ± 0	0 ± 0	3.7 ± 2.3	0 ± 0	2.4 ± 1.8
sp.2	0 ± 0	1.8 ± 1.8	0 ± 0	2.4 ± 1.8	1.8 ± 1.8	0 ± 0
Hymenoptera						
Symphyla	0 ± 0	0 ± 0	7.3 ± 3.7	0 ± 0	0 ± 0	0 ± 0
Tethredinidae	0 ± 0	0 ± 0	7.3 ± 4.6	0 ± 0	0 ± 0	0 ± 0
Apocrita						
sp.1	0 ± 0	1.8 ± 1.8	0 ± 0	0 ± 0	1.8 ± 1.8	0 ± 0
sp.2	1.8 ± 1.8	0 ± 0	1.8 ± 1.8	0 ± 0	0 ± 0	0 ± 0
Lepidoptera	1.8 ± 1.8	1.8 ± 1.8	0 ± 0	0 ± 0	0 ± 0	2.4 ± 1.8

Appendix 3: Mean density (No. per sq.m \pm 1 s.e.) of benthic invertebrate taxa collected from the treated and control enclosures in the aquatic zone on separate sampling occasions

TAXA	TREATED			CONTROLS		
	Day -1	Day 2	Day 6	Day -1	Day 2	Day 6
MOLLUSCA						
Mytilidae						
<i>Xenostrobus securis</i>	200 \pm 68.3	183.3 \pm 40.14	233.3 \pm 80.3	400 \pm 106.5	350 \pm 95.7	150 \pm 80.6
<i>Musculista senhousia</i>	116.7 \pm 30.7	200 \pm 161.2	166.7 \pm 66.7	50 \pm 22.4	50 \pm 22.4	150 \pm 22.4
Trapeziidae						
<i>Fluviolanatus subtorta</i>	83.3 \pm 30.7	300 \pm 89.4	166.7 \pm 80.3	133.3 \pm 61.5	383.3 \pm 70.3	416.7 \pm 130.2
Sanguinolariidae						
<i>Sanguinolaria biradiata</i>	16.7 \pm 16.7	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
POLYCHAETA						
Spionidae						
<i>Boccardiella limnicola</i>	5233.3 \pm 1484.5	3450 \pm 663.7	4000 \pm 417.9	8200 \pm 1559.1	4983.3 \pm 502.3	3966.7 \pm 493.1
Orbiniidae						
<i>Leitoscoloplos normalis</i>	433.3 \pm 147.6	733.3 \pm 185.6	466.7 \pm 122.9	150 \pm 61.9	266.7 \pm 84.3	216.7 \pm 119.5
Capitellidae						
<i>Capitella capitata</i>	100 \pm 68.3	116.7 \pm 54.3	33.3 \pm 21.1	0 \pm 0	0 \pm 0	0 \pm 0
Nereidae						
<i>Ceratonereis aequisetus</i>	3183.3 \pm 726.8	3700 \pm 687.5	3383.3 \pm 700.2	3583.3 \pm 406.1	4433.3 \pm 666.7	2733.3 \pm 347.1
Serpulidae						
<i>Ficopomatus enigmaticus</i>	16.7 \pm 16.7	0 \pm 0	0 \pm 0	0 \pm 0	50 \pm 22.4	133.3 \pm 133.3
Eunicidae						
<i>Marphysa sanguinea</i>	0 \pm 0	33 \pm 21.1	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
CRUSTACEA						
Amphipoda						
Gammaridae						
<i>Melita matilda</i>	100 \pm 81.6	366.7 \pm 290.6	83.3 \pm 83.3	283.3 \pm 188.7	316.7 \pm 147	83.3 \pm 47.7
Ischyroceridae						
<i>Erichthonius sp.</i>	583.3 \pm 350.6	1400 \pm 337.6	1966.7 \pm 688.3	1400 \pm 357.7	2883.3 \pm 662	2166.7 \pm 783
Corophiidae						
<i>Corophium minor</i>	0 \pm 0	16.7 \pm 16.7	216.7 \pm 179.7	50 \pm 34.2	33.3 \pm 21.1	66.7 \pm 21.1
<i>Paracorophium excavatum</i>	0 \pm 0	0 \pm 0	0 \pm 0	16.7 \pm 16.7	0 \pm 0	0 \pm 0
Isopoda						
Sphaeromatidae						
<i>Syncassidina aestuaria</i>	166.7 \pm 49.4	333.3 \pm 80.3	283.3 \pm 142.4	216.7 \pm 87.2	300 \pm 81.7	183.3 \pm 60.1
sp. 1	33.33 \pm 21.1	16.7 \pm 16.7	0 \pm 0	50 \pm 22.4	0 \pm 0	16.7 \pm 16.7
sp. 2	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	16.7 \pm 16.7
Decapoda						
Paleamonidae						
<i>Palaemonetes australis</i>	0 \pm 0	16.7 \pm 16.7	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
Hymenosomatidae						
<i>Halicarcinus bedfordi</i>	0 \pm 0	16.7 \pm 16.7	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
INSECTA						
Neuroptera						
Sisyridae						
	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	16.7 \pm 16.7	0 \pm 0