

IMPACT OF RICE GRASS *SPARTINA ANGLICA*, AND THE EFFECT OF TREATING RICE GRASS
WITH THE HERBICIDE FUSILADE FORTE® ON BENTHIC MACRO-INVERTEBRATE
COMMUNITIES IN A NORTHERN TASMANIAN ESTUARY

COLIN SHEPHERD (BSC. HONS)

**Submitted in fulfillment of the requirements for the Degree of Master of
Science**

**SCHOOL OF ZOOLOGY
UNIVERSITY OF TASMANIA**

NOVEMBER 2011

STATEMENT OF ORIGINALITY

This thesis contains no material which has been accepted for a degree or diploma by the University of Tasmania or any other institution, and to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due acknowledgement is made in the text of this thesis.

Colin James Shepherd

STATEMENT OF AUTHORITY OF ACCESS

This thesis may be made available for loan and limited copying in accordance with the *Copyright Act 1968*.

Colin James Shepherd

ABSTRACT

The deliberate introduction of *Spartina anglica* into Tasmania has resulted in several estuaries becoming infested with *S. anglica*, with growing concerns that this highly invasive species is causing adverse impacts on estuarine ecosystems.

A strategy for the management of rice grass in Tasmania was developed in 1995, determining that the most cost effective and environmentally least damaging treatment for the control of *S. anglica* was the use of the herbicide Fusilade®, which was believed to not affect native saltmarsh species or seagrasses, is rapidly degraded and appeared to have low toxicity to estuarine fauna.

The strategy was largely based on two pilot studies, both of which acknowledged limitations because of their short-term sampling designs. Consequently, this study was designed to examine the possible impacts on benthic macro-invertebrate communities of using Fusilade® over a longer time period. During the course of my research, Fusilade® was replaced by Fusilade Forte®. As a result of this change this study involved the impact of Fusilade Forte®.

This study aimed to 1) investigate the biological differences between benthic macro-invertebrate assemblages that inhabit rice grass and mudflat communities, and 2) examine the potential acute and chronic impacts on benthic macro-invertebrate

communities of rice grass treated with the herbicide Fusilade Forte®, and the potential residence time of its chemical constituents post spraying.

Multivariate analyses (nMDS and PERMANOVA) showed that colonisation by rice grass changes the benthic macro-invertebrate community structure. Univariate ANOVA indicated mudflats generally exhibited lower total faunal abundances and diversity compared to rice grass habitats, and tended to be dominated by filter feeders and opportunistic scavengers rather than grazers. Differences in community structure correlated with differences in sediment size structure and organic matter content.

No residues of the active constituent or any breakdown products of Fusilade Forte® were detectable in oysters or water after 1 day post spraying, but Fluazifop-P (acid) is detectable in sediments up to 30 days post spraying.

Multivariate analyses showed that after spraying the community was significantly different to both the rice grass community pre-spray and the mudflat communities. Univariate analyses indicated that this difference was largely driven by an explosion of grazing gastropods within the sprayed rice grass communities. By the last round of sampling (12 months post-spraying) the community structure in the sprayed rice grass area began to resemble that which occurs in mudflats.

These findings support previous studies that have shown that rice grass colonisation changes the community structure of benthic macro-invertebrate assemblages.

Following spraying with Fusilade Forte®, the benthic macro-invertebrate community structure reverts to a pre-rice grass condition that is analogous with a mudflat community structure.

The work showed that spraying rice grass with Fusilade Forte® appeared to result in acute toxic impacts to the benthic macro-invertebrate communities but within months these communities appear to recover with limited detectable long-term impacts. As this work was entirely field-based and no laboratory experiments were specifically conducted on targeted macro-invertebrate taxa, direct toxicity to in situ organisms was not explicitly established. Nevertheless, there is a weight of evidence from the research, to suggest that it is indeed possible.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my long suffering supervisor Alastair Richardson for his unwavering support, enthusiasm and encouragement throughout the long time period it took for me to complete this project.

I would like to pay special tribute to my Dad who passed away during my research but who always inspired me to continue to seek answers and to remain actively involved in research in some form. I also sincerely thank my gracious mother for her company on the road and my three older brothers who made me realise the importance of education as I didn't wish to end up like any of them.

To my beautiful wife, Rebecca, and my two amazing boys who arrived during the course of this work I am indebted to you all for your support and understanding during the long nights away and for your patience and tolerance as I pursued this long awaited end point.

This project could not have been possible without the assistance of friends and co-workers at the Department of Primary Industries, Parks, Water and Environment (DPIPWE) and I would like to acknowledge the funding and in-kind support that was extended to me by Wes Ford and Robert Gott in their respective roles of General Manager, Primary Industries and Water Division and Director of Marine Resources respectively.

Field helpers are too numerous to name however, special thanks must also go to Andrew Sullivan, Andrew Harvey, Anthony Reid and Alastair Morton for their efforts in sorting samples and general willingness to always help when I asked.

I would also like to thank Paul Webb of Shearwater Oysters in the Port Sorell estuary for his long-term support for the project and for supplying the sentinel oysters used in some of the work and Jamie Cooper from Feral Management Solutions Pty Ltd for assistance with the spraying of the rice grass.

Finally I would like to acknowledge the enormous contribution Anthony Reid has made to the project through his statistical wizardry but more importantly his humour and enthusiasm that especially helped me towards the end of my work.

TABLE OF CONTENTS

ABSTRACT	III
ACKNOWLEDGEMENTS	VI
TABLE OF CONTENTS.....	VIII
CHAPTER 1. GENERAL INTRODUCTION.....	1
1.1 OVERVIEW OF THESIS	1
1.2 BACKGROUND.....	1
1.3 HISTORY OF <i>SPARTINA ANGLICA</i>	3
1.4 IMPACTS OF <i>SPARTINA</i>	4
1.5 <i>SPARTINA</i> CONTROL.....	5
1.6 RATIONALE OF THIS STUDY	6
1.7 AIMS AND OBJECTIVES.....	7
1.8 STRUCTURE OF THESIS	8
CHAPTER 2. A COMPARISON OF BENTHIC MACRO-INVERTEBRATE COMMUNITIES IN <i>SPARTINA ANGLICA</i> MEADOWS TO THOSE INHABITING ADJACENT BARE MUDFLATS.	10
2.1 INTRODUCTION.....	10
2.2 METHODS	11
2.2.1 <i>Location.....</i>	11
2.2.2 <i>Invertebrate sampling.....</i>	12
2.2.3 <i>Sediment geo-chemistry</i>	14
2.2.3.1 <i>Visual Assessment.....</i>	15
2.2.3.2 <i>Redox potential</i>	15
2.2.3.3 <i>Loss on Ignition</i>	16
2.2.3.4 <i>Particle Size Analysis</i>	16
2.2.4 <i>Statistical analysis.....</i>	17
2.2.4.1 <i>Comparison of fauna across treatments.....</i>	17
2.2.4.2 <i>Analysis of geochemical data</i>	19
2.3. RESULTS	20
2.3.1 <i>Differences between inlets.....</i>	20
2.3.2 <i>Differences between habitats</i>	24
2.3.3 <i>Effect of season</i>	24
2.3.4 <i>Habitat characteristics</i>	27
2.4 DISCUSSION	32
2.4.1 <i>Effect of habitat</i>	32

2.4.2 Differences between the Inlets.....	33
2.4.3 Effect of Season.....	34
2.4.4 Conclusions	35
CHAPTER 3. DETECTION OF THE ACTIVE INGREDIENT OF THE HERBICIDE FUSILADE FORTE® (AND ITS DEGRADATES) FOLLOWING SPRAYING OF RICE GRASS (<i>SPARTINA ANGLICA</i>) IN THE RUBICON ESTUARY, PORT SORELL, TASMANIA.....	38
3.1 INTRODUCTION.....	38
3.2 METHODS	39
3.2.1 Location.....	39
3.2.2 Survey design	40
3.2.3 Analysis	41
3.3 RESULTS	42
3.3.1 Oysters	42
3.3.2 Sediment	43
3.3.3 Water	43
3.4 DISCUSSION	45
CHAPTER 4. THE EFFECT OF FUSILADE FORTE® ON BENTHIC MACRO-INVERTEBRATE COMMUNITIES IN <i>SPARTINA ANGLICA</i> MEADOWS AND ADJACENT BARE MUDFLATS.....	52
4.1 INTRODUCTION.....	52
4.2 METHODS	54
4.2.1 Field sampling and laboratory analyses.....	54
4.2.2 Statistical analysis.....	56
4.2.2.1 Comparison of fauna across treatments	56
4.3. RESULTS	58
4.3.1 Differences between habitats	58
4.3.2 The Impacts of Spraying Fusilade Forte®	61
4.4 DISCUSSION	67
CHAPTER 5. GENERAL DISCUSSION	71
REFERENCES.....	79

CHAPTER 1

GENERAL INTRODUCTION

1.1 Overview of thesis

This study examines the ecological impact that *Spartina anglica* is causing in a northern Tasmanian estuary on benthic macro-invertebrate communities as the plant establishes and proliferates, resulting in changes to the physiography of the intertidal zone. It also investigates the fate of the herbicide Fusilade Forte® (and its degradation products) post spraying, and the short and long-term impacts the chemical poses to the macro-invertebrate communities within estuaries where the chemical is being used in an attempt to control the spread of rice grass.

1.2 Background

Estuaries are recognised globally as ecosystems of high ecological values which are increasingly under threat (Adams 2002, Valiela 2006). Globally the major exotic plant species in temperate estuaries are grasses in the genus *Spartina* (Adams 2009). Although several species of *Spartina* have been introduced around the world, in Australia only one species, *Spartina anglica*, appears to have been successfully introduced although consignment records suggest *S. townsendii* and *S. maritima* may have been brought into the country (Bridgewater 1995).

S. anglica was deliberately introduced into Tasmania on several occasions between 1930 and 1977. Import and consignment records indicate that during this period, rice grass was planted at 11 locations within Tasmania (Figure 1.1).

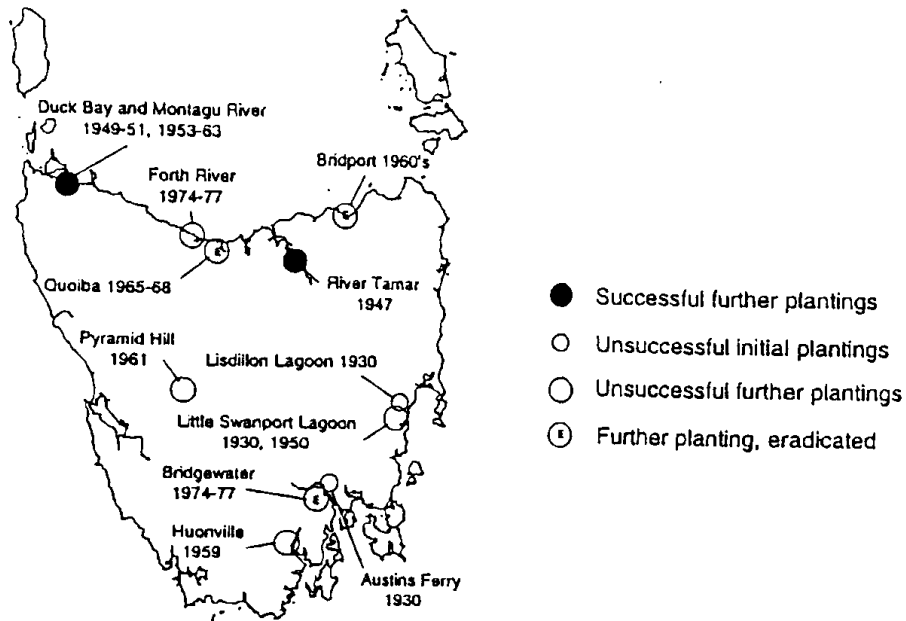


Figure 1.1. Rice grass introduction sites in Tasmania (modified from Boston, 1981).

There does not appear to have been any single reason for introducing rice grass to Tasmania (Boston 1981) and it is thought the reasons differed between regions. In some areas, such as the Little Swanport estuary and the Derwent River, it was probably to reclaim land or provide fodder, while in the Tamar Estuary it was hoped to stabilise mudflats, reclaim land and improve the navigability of shipping channels (Boston 1981, Pringle 1993).

1.3 History of *Spartina anglica*

Spartina anglica, commonly referred to as rice grass, is a saltmarsh grass that typically inhabits the upper intertidal zone of temperate estuaries. The genus *Spartina* contains 14 halophytic species in the family Chloridoideae, a monophyletic lineage of Poaceae (Hsiao et al. 1990, Strong and Ayres 2009). The natural distribution of *Spartina* species is mainly the Americas with only one native species, *S. maritima* (Curtis) Fernald, occurring in southern and western Europe (Strong and Ayres 2009). It is also found on the west coast of Africa and in South Africa, although there is debate about whether these are natural populations (Pierce 1982, Adams and Bate 1995).

Spartina anglica is believed to have arisen from chromosome doubling (allopolyploidy) of *S. alterniflora* and *S. maritima* on marshes in Brittany and the south-west of France and Southampton Water in England (Thompson, 1991). The allopolyploidy that occurred to form *S. anglica* means the species contains two copies of each of the parental chromosomes, which allows for maximum genetic diversity (Thompson, 1991). This diversity appears to have conferred beneficial characteristics on *S. anglica* that allow it to thrive in waterlogged estuarine environments. For example, Lee (2003) showed that the rhizomes of *S. anglica* exhibit higher rates of O₂ transport, lower O₂ requirements and higher rates of H₂S removal than those of *S. alterniflora*.

S. anglica has become established in a number of countries including Germany, Ireland and the Netherlands, where it is thought to have spread unaided (Strong and Ayres 2009), and in China, New Zealand and Australia where it has been deliberately introduced (Boston 1981, Chung 1983, Partridge 1987, Lambert 2005). In all these places it is now recognised as causing significant negative impacts that include ecological and economic threats to the integrity of intertidal coastal ecosystems (Doody 1990, Shaw and Gosling 1995, Williamson 1995, Xie et al. 2001, Zang et al. 2004 and Wang et al. 2006).

The current distribution of *S. anglica* in Australia generally suggests it does not readily spread from infested estuaries to nearby uninfested estuaries (Hedge 1997). Aside from humans, wind and tide generated currents appear to be the primary vectors for *S. anglica* propagules. Many infestations exhibit rapid expansion after an initial colonising phase marked by slow but progressive growth (Gray et al. 1991, Pringle 1993) and in Tasmania the populations in both the Port Sorell and Tamar estuaries have exhibited the explosive growth rate (Hedge 1997).

1.4 Impacts of *Spartina*

S. anglica modifies hydrodynamics and sediment dynamics in waterways (Gray et al. 1991, Pringle 1993). Its dense growth habit and rhizome and root network act as a trap for sediments and debris, changing the natural rate, magnitude and location of sediment deposition and erosion. These processes elevate shorelines and river banks to create terraces and marsh islands that have resulted in negative impacts in Tasmania to aquaculture (Hedge 1997), and tourism and recreation (Pringle 1993).

The rapid colonisation of mudflats by rice grass elsewhere has been blamed for the displacement of migratory and wader birds in infested estuaries (Gibbs and Phillips 1995, Simpson 1995).

1.5 *Spartina* control

World-wide, there have been numerous efforts to manage and eradicate *S. anglica* and other *Spartina* spp, and this work is still on-going for many land managers today. In recent times many of these programs have resulted in very positive outcomes with infestations in Washington State and San Francisco Bay in the USA and parts of Europe, New Zealand and Australia approaching eradication (Guenegou et al. 1991, Hedge and Kriwoken 2000, Reeder and Hacker 2004, Taylor and Hastings 2004, Grevstad 2005, Bortolus 2006).

The most common control method is now through herbicide use, although mechanical removal of small infestations is also used in areas such as Canada, the USA and Europe (Patten and O’Casey 2006, Boe 2007). Investigations into the use of a sap-feeding planthopper (*Prokelisia* sp., a *Spartina* specialist) as a biological agent have shown that under greenhouse conditions, over 90% of *S. anglica* died at high *Prokelisia* densities (Wu et al. 1999). However, the introduction of such biological control agents is fraught with risks, and would take many years to research and justify in Australia.

In Tasmania a variety of methods have been used to try to control rice grass in the past, including physical removal using both manual and mechanical techniques,

smothering using black plastic and weed matting, heat treatment using steam, infrared and burning, grazing using sheep and cattle, and chemical herbicides using Roundup and Fusilade®.

All available control techniques have inherent advantages and disadvantages; however it is evident that application of the herbicide Fusilade® is currently the only cost-effective technique for controlling and eradicating rice grass infestations at all scales (RGAG 2002).

1.6 Rationale of this study

While there have been many studies on the biological impact of *S. anglica*, including two on the impact on macro-invertebrate infaunal communities in Tasmania (Hedge and Kriwoken 2000, Davies 2001), and two on the toxicological impacts of Fusilade® on specific Australian biota (Palmer et al. 1995 and Hedge et al. 1999), all of these have been limited by the short time scales employed during the research, and little work has been carried out to quantify any undesirable long-term ecological impacts including what happens once the rice grass is actually removed (Sheehan 2008). Most researchers acknowledge this fact, admitting that their results should only be considered a “snap shot” and that further monitoring should be carried out to allow for the temporal and spatial variation that characterise benthic macro-invertebrate communities of soft-bottom habitats.

Given these limitations of previous work, this study is, to the best of my knowledge, the first of its kind in Australia to use a Before and After Control Impact (BACI)

experimental design to examine the impact of *S. anglica* on benthic macro-invertebrate infaunal communities, and the acute and chronic toxicological effects of applying Fusilade Forte® in the marine environment over an extended period.

1.7 Aims and Objectives

The overall objective of this study was to investigate the ecological impact of *S. anglica* on the benthic macro-invertebrate community in an estuary on the north coast of Tasmania and to examine the potential ecological and toxicological impacts of using the chemical Fusilade Forte® to control the rice grass on the benthic macro-invertebrate assemblages in both the short and longer term.

The aims of the research were to:

1. Compare the benthic macro-invertebrate communities that inhabit unvegetated natural mudflats with those that exist in adjacent rice grass infested mudflats.
2. Investigate the movement of the active constituent of the herbicide Fusilade Forte® (and its degradation products) post-spraying through the estuary, and whether any residues of the chemical could be detected in the water column, sediment or sentinel oysters.
3. Examine the short term and longer term impacts of treating the rice grass with Fusilade Forte ® on the benthic macro-invertebrate communities.

This work will be reported to the Tasmanian Rice Grass Advisory Group (RGAG) and regional Natural Resource Management (NRM) groups to assist them with the

future direction of *S. anglica* management in Tasmania, and potentially elsewhere, as land managers in other Australian States and in New Zealand are using chemical treatment (including Fusilade Forte®) to help them control rice grass infestations in their respective areas.

1.8 Structure of thesis

Chapter 1 (this chapter) is a general introduction describing relevant background information on rice grass and the specific aims and objectives of the study.

Chapter 2 includes some basic information on the Port Sorell Estuary in Tasmania and is an assessment of the different biological benthic macro-invertebrate communities that were observed within unvegetated natural mudflats compared to areas colonised by rice grass. These results are derived from two separate arms within the Port Sorell Estuary and the sampling regime allowed for temporal analysis over a full twelve month period.

Chapter 3 is an investigation of the fate of the chemical Fusilade Forte® and its breakdown products post-spraying through residue sampling from water, sediment and sentinel Pacific oysters deployed throughout one arm of the Port Sorell estuary. This section follows the movement of the active constituent and three breakdown products over a two month period following a large scale spraying event in the west arm of the estuary. This work was not originally planned as part of my research.

However during the course of my studies the Director of the Tasmanian Environment Protection Agency (EPA) requested additional monitoring be undertaken to test the potential impact of Fusilade Forte® (which had replaced Fusilade®) and hence I added this component of research to my thesis.

Chapter 4 is an investigation of the acute and chronic impacts of the herbicide Fusilade Forte® on the benthic macro-invertebrate community in the west arm of the Port Sorell estuary following a large-scale spraying event. Multivariate analyses (nMDS and PERMANOVA) of the data collected in Chapter 2 showed significant differences existed in the community structure between the east and west inlets pre-spray. As a result analyses of the post-spraying data were only conducted within the west inlet. The sampling regime allowed for temporal analysis over a full twelve month period.

Chapter 5 is a general discussion of the results from the research and provides some conclusions on both the impact that rice grass is having on estuarine benthic macro-invertebrate communities, and the long-term effects of using Fusilade Forte® to control the spread of this highly invasive introduced intertidal grass species.

CHAPTER 2

A COMPARISON OF BENTHIC MACRO-INVERTEBRATE COMMUNITIES IN *SPARTINA* *ANGLICA* MEADOWS TO THOSE INHABITING ADJACENT BARE MUDFLATS.

2.1 Introduction

Despite increased world-wide recognition of the ecological importance of some invasive species, the majority of research on marine non indigenous species (NIS) in Australia has focused on recent (within 10-20 years) arrivals perceived to pose a significant threat to their recipient environment (Reid 2010). Relatively little attention has been given to the effects of long established and wide-spread introduced species.

While some authors argue that the majority of NIS have little effect on the structure or function of their recipient communities (Johnson 2007) preliminary research in Tasmania by Hedge (1997) and Davies (2001) suggests this is not the case for *Spartina anglica*. In fact, their observations, supported with data elsewhere (Carr 1993, Wang et al. 2006), indicate that *S. anglica* can have a significant impact on benthic macro-invertebrate community structure and function, as well as the geochemical components of intertidal marine habitats.

In this chapter I quantify the impact of *S. anglica* on soft-sediment assemblages through 12 months of observations carried out in the Port Sorell estuary on the north

coast of Tasmania. I link differences in community structure to the alteration of habitat characteristics by *S. anglica*, in particular, to changes in sediment size, redox potential and organic matter. The degree of impact of *S. anglica* and the potential mechanisms of impact are also discussed.

2.2 Methods

2.2.1 Location

The Port Sorell Estuary is situated on Tasmania's north coast (146°39', 41°13 south) and has a relatively large tidal range, in the Tasmanian context, of approximately two to three metres. The estuary has a single narrow outlet approximately 150 m wide at high tide but considerably narrower at low tide (See Figure 2.1). Most of the estuary is very shallow, less than 2 m in depth, although some deeper water (up to 8 m) is found in the channels at the mouth of the estuary. The upper estuary is dominated by mud flats with sediment derived from the upper catchment while the lower estuary is chiefly marine and contains extensive seagrass beds and sand flats (Beard et al. 2008).

Port Sorell is described as an open marine inlet with a strong freshwater influence (Edgar et al. 2000). Edgar et al. classified the conservation significance of estuaries around Tasmania by examining their physical attributes, the degree of human development and assessing the diversity of invertebrate fauna and conservation status of identified taxa. Due to the high population density and associated human induced changes, the Port Sorell estuary was considered to be Class D, i.e. degraded and of low conservation significance (Edgar et al. 2000).



Figure 2.1. Map showing the Port Sorell Estuary.

The Greater Rubicon catchment covers an area of approximately 610 km² and incorporates a number of waterways which drain into the Port Sorell estuary. The two main river systems draining into the estuary are the Rubicon River and the Franklin Rivulet. There are also a number of smaller catchments on each side of the estuary which have intermittent flows. These include Little Branches Creek, Marshalls Creek, Little Browns Creek, Panatana Rivulet and Greens River (Krasnicki, 2002).

2.2.2 Invertebrate sampling

To investigate the effect of *S. anglica* invasion on benthic macro-invertebrate communities five locations were randomly selected from within the upper reaches of the east and west arms of the Port Sorell estuary (See Figure 2.2). At each location triplicate core samples were collected from both *S. anglica* meadows and adjacent

mudflats habitats at the same tidal height. Samples in the *S. anglica* habitats were taken in meadows (>1hectare in size) and at least 5 metres in from the leading edge of the meadow.

Samples were collected from both habitats every 3 months over a 12 month period using a 100 mm diameter x 150 mm deep core, at times when the low tide within the estuary was <0.3m. Raffaelli and Hawkins (1996) showed that the diameter of a sediment core must be bigger than the largest animal; since the crustacean and polychaete species known from the estuary reaches sizes of 80-100 mm the core size used here was considered suitable.



Figure 2.2. Map showing the approximate location of sample sites in west and east inlet

The basic sampling design included three factors, namely ‘season’ (four levels), ‘habitat’ (two levels) and ‘inlet’ (two levels). All factors were crossed giving rise to ‘treatments’ which consisted of all combinations of season*habitat*inlet. For clarity the term ‘treatment(s)’ hereafter refer to these combinations.

All samples were collected and stored in plastic buckets before being transported back to the wet laboratory at the University of Tasmania where they were sieved through 1 mm mesh to separate out the animals using pressurised salt water to displace the mud. All samples were sieved within 48 hours of being taken.

Prior to sieving any emergent rice grass was cut off, patted dry with paper towel and wet weighed, and once any animals had been removed from the processed sample the remaining sub-surface rice grass roots and rhizomes were also patted dry and wet weighed. All animals were fixed in 70% alcohol and 2% glycerol and were classified to species level where possible using a dissecting microscope.

2.2.3 Sediment geo-chemistry

Four sediment samples were collected using 43mm diameter Perspex cores at each of 5 randomly selected sites in both the East and West inlets of the Port Sorell estuary in both mudflat and rice grass habitats. The cores were kept upright and cool, and were transported back to the laboratory for analysis.

Cores were handled carefully and retained in a vertical orientation to minimise disturbance of the sediment surface until they were described and redox readings had been taken. All samples were analysed within 24 hours of being taken and were assessed for the following variables.

2.2.3.1 Visual Assessment

The cores were described with regard to their length, colour, plant and animal life, gas vesicles, and smell. Smell was noted immediately after the water was removed from the core barrels.

2.2.3.2 Redox potential

Redox potential was measured in millivolts at 3 cm below the sediment surface using a WTW pH 320 meter with an Mettler Toledo Ag/AgCl combination pH / Redox probe. The standard potential of the Ag/AgCl reference cell of the probe is 218 mV at 10 °C, the approximate temperature of the samples during measurement. Calibration and functionality of the meter were checked before each test using a Redox Buffer Solution (220 mV at 25 °C). Measurements were made within 12 hours of the samples being collected. Corrected Redox potential values were calculated by adding the standard potential of the reference cell to the measured redox potential and are reported in millivolts.

In all cases the lowest reading observed is recorded as the Redox value. In muddy, low permeability sediments this is recorded when the reading is stable or dropping at less than 1 mV per second. In permeable, sandy sediments the lowest reading is

often observed while the probe is being worked to the measurement depth. As soon as the probe stops moving in sandy sediments with low Redox values, the readings normally start to increase due to water drawn down by the probe diluting the interstitial fluids.

2.2.3.3 Loss on Ignition

After the visual description and redox measurements were completed, two samples were divided from each core. The top 30 mm of sediment was collected in a vial for analysis of organic content and particle size. The next 70 mm was collected in another vial for particle size analysis only. The samples were homogenised in the laboratory and a 15 ml sub-sample of the 30 mm sample was oven dried at 60°C for organic analysis. The remainder of the 30 mm sample was retained for particle size analysis.

Once the sub-sample was dry it was ground to a fine powder. A portion of the dried, ground sediment was placed into a pre-weighed porcelain crucible and the weight recorded. It was then heated to 450°C in a muffle furnace for 4 hours and reweighed. The loss in weight (Loss On Ignition) was taken as the organic content and calculated as a percentage of the sample's dry weight.

2.2.3.4 Particle Size Analysis

The two samples comprising the top 100 mm of each sediment core were combined and analysed as follows. The 30 - 100 mm sample was homogenised then 35 ml was divided out and discarded. This was an equal proportion to the 15 ml sample which

was removed from the 0 - 30 mm sample earlier. The remaining material from these two samples was combined and homogenised to give a 95 ml sample representing the top 100 mm of sediment.

To obtain a consistent volume of sample, a container of known volume (77 ml) was filled with the sample material which was then packed down and scraped level. This aliquot was washed through a stack of sieves by shaking them under a moderate water spray. The sieve aperture sizes were 4 mm, 2 mm, 1 mm, 500 μm , 250 μm , 125 μm and 63 μm .

The contents of each sieve were drained then transferred to a 100 ml measuring cylinder containing 20 ml of water, starting with the coarsest fraction and working through to the finest. The cumulative volume in the measuring cylinder was recorded after each sieve's contents were transferred. These volumes were entered into a spreadsheet and the fraction's percentage by volume of the original sample calculated. The percentage by volume of the fraction of less than 63 μm diameter was calculated to make the total up to 100%.

2.2.4 Statistical analysis

2.2.4.1 Comparison of fauna across treatments

To depict and assess differences between treatments in community structure, I used non-metric multi-dimensional scaling (nMDS) and permutational multivariate analysis of variance (PERMANOVA). Analyses were based on Bray-Curtis dissimilarities derived after a square-root transformation of the data. The nMDS

ordinations were carried out using PRIMER 5 software (Clarke and Gorley 2001), while PERMANOVA routines were as described in Anderson (2001) and McArdle & Anderson (2001).

Analyses of community structure were carried out on different components of the fauna. First, an nMDS ordination was carried out including all taxa identified to the lowest taxonomic level. However, given the high number of samples derived ($n=80$) from all combinations of Season*Inlet*Habitat, it was difficult to interpret differences in community structure between the treatments. Consequently, I conducted nMDS ordinations on the east and west inlets separately to clearly distinguish where differences in community structure truly lay. Both the nMDS conducted on the whole data set and the nMDSs conducted on the separate inlets were interpreted together. The significance of patterns observed in all nMDS plots was determined using PERMANOVA (Anderson 2001, McArdle & Anderson 2001).

Univariate model I ANOVA was used to compare mean species richness, total abundances and diversity (Shannon-Wiener) and abundances of select taxonomic groups and species among treatments. ANOVAs were performed using the R statistical package (R Version 2.13.0 {2011}).

All PERMANOVA (conducted on total community structure) and univariate ANOVA models (conducted on selected taxa) had the same basic design. They included the fixed effects of ‘season’ (4 levels: summer, autumn, winter, spring),

crossed with ‘inlet’ (2 levels: east, west), crossed with ‘habitat’ (2 levels: rice grass, mudflat) and all associated interactions (i.e. season*inlet, inlet*habitat, season*habitat and season*inlet*habitat).

In the event that the main analysis yielded a significant interaction, an *a-posteriori* Ryan-Einot-Gabriel-Welsch (REGWQ) multiple range test was conducted to determine the nature of the interaction. For ANOVAs, data and residuals were checked for normality and homoscedasticity, and transformed as necessary to stabilise variances on the basis of the relationship between group standard deviations and means (Draper & Smith 1981).

2.2.4.2 Analysis of geochemical data

Sediment size structure was analysed using a PCA/biplot ordination followed by MANOVA. This enabled identification of the particular sediment size fractions contributing to the differences in sediment structure between experimental treatments. The design of the MANOVA was identical to that described above (i.e. included the effects of ‘season’ (4 levels: summer, autumn, winter, spring), crossed with ‘inlet’ (2 levels: east, west), crossed with ‘habitat’ (2 levels: rice grass, mudflat) and all associated interactions.

Univariate model I ANOVA was used to compare mean rice grass biomass. Separate analyses were conducted on total rice grass wet weight, emerged rice grass wet weight and submerged rice grass wet weight. Again, data and residuals were checked for normality and homoscedasticity, and transformed as necessary to

stabilise variances on the basis of the relationship between group standard deviations and means (Draper & Smith 1981).

2.3. Results

2.3.1 Differences between inlets

A total of 890 individuals across 26 taxa were recorded in the study, including 8 mollusc, 7 crustacean, 9 polychaete, 1 nemertean and 1 vertebrate (fish) taxa. Differences in community structure between the east and west inlets were not as distinct as overall differences in community structure between rice grass and mudflat (see section 2.3.2). None the less, there were some small differences between the east and west inlets with respect to total community metrics and the abundance of select faunal taxa.

The total abundance of individuals was consistently higher in the west inlet than in the east inlet, although this depended on the habitat type (Fig 2.3, Table 2.1). Species richness was also higher in the west inlet. Shannon Wiener diversity (H') was not significantly different across habitats or inlets, varying only with season (Fig 2.3, Table 2.1). Of the 26 individual taxa, 18 were identified in the east inlet and 21 in the west inlet. Fourteen of these taxa were identified in both inlets, while 4 taxa were observed only in the east inlet, and 8 taxa identified only within the west inlet.

Analysis of total community composition also indicated significant differences between the east and the west inlets, although these differences were dependent on the habitat type (Fig 2.4, 2.5). The differences in community structure were also reflected in different abundances of dominant taxa between inlets (Fig 2.6). The west inlet generally exhibited higher abundances of crustaceans, particularly the crab *Heloecius cordiformis*, which was on average 50% more abundant in the west inlet than in the east inlet (Fig 2.6). The west inlet also possessed higher abundances of polychaetes and gastropods, while the east inlet possessed higher abundances of bivalves (Fig 2.6, Table 2.1).

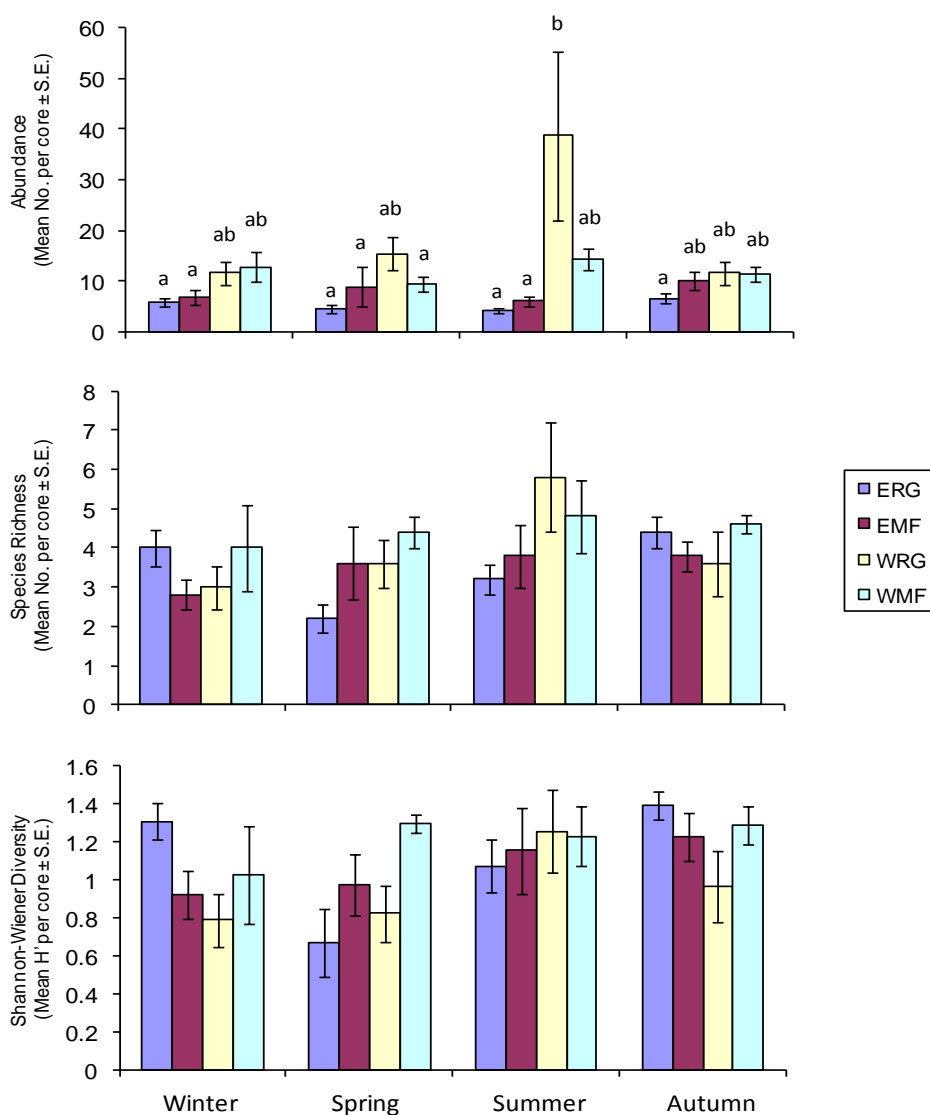


Figure 2.3. Total abundance of individuals, total taxa, Shannon-Weiner diversity (H') across all combinations of inlet, habitat and season. All values are means \pm S.E. from $n=5$ replicates. ERG: east inlet/rice grass, EMF: east inlet/mudflat, WRG: west inlet/rice grass, WMF: west inlet/mudflat. For main analyses where significant differences between treatments were detected, letters representing the REGWQ groups are positioned above the respective treatments. In the REGWQ groups, analogous letters denote the same groupings.

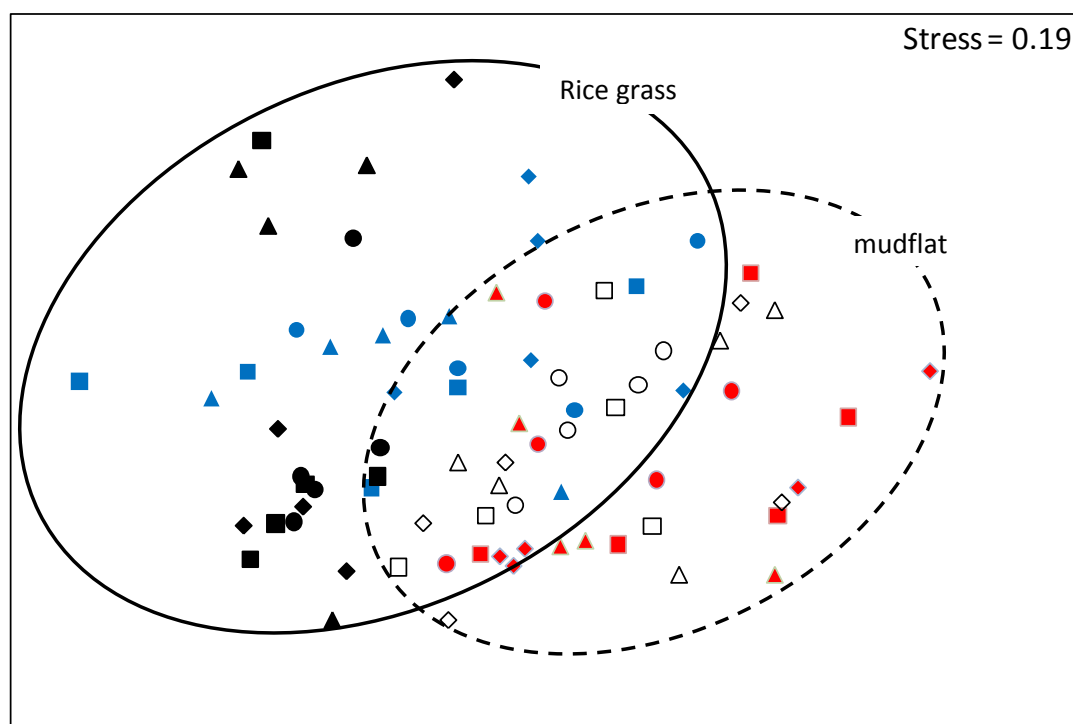


Figure 2.4. nMDS ordination showing separation of the different communities across all combinations of inlet, habitat and season. Denotation: blue icons: east inlet/rice grass, red icons: east inlet/mudflat, black icons: west inlet/rice grass, open icons: west inlet/mudflat, diamonds: summer, squares: autumn, triangle: winter, circle: spring. The ellipses indicate arbitrary separation of communities on the basis of habitat type. There were significant differences in community structure across the factors of season, habitat and inlet, although due to a significant inlet*habitat interaction, impacts of habitat and inlet could not be interpreted independently of each other (PERMANOVA, season: $MS=3256.57$, $F_{3,64}=2.16$, $P=0.016$; inlet: $MS=8963.91$, $F_{1,64}=5.95$, $P<0.001$; habitat: $MS=38608.13$, $F_{1,64}=25.65$, $P<0.001$; season*inlet $MS=2319.65$, $F_{3,64}=1.54$, $P=0.108$; season*habitat $MS=1448.18$, $F_{3,64}=0.96$, $P=0.479$; inlet*habitat $MS=7527.46$, $F_{1,64}=5.00$, $P=0.001$; season*inlet*habitat $MS=1739.91$, $F_{3,64}=1.54$, $P=0.316$).

2.3.2 *Differences between habitats*

There were also distinct and significant differences between the different habitat types (Fig 2.4), which became even more evident through individual analysis conducted on both inlets separately (Fig 2.5). Rice grass habitat supported a higher total abundance and total richness of species to areas of mudflat (Fig 2.3) although in the case of total faunal abundance, this was dependent on the inlet (Table 2.1).

Rice grass communities were dominated by high abundances of grazing and predatory gastropods (particularly within the west inlet) (Fig 2.6) while mudflat communities exhibited higher abundances of filter-feeding bivalves and sedentary and errant polychaetes, although this was also dependent on the inlet (Fig 2.6, Table 2.1). However, one of the more abundant crustacean taxa, the crab *Macrophthalmus latifrons*, was consistently more abundant in mudflat than in rice grass, irrespective of the inlet.

2.3.3 *Effect of season*

The different combinations of inlet*habitat were sampled during the four different seasons of the year to assess the spatio-temporal variability in community structure and to determine whether identified differences between rice grass and mudflat communities are consistent through time. Other than total faunal abundance, abundance of gastropods and the polychaete species *Nephtys ?australiensis*, interaction effects involving the factor of season (i.e. season*inlet, season*habitat, season*habitat*inlet) for all other measured parameters were insignificant. This

indicates that for almost all measured parameters, any impact of season was independent of the impacts of habitat and inlet.

The sampling season did however have an effect on a number of measured parameters, with a clear temporal trend leading to higher species diversity (Shannon-Wiener) in summer and autumn, a trend also reflected in the abundance of polychaetes and molluscs (Fig 2.3, 2.6, Table 2.1). Of all the groups, crustaceans were the only taxonomic group to maintain a similar abundance throughout the year, showing very little temporal variability in abundance (Fig 2.6, Table 2.1).

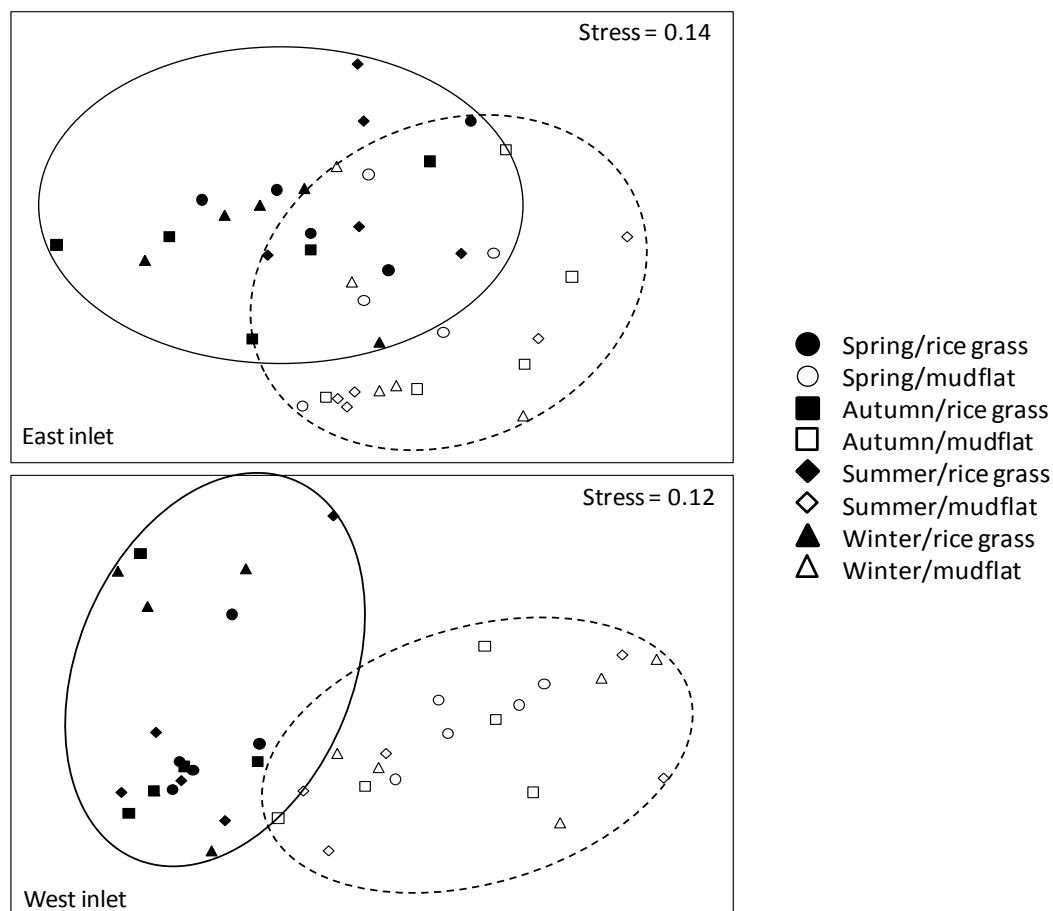


Figure 2.5. nMDS ordination showing separation of the different communities conducted on each inlet individually. The ellipses indicate arbitrary separation of communities on the basis of habitat type. There were significant differences in community structure between rice grass and mudflat in both inlets, but there was no evidence that season had any impact on community structure. (PERMANOVA for East Inlet, season: $MS=3211.29$, $F_{3,32}=1.83$, $P=0.056$; habitat: $MS=18467.26$, $F_{1,32}=10.51$, $P=0.002$, season*habitat $MS=1771.17$, $F_{3,32}=1.01$, $P=0.4280$; for West Inlet, season: $MS=2710.87$, $F_{3,32}=1.68$, $P=0.076$; habitat: $MS=27090.47$, $F_{1,32}=16.81$, $P=0.002$; season*habitat $MS=1746.98$, $F_{3,32}=1.08$, $P=0.368$)

Table 2.1. Results of fixed effects ANOVA comparing among treatments mean total abundances, species richness, Shannon-Wiener diversity and abundance of taxonomic groups and common taxa. Results are of overall ANOVAs comparing treatments. Significant P values are shown in bold face ($P \leq 0.05$).

Variable	MS _{total}	P values						
		Season	Inlet	Habitat	Season * Inlet	Season * Habitat	Inlet * Habitat	season * Inlet * Habitat
Degrees of freedom	64	3	1	1	3	3	2	3
Total number of individuals	0.24	0.435	<0.001	0.717	0.021	0.763	0.015	0.483
Species richness	2.48	0.149	0.037	0.480	0.227	0.552	0.572	0.192
Species diversity (H')	8.05	0.047	0.948	0.183	0.127	0.198	0.072	0.370
Total crustaceans	11.70	0.610	<0.001	1.000	0.553	0.330	0.082	0.790
<i>Macrophthalmus latifrons</i>	1.16	0.404	0.304	<0.001	0.589	0.702	0.536	0.866
<i>Helocius cordiformis</i>	8.76	0.283	0.003	0.453	0.309	0.312	0.074	0.808
Total Polychaetes	5.08	0.034	0.376	0.033	0.541	0.392	0.041	0.683
<i>Nephtys ?australiensis</i>	3.00	0.018	0.225	0.027	0.521	0.041	0.003	0.636
Total molluscs	5.11	0.028	0.260	0.018	0.637	0.588	0.072	0.814
gastropods	98.71	0.046	0.017	0.028	0.046	0.071	0.028	0.071
bivalves	6.15	0.506	0.788	0.007	0.357	0.323	0.590	0.334

2.3.4 Habitat characteristics

The wet weight of rice grass was strongly dependent on inlet and season, at least for the proportion that was submergent (Fig 2.8). In west inlet, there were clear spikes in the biomass of submergent rice grass during both winter and summer (although the summer spike was far larger). In contrast, the biomass of submergent rice grass in the east inlet was more consistent throughout the different seasons. There was no pattern seasonally or between inlets in the emergent biomass of rice grass (Fig 2.8), indicating constant standing biomass in both inlets through time.

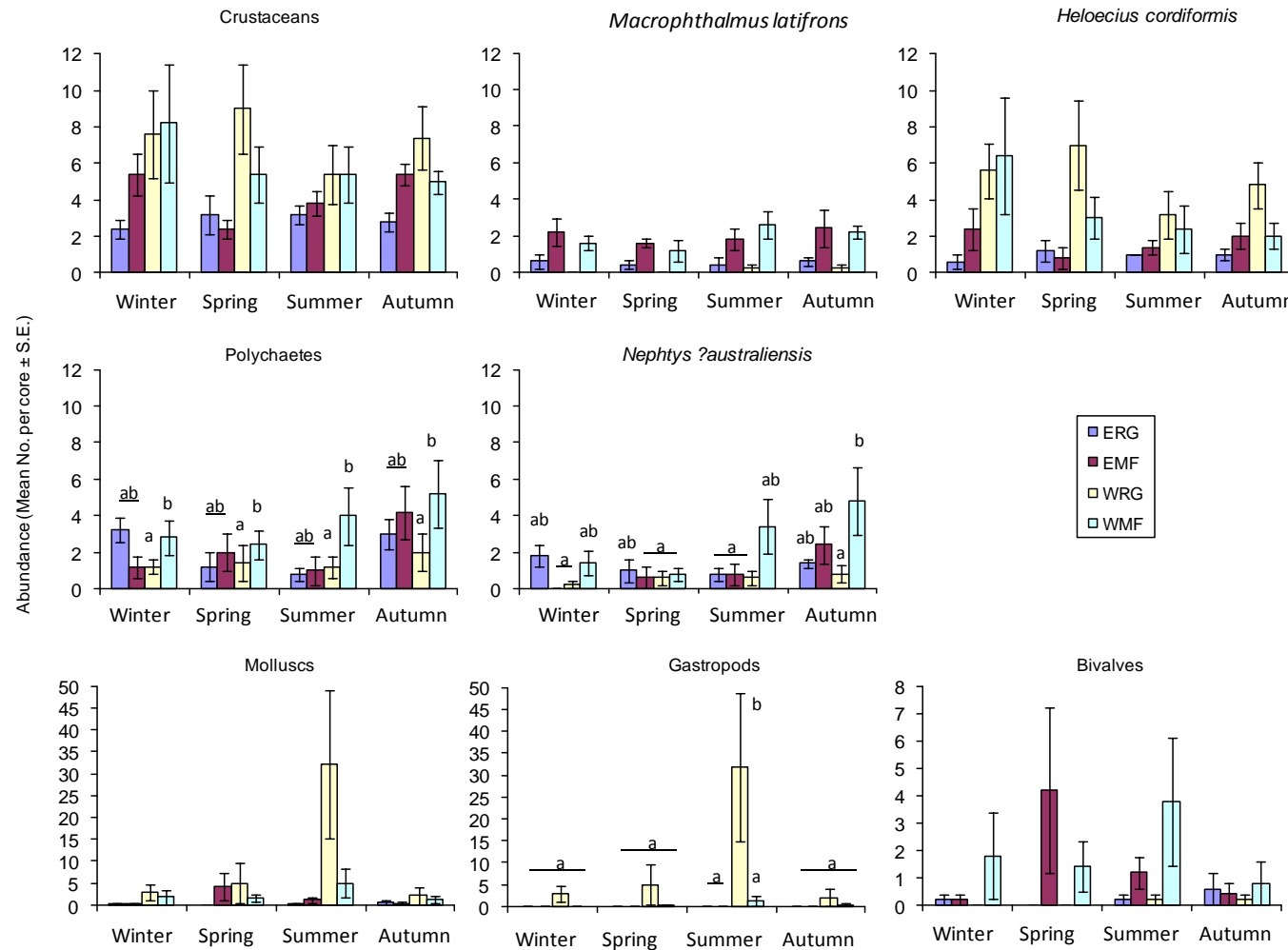


Figure 2.6. Abundance taxonomic groups and common taxa across treatments. All estimates are averages of $n=5$ replicates (\pm S.E.). ERG: East inlet/Rice grass, EMF: East inlet/Mudflat, WRG: West inlet/Rice grass, WMF: West inlet/Mudflat. For main analyses where significant inlet*season*habitat interactions were detected, the REGWQ groups are positioned above the respective treatments. In the REGWQ groups, analogous letters denote the same groupings.

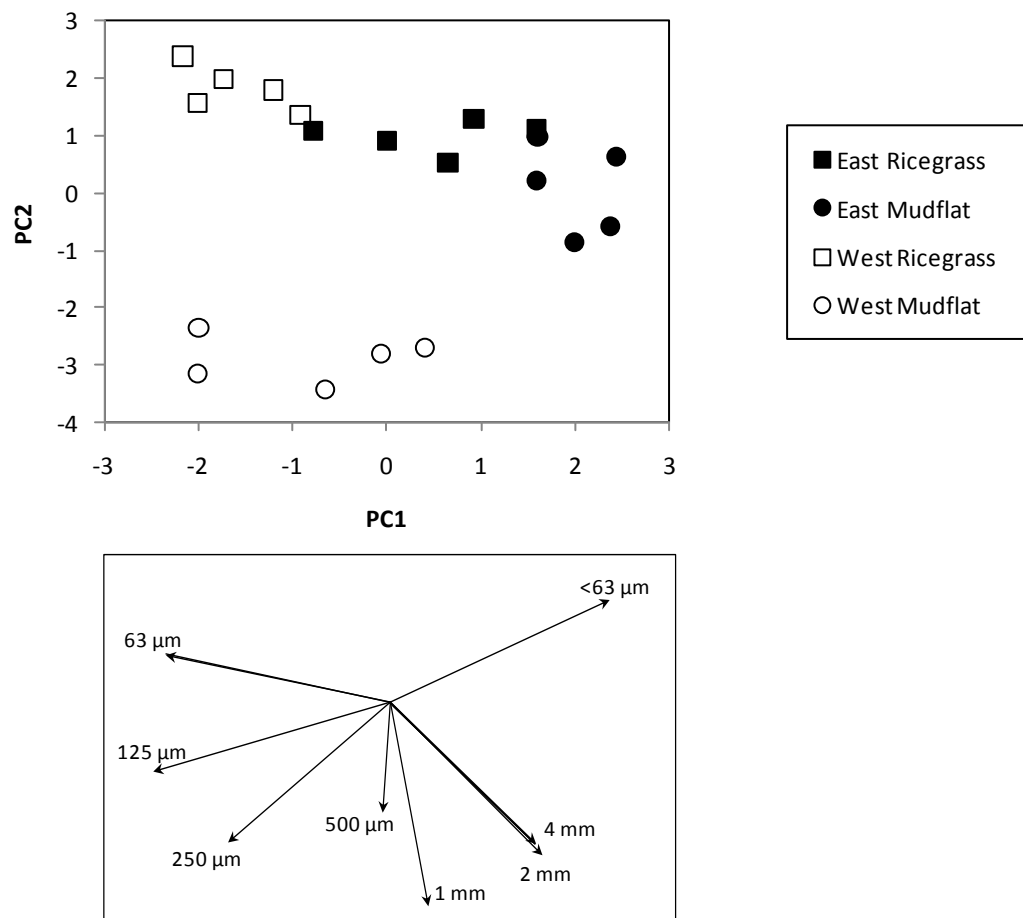


Figure 2.7. PCA ordination and associated biplot showing the separation of treatments on the basis of sediment size structure. The first two principle components accounted for 75.6% of the total variation observed. The biplots identify the sediment size fractions most important in shaping the patterns observed in the PCA ordination. The different treatments differed significantly in their sediment size composition. (2-way PERMANOVA: inlet: $MS=6427.46$ $F_{1,16}=149.71$, $P=0.002$; habitat: $MS=5557.66$ $F_{1,16}=129.45$, $P=0.002$; inlet*habitat $MS=3487.83$ $F_{1,16}=81.24$, $P=0.002$).

Although sediment size structure and benthic organic content were only measured once (hence no seasonal trends could be identified), there were some differences between habitats and inlets in these habitat characteristics. Sediment size structure depended strongly on both inlet and habitat (Fig 2.7). The PCA/biplot analysis indicated a tendency for the west inlet mudflat sediment size

structure to have a higher proportion of courser sediments, evident in the 500 μm , 250 μm and 125 μm fractions (Fig 2.7).

Rice grass habitat from both the east and west inlet exhibited a high level of finer sediment, with samples being dominated by the 63 μm and <63 μm size fractions. The difference in rice grass communities between the inlets were that of the mid range fractions, with west inlet rice grass exhibiting a slightly higher proportion of 250 μm and 125 μm sediments than rice grass communities in the east inlet. East inlet mudflat exhibited high levels of both coarse sediments (4 mm, 2 mm) and high proportions of fines (<63 μm) but lacked high quantities of the mid range sediments sizes (500 μm , 250 μm , 125 μm) which were more evident in the other sites.

The quantity of organic matter was higher in the rice grass habitats than in mudflats (Fig. 2.9), and the east inlet generally had higher organic matter content than the west inlet. The east inlet rice grass habitat exhibited the highest organic content and the west inlet mudflat the lowest. The quantity of organic matter also reflected similar patterns identified in sediment size distribution (Fig 2.7). Similar observations were identified in redox values, with rice grass having higher redox potential than mudflats, although this was more evident within the West Inlet (Fig 2.10)

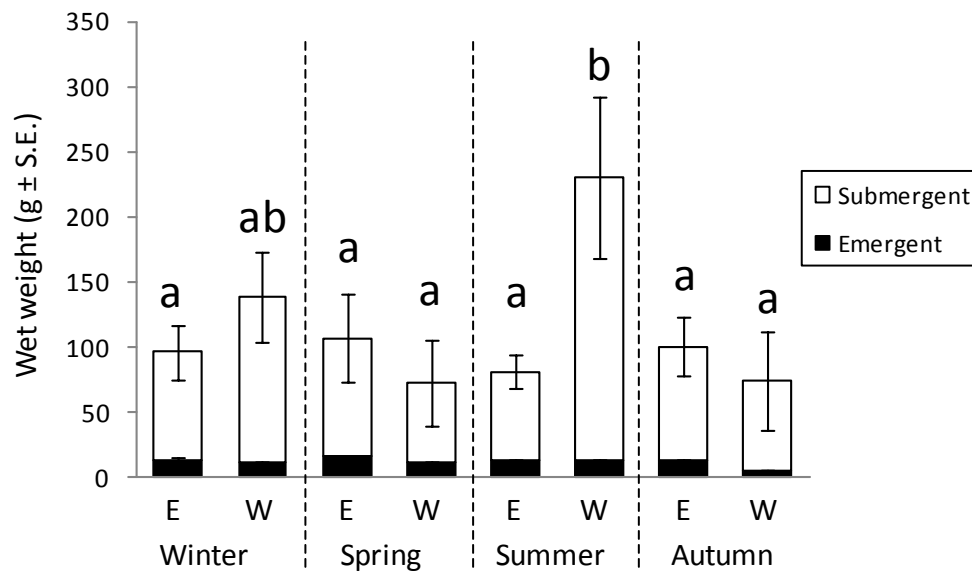


Figure 2.8. The wet weight of submergent and emergent rice grass from both east and west inlet across all for seasons. Wet weights are means (g) \pm S.E. of $n=5$ replicates. There were significant differences in the wet weight of submergent rice grass across seasons, but this depended on inlet. (2-way ANOVA: Season: MS=10491.4, $F_{3,32}=2.032$, $P=0.129$; inlet: MS=16016.1, $F_{1,32}=3.11$, $P=0.087$; season*inlet: MS=21020.8, $F_{3,32}=4.08$, $P=0.015$). Alternatively, no significant differences were identified for emergent rice grass biomass across seasons or inlets (2-way ANOVA: Season: MS=21.33, $F_{3,32}=0.52$, $P=0.672$; inlet: MS=116.15, $F_{1,32}=2.83$, $P=0.102$; season*inlet: MS=5.48, $F_{3,32}=0.1336$, $P=0.94$).

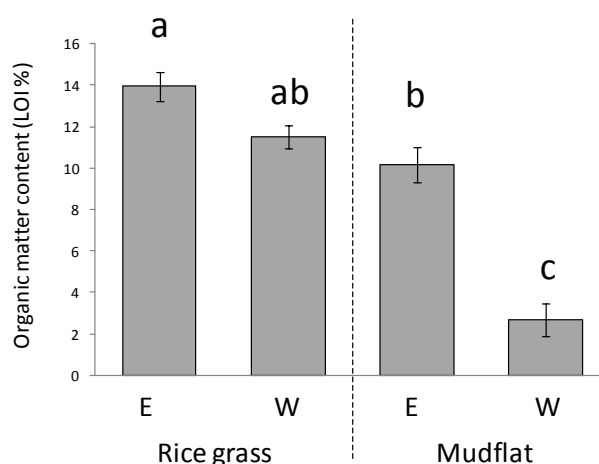


Figure 2.9. Organic matter content from rice grass and mudflat from both east and west inlets. Values are percentages (%) \pm S.E. of $n=5$ replicates. There were significant differences in the identified between habitats, but this depended on inlet (2-way ANOVA: inlet: MS=123.95, $F_{1,16}=33.14$, $P=2.9 \times 10^{-5}$; habitat: MS=199.9, $F_{1,16}=53.44$, $P=1.7 \times 10^{-6}$; inlet*habitat: MS=32.13, $F_{1,16}=8.59$, $P=0.010$).

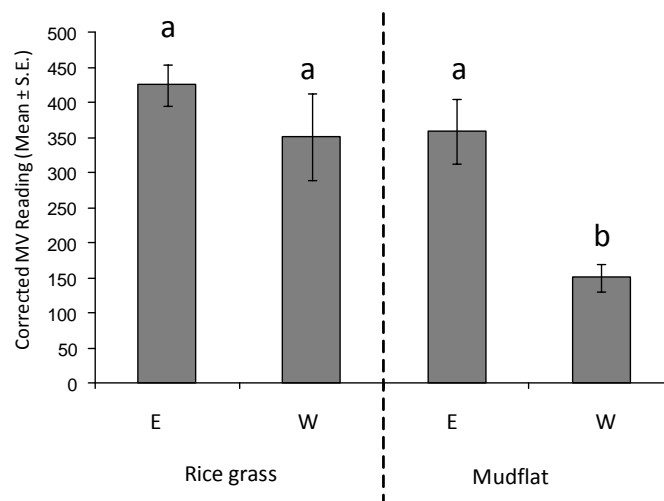


Figure 2.10. Redox potentials from rice grass and mudflat from both east and west inlets. Values are mean MV readings \pm S.E. of $n=5$ replicates. There were significant differences in the identified between habitats, but this depended on inlet (2-way ANOVA: inlet: $MS=89512.0$, $F_{1,16}=10.00$, $P=0.006$; habitat: $MS=98842.0$, $F_{1,16}=11.05$, $P=0.004$; inlet*habitat: $MS=22714$, $F_{1,16}=2.54$, $P=0.131$).

2.4 Discussion

2.4.1 Effect of habitat

The colonization of the Port Sorell estuary by *S. anglica* has dramatically altered the soft-sediment habitats in the estuary. In geomorphological terms, it appears to have transformed the intertidal zone from gently grading mudflats and sandy beaches into laterally extensive *S. anglica* monocultures (Greg Stokes, per com) composed of fine grained sediments, which is exactly the same trend that has been observed further east in the Tamar Estuary (Pringle 1975, Pringle 1993, Sheehan 2008).

The presence of rice grass has effectively ‘re-engineered’ the benthic substratum resulting in significantly increased three-dimensional complexity, accretion of fine sediments and increased organic loads. Given these changes to habitat

complexity and sediment geochemistry characteristics, it is not surprising that I observed differences in species abundance and community compositions.

My data shows that the invasion of rice grass has resulted in an increase in abundance of benthic macro-invertebrates and species richness, which has been documented as a common response following invasion by an NIS (eg: Castel et al. 1989, Posey et al. 1993, Crooks 1998, Wonham et al. 2005) especially if the presence of the exotic species alters the recipient environment (Crooks 2002, Wonham et al. 2005).

Mechanisms that may explain the increase in species richness and abundance include increased 3-dimensional structural heterogeneity (Castel et al. 1989, Stewart and Haynes 1994, Crooks 1998 and Horvath et al. 1999); alteration of sediment size composition and stability (Lenihan 1999, Crooks 2002 and Wonham et al. 2005); changes to currents, water flows and organic matter deposition (Crooks and Khim 1999, Wonham et al. 2005) and interference with biogeochemical cycling, oxygen concentration and nutrient fluxes (Vitousek 1990, Crooks and Khim 1999 and Parker et al. 1999); and all of these may account for the patterns I observed.

2.4.2 Differences between the Inlets

While differences were apparent between the two inlets, they were less evident than those between habitats. The west inlet generally exhibited higher abundances of crustaceans, particularly *Heloecius cordiformis*, and higher abundances of polychaetes and gastropods. Work by Griffin (1971) shows that some crab species have clear habitat preferences. The two ocypodid crabs I

found, namely *Heloecius cordiformis* and *Macrophthalmus latifrons* prefer muddy substrates where they build a complex network of burrows and feed by scraping up pellets of mud (Griffin 1971).

In contrast, the east inlet possessed higher abundances of bivalves. Reasons for these differences can be attributed (at least in part) to differences in the physical environments, reflected in the sediment geochemistry (Hedge and Kriwoken 2000). However, spatial variability in macro-invertebrate communities across even small spatial scales has been extensively documented for a variety of communities and is not an uncommon phenomenon, particularly in soft-sediment marine environs (Josefson 1998, Byers and Noonburg 2003, Reid 2010).

This variability has previously been attributed to many biotic and abiotic factors and processes including (but not limited to) variation in species recruitment, species habitat preferences, competition, predation and environmental factors (Stewart and Hayes 1994, Crooks 1998 and 2002, Ross et al. 2003a and b, Wonham et al. 2005). It is not surprising then that I observed a reasonably significant degree of spatial variability in community structure between inlets, particularly given the large distances between them.

2.4.3 *Effect of Season*

As with the spatial variability in macro-invertebrate communities, there was also some evidence of temporal variability. There was a clear temporal trend for higher species diversity (Shannon-Wiener) in summer and autumn, a trend also reflected in the abundance of polychaetes and molluscs. In contrast, crustaceans were the only taxonomic group to maintain a similar abundance throughout the

year, showing very little temporal variability in abundance. Reasons for this variability across time may be similar to those identified for causing observed spatial variability however without seasonal data on the sediment chemistry it is difficult to determine with any confidence what may be driving these changes (see section 2.4.2).

2.4.4 Conclusions

This study has demonstrated, perhaps unsurprisingly, that profound differences exist between benthic macro-invertebrate communities in rice grass and mudflat habitats. The presence of rice grass has resulted in higher abundances of selected species and taxonomic groups, and in particular grazing gastropods.

This trend has been demonstrated previously in Tasmania in the Little Swanport Estuary by Hedge (1997) and Hedge and Kriwoken (2000) who reported both species richness and abundance of macro-invertebrates increases in rice grass communities where *S. anglica* invades and replaces previous mudflat habitat.

Hedge and Kriwoken (2000) showed that the communities associated with *S. anglica* marsh are remarkably similar to those associated with native salt-marsh communities which might allow for speculation that *S. anglica* invasion may not constitute as significant an ecological threat to Australian temperate estuaries as previously thought, at least in terms of changes in native species.

The Little Swanport Estuary study by Hedge and Kriwoken (2000) however contained some interesting differences to my work as they reported significantly different redox values between rice grass and mudflat habitats (ANOVA $F_{2,57} =$

19.8, $P < 0.0001$), which was not seen here. They also found no significant difference in the organic content level between habitats (Kruskal-Wallis ANOVA $H_2 = 1.59$, $P = 0.452$) while I did see a significant difference in organic load between habitat, although this was dependent on inlet. Despite these differences, at both sites rice grass clearly provides a habitat that results in increased abundance of benthic macro-invertebrates with grazing gastropods being one of the key taxonomic groups to increase.

The establishment of rice grass results in a dense network of culms, rhizomes and roots that increases habitat complexity and heterogeneity in the substrate (Lana and Guiss 1991, Flynn et al. 1996). The gastropods may be grazing on epiphytic algae growing on the rice grass as many were observed in the field living on the upright shoots of the rice grass. The roots and rhizomes of *S. anglica* can actively contribute to sediment oxygenation, encouraging faunal colonization (Teal and Wieser 1996, Osenga and Coull 1983, Lana and Guiss 1991).

In contrast some species clearly prefer the mudflat habitat, particularly bivalves and ocypodid shore crab species, as demonstrated by Griffin (1971). Since the water flow across the mudflat will be largely unimpeded compared to the rice grass beds, it not surprising that filter feeders like bivalves would have a preference for this habitat type so as to maximize their exposure to the greatest water flow to deliver their food resource.

The different community structures between habitats also reflect the changes in sediment geochemistry characteristics that appear to follow with the colonisation

of rice grass. In the *S. anglica* meadows the rice grass appears to be acting as a sink that traps finer sediments. Kersten and Smedes (2002) showed there is a worldwide positive correlation between silt fractions size and increased organic loads, which results from organic material attaching to the finer sediment. This, coupled with the naturally higher organic loads that result from the accumulation of trapped decaying material that comes directly from the rice grass, results in a habitat that is better suited to supporting large numbers of grazing gastropods that may be able to exploit this habitat niche.

It is also likely that the *S. anglica* meadows provide a refuge for macro-invertebrates by providing protection from abiotic stress and predation. The dense aggregation of rice grass culms may provide shelter from wind and sunlight and hence a more stable microclimate. Resident and migratory birds exert heavy predatory pressure on mudflat macro-invertebrate communities (Long and Mason 1983, Reise 1985, Inglis 1995) but several reports from Australia (Simpson 1995) and England (Evans 1986, Goss-Custard and Moser 1990) show that wading birds avoid *S. anglica* habitats.

In conclusion, the results of this study provide additional evidence that *S. anglica* invasion of mudflat habitat promotes a more abundant and species rich benthic macro-invertebrate community structure than would normally be found on natural mudflats. The differences observed between the two arms of the estuary have implications for the design of the later parts of the study as it resulted in my post spraying analysis being limited to work in only the west arm (See Chapter 4).

CHAPTER 3

DETECTION OF THE ACTIVE INGREDIENT OF THE HERBICIDE FUSILADE FORTE® (AND ITS DEGRADATES) FOLLOWING SPRAYING OF RICE GRASS (*SPARTINA ANGLICA*) IN THE RUBICON ESTUARY, PORT SORELL, TASMANIA

3.1 Introduction

In 2005, Syngenta Pty Ltd, the manufacturers of Fusilade® replaced this product with a new herbicide called Fusilade Forte®. Fusilade Forte® contains the same active constituent as Fusilade®, namely fluazifop-P-butyl but at a lower concentration (ie 128 g/L as opposed to 212 g/L). As a consequence of this change in concentration of the active constituent the permit for the use of Fusilade Forte® was subsequently varied to state that Fusilade Forte® should be mixed at a rate of 1.65 L per 100L (as opposed to 1 L per 100 L for Fusilade®).

The Marine Environment Group of the Department of Primary Industries and Water, noting the change to the original chemical compositions, contacted the Department's Environment Division (ED) and requested they undertake a review of the potential impact of applying Fusilade Forte® into the marine environment, given this product was slightly different to the one that was originally considered ie Fusilade®.

As discussed earlier, both Fusilade Forte® and Fusilade® contain the active ingredient fluazifop-P-butyl (parent material) which metabolises and degrades to fluazifop P (acid), 5-trifluoromethyl-2-pyridone and 2-(4-hydroxyphenoxy)

propionic acid. As such the ED's review included the hazards and environmental risks of the parent material and three major metabolites.

Their review was based on contemporary literature and Chemwatch, the principal reference software for the Material Safety Data Sheet (MSDS). As a result of this review the Director of the Environment Protection Authority (EPA) requested a "thorough investigation of the persistence of Fusilade Forte® in the marine environment" as the review suggested the active constituent and its breakdown products can be toxic to aquatic fauna, may persist in the environment and may be insoluble in water, which appears to have been a contradictory position to previous risk assessments that had been undertaken for Fusilade® (See Davies 2000).

Consequently I was approached by the (then) Department of Primary Industries and Water (DPIW) to undertake this investigation, given that it fitted neatly into my research project. The following work aimed to quantify whether Fusilade Forte® (or its degradates) could be detected in samples of sediment, water, and sentinel shellfish after a single broad-acre spraying event in the Port Sorell estuary.

3.2 Methods

3.2.1 Location

The spray trial took place in the west inlet of the Port Sorell estuary on the north coast of Tasmania, where at least 120 ha of rice grass existed in the estuary at that time in 2007 (as described earlier in Chapter 2).

3.2.2 Survey design

Pre-spray sampling was conducted in late January. Sub-tidal sediment (from the first 2 cm), water (approximately 1 L of surface water taken from 30 cm below the surface), and six Pacific Oysters (*Crassostrea gigas*) were collected at each of six locations throughout the west inlet of the estuary. All the oysters sampled during the field program were locally sourced from an oyster farm operating in the east inlet of the estuary and were deployed for 2 months on purpose-built structures in traditional oyster baskets.

Pacific oysters were chosen as a sentinel shellfish because they:

- Occur naturally in the estuary where the study was conducted;
- Are abundant and easy to obtain in the estuary;
- Are sessile filter feeders that concentrate toxins;
- Have been widely used as a sentinel shellfish species in other toxicological studies (Hedge et al. 1999 and Gagnaire et al. 2006).

Two sample sites were located immediately adjacent to the area to be sprayed and the other four sites were at various longitudinal distances downstream from the spray zone (See Figure 3.1).

In March, approximately 1.5 ha of rice grass was sprayed with the herbicide Fusilade Forte® using backpack sprayers and a quick spray automated unit mounted in a small boat. Herbicide concentration and application rates were consistent with those used for all rice grass spraying activities in Tasmania. Fusilade Forte® was mixed at 16 ml/L and applied at the rate of 10 L/ha. On this basis it was estimated that 240 ml of Fusilade Forte® was applied to the

immediate area. All standard spray protocols for the use of Fusilade Forte® were followed (DPIW 2003, unpublished report).

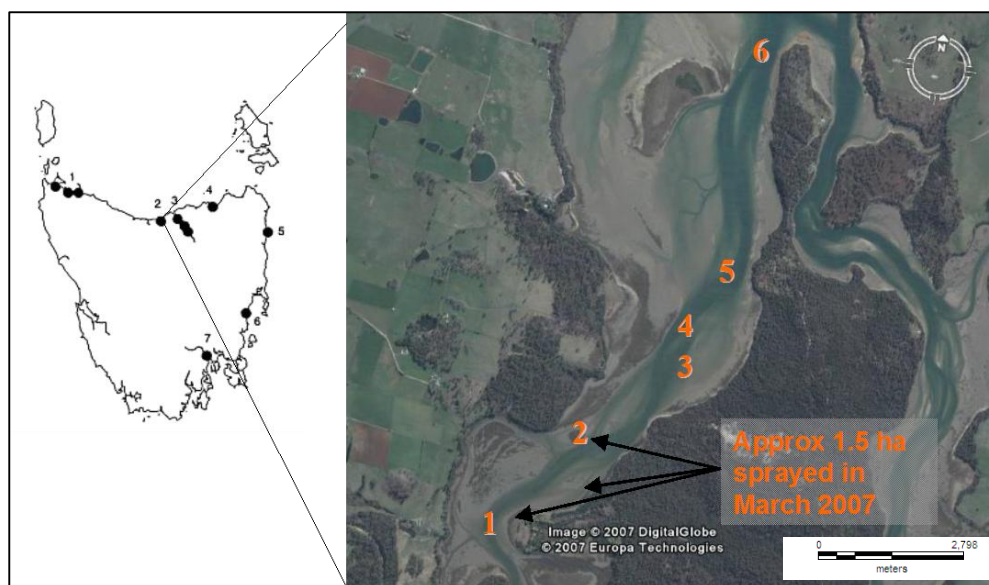


Figure 3.1. Map of Tasmania showing the Port Sorell area and the indicative positions of the six sampling sites located in the Rubicon River arm of the estuary and their position relative to the area where 1.5 ha of rice grass was sprayed.

3.2.3 Analysis

After spraying, samples of sub-tidal sediment, water and Pacific Oysters were collected at each of the six sites on days 1, 3, 7, 15, 30, and 60 (see Table 3.1). Sediment and water samples were sent to Analytical Services Tasmania (AST) and oyster samples were sent to Advanced Analytical Sydney (AA) for analysis. All samples were analysed for the active constituent (fluazifop-P-butyl) and the breakdown products (fluazifop-P, 2-(4-Hydroxyphenoxy) propanoic acid {HPPA} and 5-trifluoromethyl-2-pyridone {TFMP}). Table 3.2 shows the detectable limits for the active constituent and its breakdown products achieved by AST and AA.

Table 3.1 Days and dates when samples of oysters, soil and water were collected.

Day	Date	Advanced Analytical (AA)	Analytical Services Tasmania (AST)	
		Oysters	Sediment	Water
-1	29/01/2007	Y	Y	Y
0	14/03/2007	Spray day		
1	15/03/2007	Y	Y	Y
3	18/03/2007	Y	Y	Y
7	22/03/2007	Y	Y	Y
15	29/03/2007	Y	Y	Y
30	16/04/2007	Y	Y	Y
60	25/05/2007	Y	Y	Y

Table 3.2 Detectable limits (mg/kg and µg/L) for the active constituent of Fusilade Forte® and three breakdown products.

Sample Type		Oysters	Water	Sediment
Laboratory		AA	AST	AST
Detectable limit	Type	mg/kg	µg/L	mg/kg
Fluazifop-P-butyl	Active	<0.01	<1.0	<0.008
Fluazifop-P	Degradant 1	<0.01	<0.2	<0.002
HPPA*	Degradant 2	<0.01	<1.5	<0.012
TFMP[#]	Degradant 3	<0.01	<1.5	<0.012

* 2-(4-Hydroxyphenoxy)
propanoic acid

[#] 5-trifluoromethyl-2-pyridone

3.3 Results

3.3.1 Oysters

Neither the active constituent nor any of the other three degradates were found above the detectable levels in any samples of Pacific Oysters.

3.3.2 Sediment

Samples analysed by Analytical Services Tasmania show that only the acid breakdown product (fluazifop-P) was recorded above detectable levels (i.e. < 0.002 mg/kg), and then only at site 1 (which was located adjacent to the spray area)(see Figure 3.1, 3.2). However this compound was detected in the sediment up to 30 days post spraying.

3.3.3 Water

Samples analysed by Analytical Services Tasmania show that only the acid breakdown product (fluazifop-P) was recorded above detectable levels (i.e. < 0.2 $\mu\text{g/L}$), and then only on day 1 after spraying (see Figure 3.3). On day 1, however all sites except site 1 recorded fluazifop-P above the detectable levels.

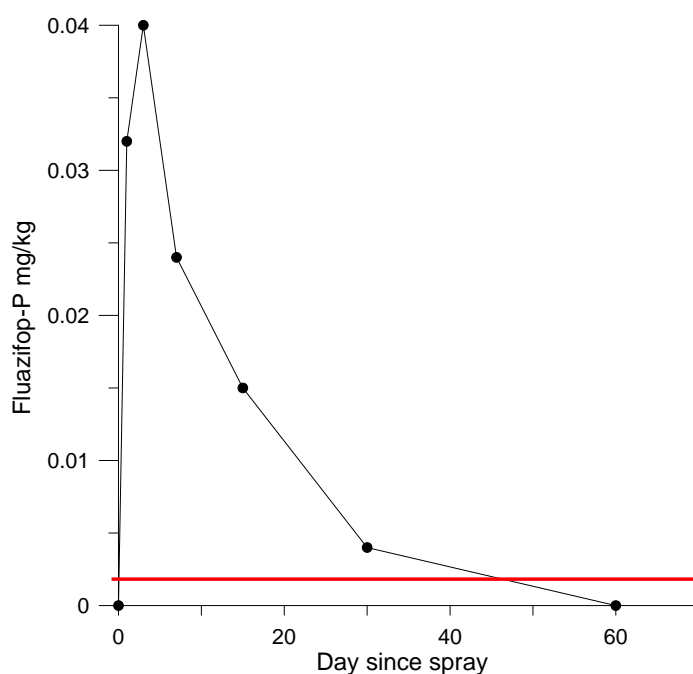


Figure 3.2. Levels of the acid breakdown product (fluazifop-P mg/kg) in sediment samples at site 1 in samples analysed by Analytical Services Tasmania. DL (red line) denotes Detectable Limit of 0.002 mg/kg for AST.

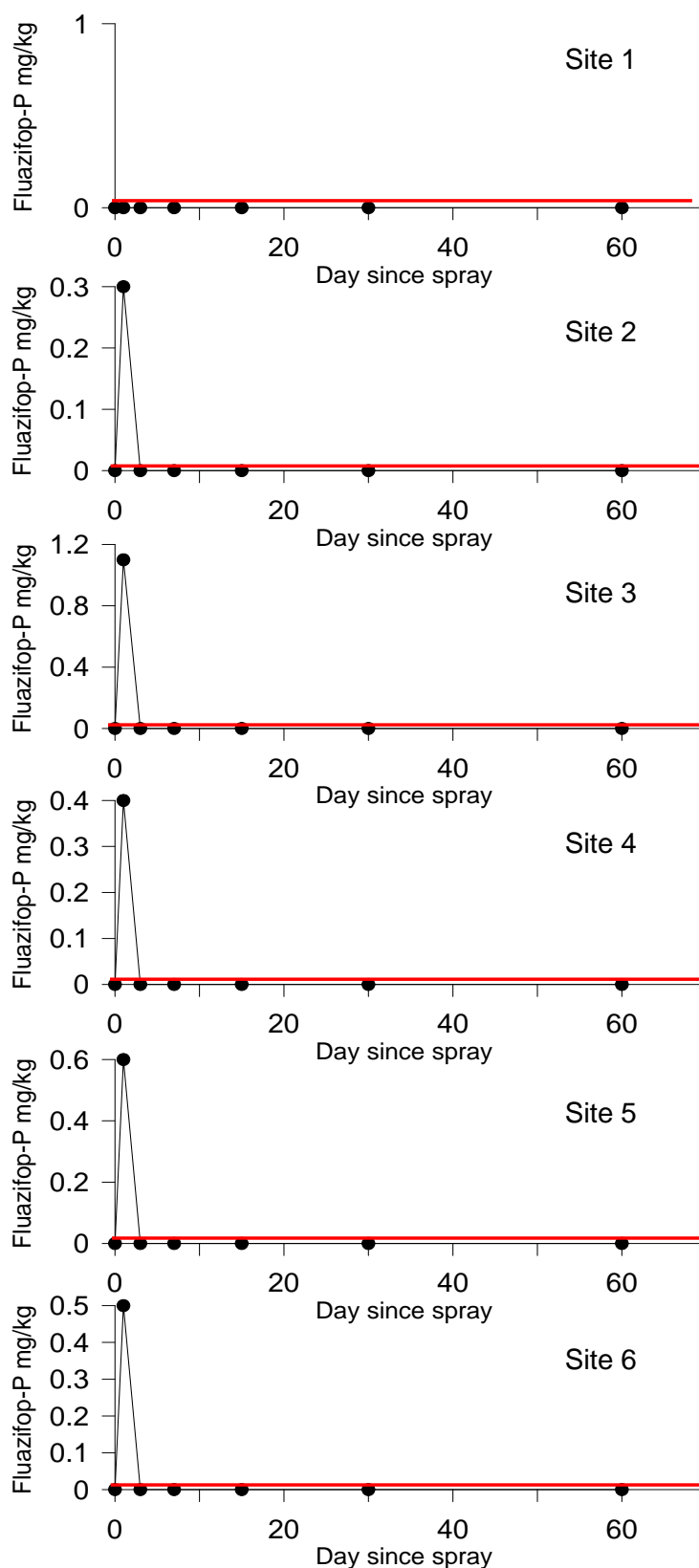


Figure 3.3. Levels of the acid breakdown product (fluazifop-P mg/kg) in water samples analysed by Analytical Services Tasmania. DL (red line) denotes Detectable Limit (0.002 µg/L).

3.4 Discussion

There is a reasonable body of both technical and commercial literature that relates to the environmental and toxicological properties of fluazifop-P butyl ester (FPB) and the less toxic fluazifop-P (FP) (the acid form) and some of this literature is Australian in origin (See Palmer et al. 1995, Hedge 1997).

The Material Safety Data Sheet (MSDS) for Fusilade Forte®128 EC Selective herbicide classifies the product as an “environmentally hazardous substance” that is slightly toxic to fish, highly toxic to algae, slightly toxic to aquatic invertebrates and practically non-toxic to soil dwelling organisms.

The algal and invertebrate toxicity assessment was conducted on the alga *Scenedesmus subspicatus* and the micro-crustacean *Daphnia magna*; both of these are freshwater species so the results from this work should be treated with caution when trying to make comparisons with estuarine species.

These conclusions are however based on internal studies undertaken by Syngenta Crop Protection Pty Ltd (the company who supply Fusilade Forte® {and supplied Fusilade®} into Australia) that have been conducted according to regulatory requirements, including OECD and CIPAC Guidelines and EC Directives. The company also states that “a comprehensive package of toxicology and environmental data for the active ingredients of Fusilade Forte® has been submitted to the government health and environment authorities and has been evaluated by expert toxicologists and environmental scientists” (Syngenta Crop Protection Pty Ltd 2010).

The MSDS also states that FPB is not persistent in soil or water, claiming there is evidence of rapid hydrolysis in water and soil to FP, which also rapidly degrades and is of lower intrinsic toxicity. Humburg and Colby (1989) suggest that the half-life of FP is approximately three weeks.

Both Hedge (1997) and Palmer et al. (1995) undertook similar work to the present study as their research included field-based monitoring in estuarine environments for the persistence of the active ingredient and the primary breakdown product of Fusilade®, i.e. FPB (ester) and FP (acid) respectively.

Hedge (1997) conducted his work in the Little Swanport estuary on the east coast of Tasmanian where he monitored the degradation of FPB in water, sediment and soft tissue of Pacific oysters following spraying with Fusilade® while Palmer et al. (1995) worked in the intertidal zone in the Albert River estuary in Victoria, where he measured fluazifop in sediment and water post spraying.

In Hedge's work, water and sediment samples were collected from three sites daily for six days post-spraying. Water was collected in glass bottles (approximately 1 L) by submerging the bottles in water before replacing the cap. Sediment was collected by scraping the surface (approximately 3 cm deep) with sticks collected from the shoreline.

Oysters sampled were placed in a light mesh bag which was positioned 15 m from the Fusilade® treated area at one site. Samples from the bag were then collected at day 9 and day 60 from this site and additional oysters were also sampled from an oyster lease in the estuary 4.5 km from the sprayed site at

day 60. Hedge mixed Fusilade® at 10ml/L and applied it to the area at a rate of 10 L/ha.

The field work by Palmer et al. (1995) examined the persistence of fluazifop in the field by sampling the sediment and water from two different areas for four days and seven days post-spraying respectively. At one site ten sediment samples were collected (which were pooled) from within a 15 cm depth while five 1 L water samples were also taken, including two from shallow tidal pools, immediately post spraying and at 3.5, 6, 11 and 24 hours post spraying respectively.

At the other site 15 sediment samples were collected (that were pooled) from three depth: 0-5 cm, 5-15 cm and 15-25 cm and an integrated water sample was also taken on nine occasions post spraying. These samples were collected 20 minutes, 4.5, 10.5, 11, 23 and 48 hours post spraying and 4 days and 7 days post spraying with some samples being conducted to correspond with first incoming and outgoing tides. Palmer et al. applied the Fusilade® at a rate of 12.3 L/ha and 10L/ha respectively.

Hedge's results, albeit for Fusilade® not Fusilade Forte®, were similar to mine. He detected FPB at varying concentrations (0.016-0.026 µg/L) in water samples at all sites up to 22 hrs post spraying but nothing thereafter. In sediments FPB was detected at two sites in varying concentrations (0.029 -0.014mg/kg) but was not detected until 84 and 133 hrs post-spraying and the decrease of the concentration over time suggests that the active ingredient is progressively

degrading to low levels within the term of its half life (predicted to be less than one week in moist conditions according to the Royal Society of Science {1991}).

In nearby oysters (within 15 m of the spray site) Hedge detected FPB nine days after spraying but could not detect anything in nearby or distant oysters (4.5 km down the estuary) two months after spraying.

Work by Thompson and Comber (1982) and Hedge et al. (1999) showed that toxicity of FPB and its degradate FB on Pacific oyster (*Crassostrea gigas*) D-veliger larva and juveniles respectively was low to moderate at expected field concentrations. While Thompson and Comber did report some developmental abnormality on exposure to FPB the form of abnormality was not described.

Hedge showed that Fusilade® concentrations (based on worse case scenarios) do not affect the survival of juvenile oysters at 2 and 4 mm in length under test conditions and that the growth rate of oysters over a 15 week period was not affected by greater than expected field concentrations of Fusilade®. Furthermore, when exposed to concentrations of Fusilade® that clearly exceeded expected concentrations following a spray event, oysters of 45 and 87 mm in length rapidly depurated the active ingredient (ie 80% within 24 hours) and the active ingredient was below detectable levels within 7 days of the oysters being treated.

In contrast Palmer et al. showed that fluazifop concentrations in sediment fell below detection limits within one day and concentrations in the water column were still detected after seven days. Unfortunately Palmer et al. did not

differentiate between the active FPB (ester) and the primary break-down product FP (acid) in their field work.

Interestingly they did conduct several laboratory-based experiments where they investigated the decomposition of Fusilade® by examining the concentrations of the FPB and FP during static toxicity trials in seawater. In this work they showed that most of the FPB decomposed to the less toxic FP acid after 3 days in the static toxicity test with the Smallmouth Hardyhead *Atherinosoma microstoma*, after 4 days with the gammarid amphipod *Allorchestes compressa* and in 1 day in degradation trials when exposed to natural light, whether in contact with mud substrate or not.

In this study the active ingredient of Fusilade Forte® (ie FPB) or any of the three degradates were not detected in any sample of Pacific Oysters (at least above the Detectable Limit < 0.01 mg/kg). As previously discussed tank-based toxicity trials on Pacific Oysters have concluded that Fusilade® does not appear to affect the survival, mortality or growth rates of juveniles or adults, and that the active ingredient FPB, does not bio-accumulate in oyster tissue and is rapidly depurated (Hedge et al. 1999).

For sediment samples only the acid breakdown product (FP) was recorded above detectable levels (i.e < 0.002 mg/kg), and then only immediately adjacent to the spray area although it may persist for up to 30 days.

For water samples only the acid breakdown product (FP) was recorded above detectable levels (i.e < 0.2 µg/L), and then only on day 1 after spraying. On day

1 however, it was recorded at detectable levels at all sites except site 1. The fact that FP was recorded across the estuary but only on day 1 suggests that tidal flushing within the estuary rapidly removes the chemical although it is also possible that it was broken down and absorbed into the sediment elsewhere within the estuary.

In conclusion this trial suggests that the chemical constituents of Fusilade Forte® were quickly removed from the water column of the west arm of the Port Sorell estuary and if it did enter into feeding Pacific Oysters it appears to have been rapidly depurated from this species.

In contrast one of the chemical constituents of Fusilade Forte® namely fluazifop-P (acid), was detected within sediment immediately adjacent to the spray site for at least several weeks post spraying. The persistence of the FP could be as a result of the degradate being trapped around the rice grass roots or captured in some of the crab burrows evident in the rice grass. The two species of ocypodid crabs commonly found on the mudflats but also within the rice grass meadows always construct burrows, *Heloecius cordiformis* vertical ones and *Macrophthalmus latifrons* sloping ones (Griffin 1971), and it is possible the FP is retained within these burrows as they may be poorly flushed during incoming tides.

The fact that the active ingredient and the three main degradates appear to be fairly rapidly removed from the water column and sediment i.e. within 3-4 weeks suggests that any impact from spraying can be expected to be acute immediately post-spraying while the concentrations of the chemical constituents is quite high.

Obviously through time as these concentrations diminish; it is likely that any biological impact will also reduce and evidence to show this will be described in the following chapter where the impact on benthic macro-invertebrates post-spraying is specifically investigated.

CHAPTER 4

THE EFFECT OF FUSILADE FORTE® ON BENTHIC MACRO-INVERTEBRATE COMMUNITIES IN *SPARTINA ANGLICA* MEADOWS AND ADJACENT BARE MUDFLATS

4.1 Introduction

Fusilade Forte® is a selective herbicide that is recognised as having potentially toxic impacts on aquatic invertebrates. As described in Chapter 3 the Material Safety Data Sheet (MSDS) for Fusilade Forte® notes the chemical constituents of this product are highly toxic to algae, slightly toxic to aquatic invertebrates and practically non-toxic to soil dwelling organisms (although these conclusions are based on research on freshwater and terrestrial species).

The MSDS states that the active ingredient, fluazifop-P-butyl (FPB), is not persistent in soil or water, becomes incorporated into organic molecules (ie binds with chelates) and does not bioaccumulate (Syngenta Crop Protection Pty Ltd 2010). Fluazifop-P-butyl is thought to hydrolyse rapidly (<24 hours) to fluazifop acid (FP), with the rate increasing with pH (Chemwatch 2002). This hydrolysis product is reported to have moderate toxicity to aquatic life, and to be immiscible and stable in water (Chemwatch 2005).

However, in assessing whether to permit the use of Fusilade Forte® in 2006, the Environment Division of the (then) Department of Primary Industries and Water (DPIW) took a conservative position based on a review of contemporary literature.

They noted that fluazifop-P-butyl is not registered for use in aquatic systems (Weed Control Methods Handbook 2001) and felt there was sufficient evidence to suggest that “the product and break-down products” do persist in the environment, are insoluble in water and can be toxic to aquatic fauna and flora (See ICI internal memos 1982 and 1985).

In light of these conflicting opinions, my work aimed to quantify the potential acute and chronic effects on benthic macro-invertebrate communities following the application of Fusilade Forte® to treat rice grass in a Tasmanian estuarine environment. Other work investigating the effects of toxicity is normally undertaken in controlled laboratory environments and at small scales. Moreover, these laboratory tests are typically conducted on individual species of interest, rather than communities or macro-invertebrate assemblages (Palmer et al. 1995). While some work attempts to compliment laboratory tests with field assessments, this is not usually the case.

This study was devised to assess impacts on benthic macro-invertebrate community structure of spraying rice grass with Fusilade Forte® under field conditions, and at a number of spatial and temporal scales. Therefore, my research addresses some

aspects of toxicity testing and a holistic community level assessment of the impacts of herbicide spraying.

4.2 Methods

4.2.1 Field sampling and laboratory analyses

In March 2007 an area of approximately 1.5 ha of rice grass was sprayed within the West Inlet of the Port Sorell Estuary with the herbicide Fusilade Forte® using backpack sprayers and a quick spray automated unit mounted in a small boat (as described in Chapter 3). At the time of spraying the tide was very low at <0.2 m and due to a combination of tides and a localised high pressure system over Tasmania, it is estimated that the rice grass was exposed by the tide for at least 12 hours. Climatic conditions at the time of spraying were considered favourable, ie the plants were dry, there was clear weather, the temperature was 18-21 °C, the relative humidity was 64% and there were only light winds.

Fusilade Forte® was mixed at the recommended rate of 16 ml/L and applied at the rate of 10 L/ha and on this basis it was estimated that 240 ml of Fusilade Forte® was applied to the immediate area. All standard spray protocols for the use of Fusilade Forte® were followed (DPIW 2003, unpublished report). Only the West Inlet was treated with Fusilade Forte® as earlier work (See Chapter 2) had shown there was a significant difference in the rice grass benthic macro-invertebrate communities between the West and East Inlets.

Following the application, the area sprayed and the adjacent mudflat habitat was sampled for benthic macro-invertebrates on a 3 monthly basis for a period of 12 months. As work had already been undertaken examining the immediate effects post spraying (Davies 2001) and as I was interested in investigating the impact at a community level I decided to allow a residual time for the community to respond to the effects of spraying. Each sampling event used the same methodology as had been applied in the pre-spraying sampling and is described in Chapter 2.

The benthic macro-invertebrate communities were sampled at five randomly selected locations from within the upper reaches of only the west arm of the Port Sorell estuary in the same general area as had been sampled pre-spraying. At each location triplicate core samples were collected from both the treated *S. anglica* meadows and mudflats habitats located approximately 50 m away from the treated area. Samples in the *S. anglica* habitats were still taken at least 5 m in from the leading edge of the rice grass meadow. All samples were taken using a circular 100 mm diameter x 150 mm deep core.

All samples collected were stored in plastic buckets before being transported back to the wet laboratory at the University of Tasmania where they were sieved through 1 mm mesh to separate out the animals using pressurised salt water to displace the mud. All samples were sieved within 48 hours of being taken.

Prior to sieving any emergent rice grass was cut off, patted dry with paper towel and wet weighed and once any animals had been removed from the processed sample the

remaining sub-surface rice grass roots and rhizomes were also patted dry and wet weighed. All animals were fixed in 70% alcohol and 2% glycerol and were classified to species level where possible using a dissecting microscope.

4.2.2 Statistical analysis

4.2.2.1 Comparison of fauna across treatments

To depict and assess differences in community structure between treatments, I used non-metric multi-dimensional scaling (nMDS) and permutational multivariate analysis of variance (PERMANOVA). Analyses were based on Bray-Curtis dissimilarities calculated after a square-root transformation of the data. The nMDS ordinations were developed using PRIMER 5 software (Clarke and Gorley 2001), while PERMANOVA routines were as described in Anderson (2001) and McArdle & Anderson (2001).

Analyses of community structure were carried out on different components of the fauna. First, an nMDS ordination was carried out including all taxa identified to the lowest taxonomic level. Given that the rice grass communities were subject to a single spray of Fusilade Forte®, I also conducted nMDS ordinations on the mudflat and rice grass separately to clearly distinguish impacts associated with the spray from those related to temporal variability. The nMDS conducted on the whole data set and the nMDSs conducted on the separate habitats were interpreted together. The significance of patterns observed in all nMDS plots was determined using PERMANOVA (Anderson 2001, McArdle & Anderson 2001).

Univariate model I ANOVA was used to compare mean species richness, total abundances and diversity (Shannon-Wiener) and abundances of select taxonomic groups and species among treatments. ANOVAs were performed using the R statistical package (R Version 2.13.0 {2011}).

All PERMANOVA (conducted on total community structure) and univariate ANOVA models (conducted on selected taxa) had the same basic design. They included the fixed effects of ‘sampling time’ (8 levels: 3 monthly intervals from 12 months prior to spray event, to 12 months post the spray event), crossed with ‘habitat’ (2 levels: rice grass, mudflat) and the sample time*habitat interactions.

In the event that the main analysis yielded a significant interaction, an *a-posteriori* Ryan-Einot-Gabriel-Welsch (REGWQ) multiple range test was conducted to determine the nature of the interaction. For ANOVAs, data and residuals were checked for normality and homoscedasticity, and transformed as necessary to stabilise variances on the basis of the relationship between group standard deviations and means (Draper & Smith 1981).

Separate analyses were conducted on total rice grass wet weight, emerged rice grass wet weight and submerged rice grass wet weight. Again, data and residuals were checked for normality and homoscedasticity, and transformed as necessary to stabilise variances on the basis of the relationship between group standard deviations and means (Draper & Smith 1981). Differences in community structure between treatments were assessed in light of patterns in the biomass of rice grass.

4.3. Results

4.3.1 *Differences between habitats*

A total of 2697 individuals across 26 taxa were recorded in the study, including 8 mollusc, 9 crustacean and 8 polychaete taxa. Differences in community structure between habitats (i.e. rice grass vs. mudflat) for the four sampling events prior to spraying were assessed and discussed in another chapter of this thesis (Chapter 2) and therefore are not discussed here. However, differences in community structure between rice grass and mudflat during the post-spray sampling periods were also clearly evident.

For the post-spray sampling periods, the total abundance of individuals was consistently higher in rice grass than in mudflat, although this depended on the sampling time (Fig 4.1, Table 4.1). During the last sampling period (12 months post-spray), similar estimates were recorded for all community metrics in both rice grass and mudflat (Figure 4.1).

Shannon-Wiener diversity (H') and species richness was not significantly different across habitats but was significant across sampling time (Fig 4.1, Table 4.1). Of the 26 individual taxa, 22 were identified in mudflat and 17 in rice grass. Thirteen of these taxa were identified in both habitats, while 4 taxa were observed only in rice grass, and 9 taxa identified only within mudflat.

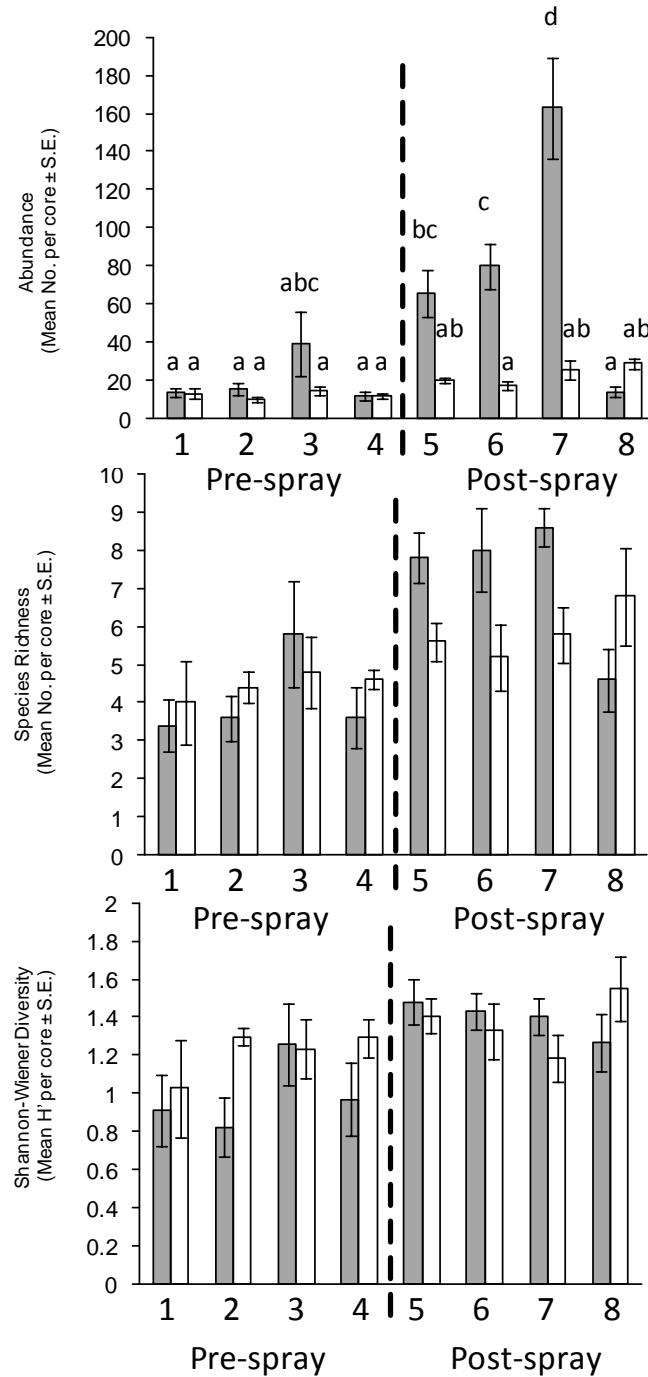


Figure 4.1. Total abundance of individuals, total taxa and Shannon-Weiner diversity (H') across all combinations of sampling time and habitat. All values are means \pm S.E. from $n=5$ replicates. Pre-spray 1-4 are the four baseline samples from 12, 9, 6 and 3 months prior to spray event; post-spray 5-8 are the four samples from 3, 6, 9 and 12 months post spray. Grey bars denote rice grass and open bars denote mudflat. For main analyses where significant interactions were detected, the REGWQ groups are positioned above the respective treatments. In the REGWQ groups, analogous letters denote the same groupings.

Analysis of total community composition also indicated significant differences between mudflat and rice grass, although these differences were dependent on the sampling time (Fig 4.2). The differences in community structure were most strongly evident in the 3 and 6 month samples post-spray, where community structure in rice grass was distinctly different from pre-spray rice grass communities, and all communities associated with mudflat.

The communities within rice grass sampled at 9 months and 12 months after spraying were also distinctly different from other communities, being more similar to mudflat communities than the other post-spray rice grass communities (Fig 4.2). The 9 month post-spray sample was similar to the pre-spray rice grass communities, but the 12 month post-spray sample was more closely related to mudflat than any of the rice grass communities. This perhaps indicates that the rice grass habitat (and therefore the community) is beginning to more closely represent a mudflat habitat after 12 months post-spray with Fusilade Forte®.

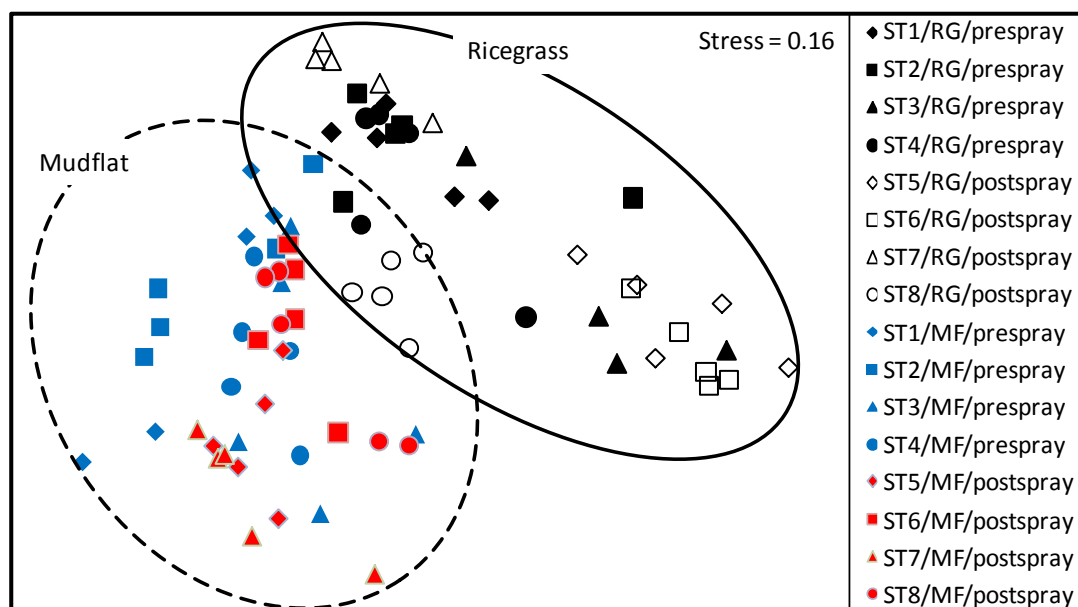


Figure 4.2. nMDS ordination showing separation of the different communities across all combinations of sampling time and habitat. ST1-4 denote the first four sampling times prior to spray at 12, 9, 6 and 3 months pre-spray respectively. ST5-8 denote the four sampling times post spray at 3, 6, 9 and 12 months post-spray respectively. The ellipses indicate arbitrary separation of communities based on habitat type. There were significant differences in community structure between habitats and sampling times (PERMANOVA, sampling time: $MS=1575.65$, $F_{7,64}=4.72$, $P<0.001$; habitat: $MS= 54802.65$, $F_{1,64}=34.78$, $P<0.001$; sampling time*habitat: $MS=4906.5$, $F_{7,64}=3.11$, $P<0.001$)

4.3.2 The Impacts of Spraying Fusilade Forte®

There were clear and significant impacts of spraying Fusilade Forte®. Unsurprisingly, the rice grass habitat underwent large alterations in community structure and the abundance of some taxonomic groups and select fauna, while the analogous community parameters remained relatively constant within mudflat habitat. Firstly, rice grass communities exhibited higher total abundance and species richness after spraying than before (Fig 4.1, Table 4.1).

There was also a small increase in the total abundance and species richness in the mudflat habitat, but this difference was far more prevalent in rice grass (Fig 4.1, Table 4.1). The impact of spraying Fusilade Forte® was also evident in the multivariate analysis, with community structure being clearly different in rice grass post-spray compared to analogous rice grass habitats pre-spray (Fig 4.2, 4.3). Only after 12 months post-spray did the community structure begin to resemble communities associated with mudflat adjacent to the sprayed rice grass (Fig 4.2).

An assessment of the major taxonomic groups and the abundance of selected taxa best describes the differences in community structure resulting from the spraying of Fusilade Forte®. Rice grass communities at 3, 6 and 9 months post-spray exhibited lower abundances of crustaceans and higher abundances of molluscs, particularly grazing gastropods, than rice grass communities prior to spraying (Fig 4.4, Table 4.1).

The abundance of polychaetes and bivalve molluscs did not change from their respective abundances pre-spray (Fig 4.4, Table 4.1). However, the abundances of gastropods and crustaceans for the rice grass communities at 12 months post-spray were different to those for 3, 6 and 9 month post-spray, but similar to mudflat communities 12 month post-spray, indicating that the community structure of sprayed rice grass is possibly beginning to resemble that observed within mudflat, at least for these taxonomic groups.

The anticipated response of the rice grass habitat after spraying is that the biomass of rice grass would decline and as a result, there would be an alteration to community structure and abundance of certain taxonomic groups. While there was a clear decline in the emergent component of rice grass during sampling events post-spray, the proportion of submerged rice grass remained consistently high (Fig 4.5).

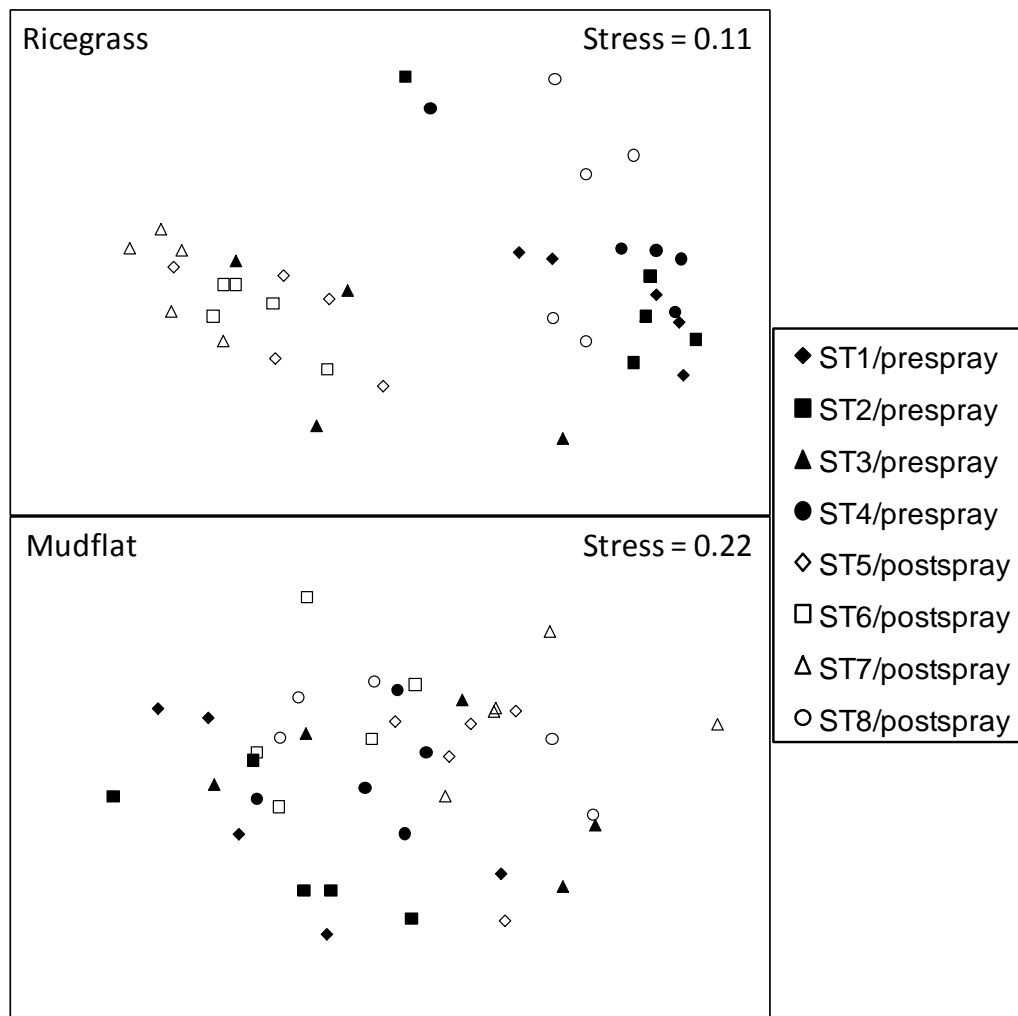


Figure 4.3. nMDS ordination showing separation of the different communities conducted on each habitat type individually. ST1-4 denote the first four sampling times prior to spray at 12, 9, 6 and 3 months pre-spray respectively. ST5-8 denote the four sampling times post spray at 3, 6, 9 and 12 months post-spray respectively. There were significant differences (1-way PERMANOVA for Rice grass: sampling time: $MS = 8891.08$, $F_{7,39} = 6.95$, $P = 0.002$; 1-way PERMANOVA for Mudflat $MS = 3458.01$, $F_{7,39} = 1.85$, $P = 0.008$).

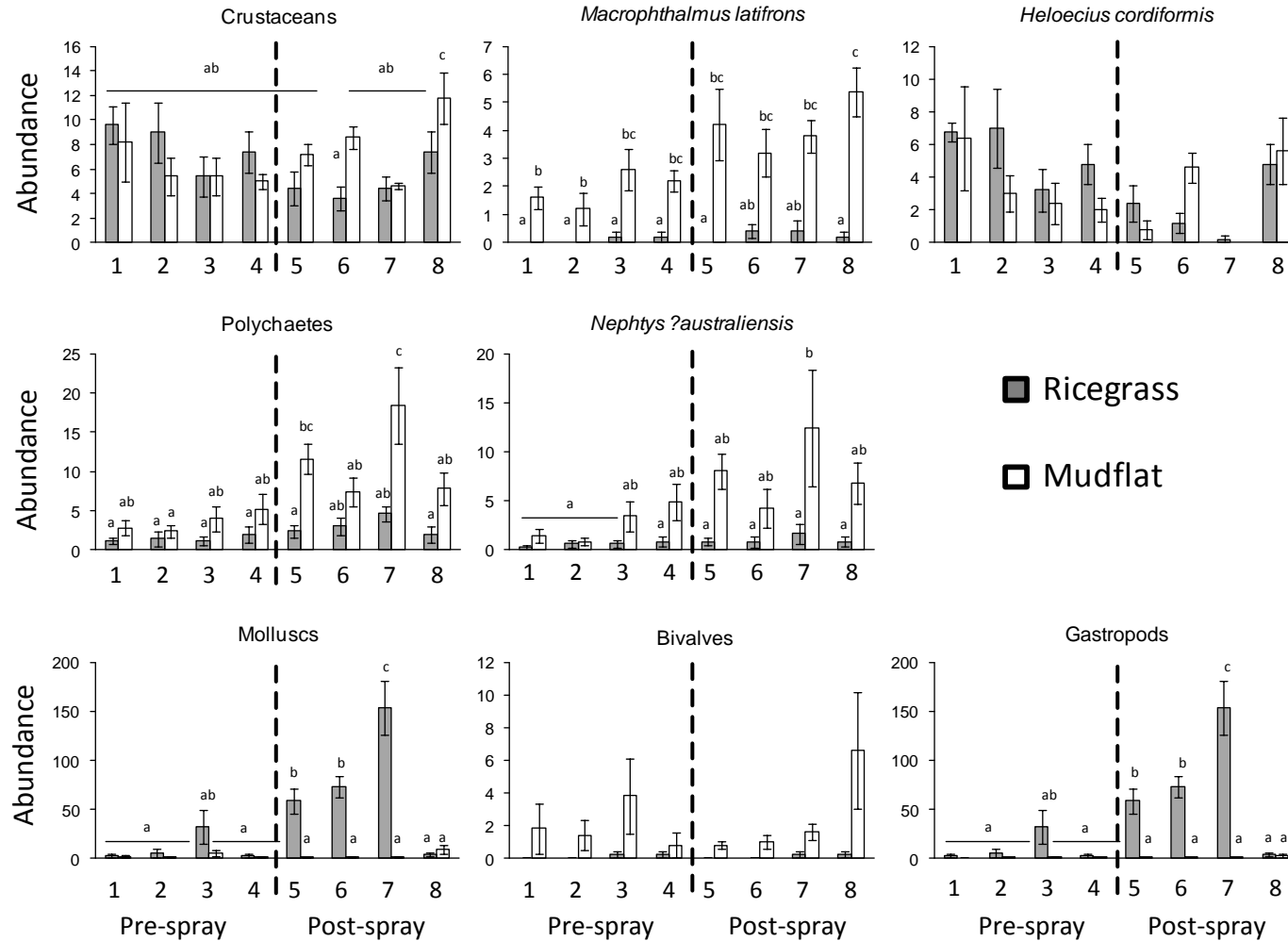


Figure 4.4. Relative abundance of common taxonomic groups and abundance taxa. The numbers (1-8) on the x-axis correspond to sampling time in 3 monthly intervals from 12 months pre-spray (1) to 12 months post-spray (8). The 4 sampling times pre-spray (1-4) are separated from the 4 post-spray sampling events (5-8) by the dashed vertical line on each graph. All values are means \pm S.E. for $n=5$ sample replicates. REGWQ groupings from ANOVA with significant sampling time*habitat interaction are positioned above the respective groups, where analogous letters denote the same REGWQ groups.

Table 4.1. Results of fixed effects ANOVA comparing among treatments for mean total abundances, species richness, Shannon-Wiener diversity and abundance of taxonomic groups and common taxa. Results are of overall ANOVAs comparing among treatments. Significant P values are shown in bold face ($P \leq 0.05$).

Variable	MS _{resid}	P values		
		Sampling time	Habitat	Sampling time*Habitat
Degrees of freedom	76	1	1	1
Total number of individuals	1300.70	3×10^{-4}	1.1×10^{-4}	0.019
Species richness	4.49	7.1×10^{-5}	0.271	0.378
Species diversity (H')	8.05	0.047	0.183	0.127
Total crustaceans	14.93	0.664	0.471	0.014
<i>Macrophthalmus latifrons</i>	1.50	1.16×10^{-5}	2.99×10^{-16}	1.8×10^{-4}
<i>Heloeccius cordiformis</i>	12.22	0.015	0.373	0.150
Total Polychaetes	19.72	3.93×10^{-5}	1.28×10^{-6}	5.44×10^{-3}
<i>Nephtys ?australiensis</i>	17.15	1.59×10^{-3}	7.58×10^{-6}	0.009
Total molluscs	1335.70	1.77×10^{-3}	1.03×10^{-5}	0.004
gastropods	1325.00	0.002	3.49×10^{-6}	0.003
bivalves	7.66	0.022	9.66×10^{-4}	0.282

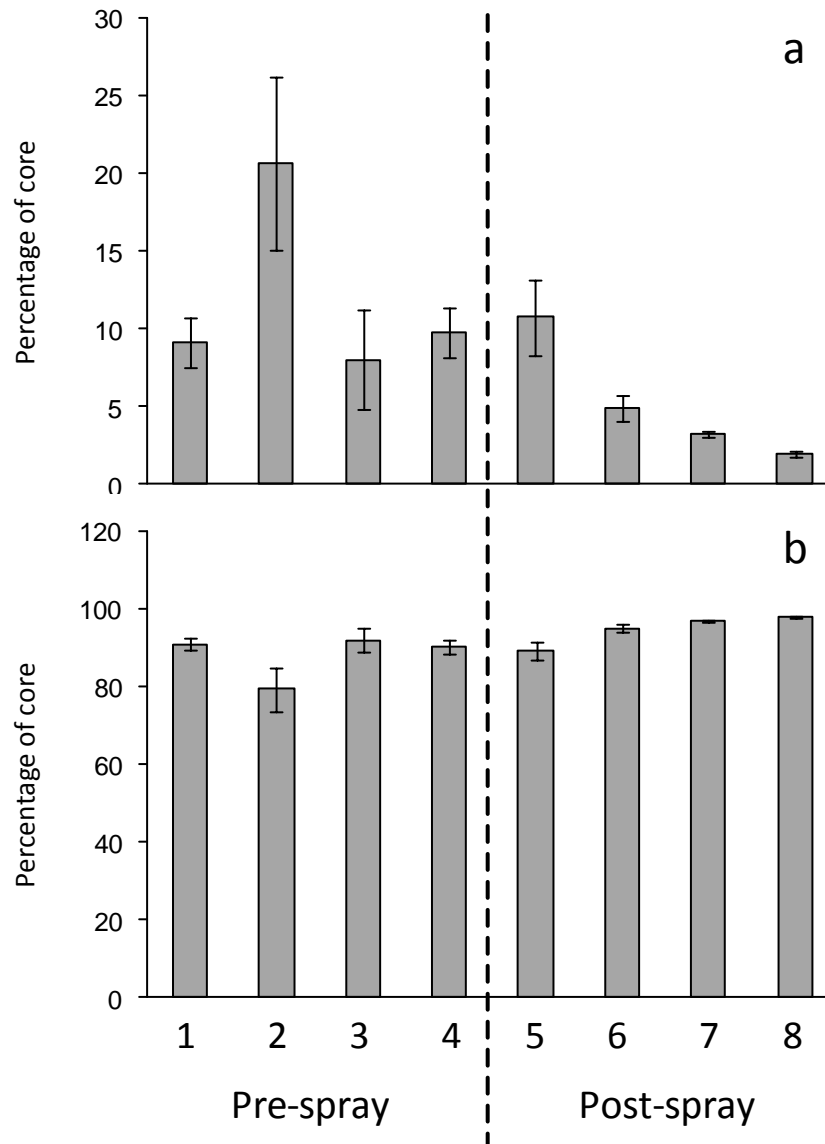


Figure 4.5. Relative percentages of emergent (a) and submergent (b) rice grass across all sampling times both pre- and post-spray. The numbers (1-8) on the x-axis correspond to sampling time in 3 monthly intervals from 12 months pre-spray (1) to 12 months post-spray (8). The 4 sampling times pre-spray (1-4) are separated from the 4 post-spray sampling events (5-8) by the dashed vertical line on each graph. There were significant differences over the sampling times in the percentages of both submergent and emergent rice grass (1-way PERMANOVA for emergent percentage: $MS=567.89$, $F_{1,34}=13.26$, $P>0.001$; 1-way PERMANOVA for submergent percentage: $MS=567.89$, $F_{1,34}=13.26$, $P>0.001$)

4.4 Discussion

It is clear from my research that there is a dramatic effect on the benthic macro-invertebrate community after spraying with Fusilade Forte®, although it appears that within 12 months of spraying the effects decline and benthic community structure starts to head back towards that generally associated with a mudflat habitat and this is likely to be a result of the emerged component of rice grass disappearing.

Rice grass communities at 3, 6 and 9 months post-spray exhibited lower abundances of crustaceans, and higher abundances of molluscs, particularly grazing gastropods, than rice grass communities prior to spraying. The abundances of gastropods and crustaceans for the rice grass communities at 12 months post-spray were different to those for 3, 6 and 9 month post-spray, but similar to mudflat communities 12 month post-spray.

Clearly, the anticipated response of rice grass habitat to spraying is that the biomass of rice grass would decline (from decay) as the plant died, resulting in an alteration to community structure and changes in the abundance of certain taxonomic groups; this was evident from my data. While there was a clear decline in the emergent rice grass following spraying, the proportion of submerged rice grass remained consistently high. This is not surprising as the anaerobic conditions that exist below the sediment-water interface (Saintilan 2009) within the rice grass meadows would reduce the capacity for the plant material to be broken down during the 12 months post-spraying.

While the sampling design used here does not show how soon after spraying the impact actually begins, it is evident that within 3 months post-spray, the community structure has undergone significant change and this change appears to be largely driven by an increase in the total abundance of molluscs; in particular grazing gastropods.

In contrast, Davies' (2001) concluded that there was "no detectable change in abundance or diversity of macro-invertebrates associated with the immediate effects of *Spartina* spraying over either *Spartina* or adjacent mudflats". His experimental design was similar to the one used here, i.e. a before/after control impact experimental design. However, Davies (2001) only sampled once post-spray (at day 8) and was investigating the effect of spraying with Fusilade®, not Fusilade Forte®.

In determining his experimental design Davies had conducted power analysis on sediment core-derived benthic macro-invertebrate data collected in Little Swanport estuary by Hedge (1998) and from this concluded that a total of 5 sites in each estuary would allow for reasonable power ($\beta \geq 0.8$) to detect changes of 30-50% or less in abundance and ca. 25% or less in family level diversity.

Thus, while Davies work provides only limited information due to its small temporal sampling range, it was based on a reliable dataset and extensive analysis. From this and data collected in my study, it is not unreasonable to conclude that after spraying with Fusilade Forte® it may take at least a number of weeks before a response is detected in the benthic macro-invertebrate community.

Palmer et al. (1995) and Hedge (1997) focused on key environmental issues associated with control of rice grass by Fusilade®, including the effect on macro-invertebrate community structure. Their work has been criticised for having insufficient replication for robust statistical conclusions, and treatments areas that were too small to avoid confounding of spray effects with spatio-temporal heterogeneity of macro-invertebrate distribution, and edge effects (Davies 2000). However, they still provide a useful comparison with this study.

The field studies by Palmer et al. (1995) indicated that mortality rates were too low to allow detection of a significant decrease in the abundance of any taxa (even when Fusilade® was applied at double the recommended rate). However, they did report an increase in the abundance of five species of dead mollusc shells, and of the remaining dead molluscs when considered collectively. They suggest that it appears that limited mortality (~10%) of fauna occurred following the treatment, but this was only detectable where dead animals could be identified.

Other work in Tasmania (Hedge 1997) involved monitoring of macro-invertebrate communities in rice grass meadows after spraying with Fusilade®. His results suggested that Fusilade® did not have a statistically significant effect on the abundance of molluscs or polychaete worms inhabiting *S. anglica* marsh. He did however, detect a significant difference in amphipod abundance between Fusilade® and control treatments, but it was difficult to determine whether this was due to the effects of Fusilade® or random sampling variation (Hedge 1997).

Hedge's results for molluscs were similar to those identified in this work, but less so for polychaetes. Hedge (1997) observed increased abundances in both molluscs and polychaetes after spraying, whereas the results of this study indicated an increase in mollusc abundance post-spray but no such change in polychaete abundances. The increase in molluscs, particularly gastropods is possibly as a result of the death of the rice grass and an increase in organic matter or epiphytic algae growing on the emerged structure that is being exploited as a food resource by the grazing gastropods.

The results of Palmer et al. (1995) and Hedge (1997) appear to conflict with the work by Davies (2001), as both of the former authors considered it probable that the mortality observed in the field occurred in the first few hours following spraying. They believed that this corresponded with when the macro-invertebrate faunal communities were exposed to the highest concentrations of Fusilade® during the low tide that was present while the rice grass was being sprayed. It should be noted however that Palmer et al. (1995) were working in seagrass meadows compared to rice grass meadows and so comparisons between my work and theirs must be treated with caution.

CHAPTER 5

GENERAL DISCUSSION

Most introduced non-indigenous species (NIS) result in some ecological change in the recipient environment and for marine and estuarine environment in many instances the introduction and range expansion of NIS are regarded as major threats to the integrity, diversity and health of natural ecosystems worldwide (Carlton 1989 and 1994, Ruiz et al. 2000, Crooks 2002, Ross et al. 2002, 2003b and Ruiz and Hines 2004).

The potential impact of *Spartina anglica* is well documented internationally having been the subject of considerable research over the last three decades (Chung 1990, Frid et al. 1999, DPIWE 2002, Sheehan 2008). In most instances it is generally accepted that in areas where *S. anglica* has been deliberately introduced, the negative impacts of this exotic intertidal grass have far outweighed any positive benefits.

Typically, *S. anglica* infestations form dense aggregations of culms, rhizomes and roots that promote sediment accretion, eventually leading to the formation of intertidal terraces or saltmarsh islands (Hedge and Kriwoken 2000). The vast majority of infestations have been found to occur in intertidal mudflat habitat, although it also occurs in native saltmarsh, seagrass, mangrove and intertidal seagrass habitats (Thompson 1991, Blood 1995, Hedge 1997).

Only a limited number of comprehensive studies have examined the ecological impact of *S. anglica* on estuaries, and many studies have concentrated on its distribution (Pringle 1975, 1988) or controlling its spread (Wells et al.1991, Bishop 1995 and Pritchard 1995).

In this thesis, I attempted to examine the impact that rice grass *S. anglica* is having across a number of spatial and temporal scales using a Before and After Controlled Impact (BACI) experimental design. This is, to the best of my knowledge, the first time in Australia that an assessment has been undertaken with this sort of long-term design on benthic macro-invertebrate communities.

I also set out to investigate potential short and long-term toxicological impacts of using the herbicide Fusilade Forte® to treat the rice grass and to examine the potential residence time that the chemical constituents of the herbicide persist in the estuarine environment following spraying. As a result of the die-back of the sprayed rice grass and the 12 month sampling period post spraying that I undertook, it was also possible indirectly to start to understand the ecological effect of how the benthic macro-invertebrate community respond following the removal of the rice grass.

The results of this study shows that benthic macro-invertebrate communities associated with *S. anglica* meadows are significantly different when compared to that of adjacent mudflats. The *S. anglica* meadows have higher species richness and total abundance of benthic macro-invertebrates. The rice grass communities were dominated by high abundances of grazing and predatory gastropods while

mudflat communities exhibited higher abundances of filter-feeding bivalves, and sedentary and errant polychaetes.

The most abundant and widespread species inhabiting mudflat were the polychaete *Nephtys ?australiensis* and the crab *Macrophthalmus latifrons*. Other sub-dominant species were the bivalves *Macomona deltoidalis*, *Laternula tasmanica* and *Mysella cf denaaformis*. In contrast the rice grass habitats are dominated by grazing gastropod like *Tatea rufilabris*, *Salinator cf solidus*, *Bembicium cf auratum* and *Hydroccus brazieri*. These results are similar to utilisation work by Baxter (2001) in the Tamar Estuary who found the gastropods *Tatea rufilabris* and *Bembicium melanostomum* to be the two most abundant species inhabiting the rice grass meadows.

The data from this study suggested there was little sign of seasonal variation in either habitat, which is quite different to results of research in the more-seasonal British Isles (Jackson et al. 1985, Long and Mason 1983) and North America (Luiting et al. 1997), where studies comparing the structure of benthic macro-invertebrate communities between mudflat and adjacent vegetated communities indicated strong seasonal changes. It is likely that freedom from frost in Tasmanian estuarine habitats is the most important factor contributing to these geographical differences.

When *S. anglica* invades a mudflat the subterranean habitat structure is significantly altered. The *Spartina* increases the habitat complexity (Lana and Guiss 1991, Flynn et al. 1996) and the plants may provide a refuge from abiotic

stresses and reduce predation pressure (Reise 1985) which promotes increased species abundance and richness.

In salt-marsh soils, particularly *Spartina* dominated areas where leaf litter is mostly removed from the surface by tides (Cifuentes 1991, Dame et al. 1991) below ground production is the largest source of organic matter. However the surface layer on the rice grass is likely to provide a habitat for epiphytic algal, which in turn generates a habitat more favourable to grazers like the several gastropod species commonly found in rice grass meadows. This type of response has been documented in other places where exotic grass species have invaded aquatic habitats (See Douglas et al. 2005 and Minchin 2008)

Other sediment chemistry data analyses showed there exists a significant difference between the two habitats, with rice grass containing higher percentages of very fine material (<63 μm) and higher redox values and the increased oxygenation has been shown to enhance faunal colonisation (Osenga and Coull 1983, Teal and Wieser 1996). Chung (1990) and Doody (1990) demonstrated that mudflat and estuarine productivity is actually increased after *S. anglica* invasion and Lana and Guiss (1991) showed that *S. alterniflora* detritus promotes the abundance of macro-invertebrates.

My data showed that some taxa, especially molluscs, prefer rice grass habitat and I suggest that this is because they are exploiting the food supply of epiphytic algal that is growing on the increased three-dimensional structure that is provided by the rice grass stems. It appears that this potential food resource

increases dramatically post-spraying as the rice grass is decaying although more work is needed to clearly show this.

The other differences I observed in the benthic macro-invertebrate assemblages between the habitat types may be attributed to a variety of factors. The higher species richness and species abundance of *S. anglica* meadows, relative to adjacent mudflats, may be attributed to increases in spatial heterogeneity, sediment oxygenation, food resources and the moderation of abiotic and biotic pressures, like desiccation and predation.

The results of the work examining the persistence of the chemical constituents of Fusilade Forte® show that both the parent material (fluazifop-P-butyl){FPB} and the degradates [fluazifop-P{FP}, 5-trifluoromethyl-2-pyridone and 2-(4-hydroxyphenoxy) propionic acid] all seem to decrease to below detectable limits from within the estuary rapidly after spraying, although the residence time was dependent upon the matrices being investigated.

None of the chemical degradates were ever detected in any sentinel Pacific oysters deployed throughout the estuary. In the water and sediment the primary breakdown product FB was the only degradate detectable and this could only be found for one and thirty days post spraying respectively. In the water column FB was detected right throughout the estuary on the first tidal flush but not again after day 1, while it was only ever detected in the sediment sampled immediately adjacent to the largest area of rice grass that had been treated with the Fusilade Forte®.

Results from the analysis of water, sediment and oysters suggest that when Fusilade Forte® is applied at the recommended rate for the control of rice grass, the active ingredient, fluazifop-P-butyl (ester), quickly degrades to fluazifop-P (acid) which is detectable on the first outgoing tide throughout the estuary. However within 24 hours, the acid has further degraded to very low levels that are below detectable limits or has been largely diluted by the large volume of incoming water that has entered the estuary of the next flood tide.

Spraying with the herbicide Fusilade Forte® is claimed to be an environmentally responsible and safe approach to controlling *S. anglica* infestations in Tasmania by the State Government. Given the high efficacy of using this chemical to treat the rice grass - predominantly 90% or better kill rates (DPIW 2006) and the associated impacts on other non-target species using alternate methods like burning, smothering or mechanical removal, the results of this study support the assumption that when applied at the recommended rate, Fusilade Forte® poses an acceptable environmental risk for the treatment of *S.anglica*.

While this study has been able to demonstrate what the impacts of spraying with Fusilade Forte® are on the area treated, it was unable to show to what extent the Fusilade Forte® actually impacts spatially on adjacent areas. Given the results clearly show there is a dramatic ecological shift within three months of treating the rice grass with the Fusilade Forte® it is recommended that additional work be undertaken to try and define the total spatial extent of spraying with the chemical, especially if large scale spraying events were planned as appears to be what the Port Sorell community wants to allow for the rice grass to be effectively treated within their estuary.

The results of my work however are obviously only based around the potential impacts of Fusilade Forte® on benthic macro-invertebrates and in particular primary consumers. Given the herbicide is recognised as having possible effects on a range of different species, it is recommended that additional work be conducted on algae and other faunal groups to determine the toxicological impacts of the herbicide across a range of trophic levels within estuarine ecosystems.

Regardless of the effect however, the results from my work show that the use the chemical Fusilade Forte® to treat *Spartina anglica* should be accepted as a cost effective, practical and environmentally sustainable management response to try and control this highly invasive non-indigenous species. While there are obvious short-term ecological impacts from the chemical, it appears to degrade rapidly and is not detectable in either the water, the sediment or bivalve shellfish after 3-4 weeks post spraying.

As with other major chemical or pollution events (e.g. oil spills or finfish aquaculture), while there is considerable impact following these events (See Kingston 1998, Crawford 2002, Macleod 2002) within a relatively short space of time, the benthic macro-invertebrate communities appears to recover and move towards a transitional community structure that is starting to resemble the macro-invertebrate assemblage that exist prior to the disturbance event. From my work it is apparent that the same trend is happening in that 12 months post-spraying the benthic macro-invertebrate community in the treated rice grass meadow starts to look like the community structure that exist in mudflat habitats.

In conclusion the results of this study should provide land managers in Australia (and possibly internationally) with renewed confidence that the use of chemical Fusilade Forte® to treat rice grass in temperate estuaries is an environmentally sustainable product where any short-term ecologically impacts are significantly outweighed by the long-term positive environmental outcome that will accompany the removal of rice grass from estuaries where it has invaded.

REFERENCES

Adam, P., Burchmore, J., Chrystal, J., Creighton, C., Downey, J., Geary, M., Hughes, P., Leadbitter, D., Llewellyn, L. and Patten, J. (1992). *Estuary management manual*. New South Wales Government.

Adams, J. B. and Bate, G. C. (1995). Ecological implications of tolerance of salinity and inundation by *Spartina maritima*. *Aquatic Botany*, **52**(3): 183-191.

Adams, P. (2002). Saltmarshes in a time of change. *Environmental Conservation*, **29**: 39-61.

Adams, P. (2009). In *Australian Saltmarsh Ecology*. Edited by Neil Saintilan. CSIRO Publishing.

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, **26**:32-46.

Baxter, K. (2001). *Utilisation of Rice Grass (Spartina anglica) by Invertebrate Community's within the Tamar Estuary, Tasmania*. Unpublished Honours Thesis, University of Tasmania.

Beard, J., Crawford, C. and Hirst, A. (2008). *Developing a monitoring program for six key estuaries in north-west Tasmania*, Tasmanian Aquaculture and Fisheries Institute, Hobart.

-
- Bishop, A. (1995). Assessing options for *Spartina* containment in Tasmania. *Proceedings of the Australasian Conference on Spartina control*. Ed J. A. E. Rash, R. C. Williamson and S. J. Taylor. Flora, Fauna and Fisheries Division, Department of Conservation and Natural Resources, Victoria, Australia.
- Blood, K. (1995). *Spartina* in Anderson Inlet. In: J. E. Rash, R. C. Williamson and S. J. Taylor (Eds) *Proceedings for the Australasian Conference on Spartina Control*, Victorian Government Publication, Melbourne, Australia.
- Boe, J. (2007). Below ground dynamics related to mudflat restoration strategies in a chemically and mechanically controlled *Spartina alterniflora* marsh in Willapa Bay, Washington, Unpublished Masters Thesis, Western Washington University.
- Bortolus, A. (2006). The austral cordgrass *Spartina densiflora* Brong: Its taxonomy, biogeography and natural history. *Journal of Biogeography*, **33**(1): 158-168.
- Boston, K. G. (1981). *The introduction of Spartina townsendii (s.l.) to Australia*. Department of Geography, Melbourne State College.
- Bridgewater, P (1995). Opening Address. In: *Proceedings of the Australasian Conference on Spartina Control* (Eds J. E. Rash, R. C. Williamson & S. J. Taylor) pp. 2-4. Victorian Government Publication, Melbourne.
-

Byers, J. E. and Noonburg, E. G. (2003). Scale dependent effects of biotic resistance to biological invasion. *Ecology*, **84**:1428-1433.

Carlton, J. T. (1989). Man's role in changing the face of the ocean: Biological invasions and implications for conservation of near-shore environments. *Conservation Biology*, **3**: 265-73.

Carlton, J. T. (1989). Biological invasions and biodiversity in the sea: The ecological and human impacts of nonindigenous marine and estuarine species. In: Nonindigenous Estuarine and Marine Organisms (NEMO) (eds R. H. Brown, D. J. Baker and D. K. Sullivan) pp 5-12. United States Department of Commerce, National Oceanic and Atmospheric Administration, Seattle.

Carr, G. W. (1993). *Exotic flora of Victoria and its impact of indigenous biota*. In: D. B. Foreman and N. G. Walsh (Editors), *Flora of Victoria*. Inkata Press, Melbourne.

Castel J., Labourg, P. J. Escaravage, V. Auby, I. and Garcie, M. E. (1989). Influence of seagrass beds and oyster parks on the abundance and biomass patterns of meio and macrobenthos in tidal flats. *Estuarine and Coastal Shelf Science*, **28**: 71-85.

Chemwatch (2005) *MSDS Fluazifop-P*.

Chung, C. (1983). Geographical distribution of *Spartina anglica* in China. *Bulletin of Marine Science*, **33(3)**: 753-758.

-
- Chung, C. (1990). Twenty-five years of introduced *Spartina anglica* in China. *Spartina anglica: a research review*. ITE research publication no. 2 pp 72-76. Edited by A. J. Gray and P. E. M. Benham, Natural Environment Research Council, Institute of Terrestrial Ecology, London.
- Cifuentes, L. A. (1991). Spatial and temporal variation in terrestrially derived organic matter from sediments on the Delaware Estuary. *Estuaries*, **14**: 414-429.
- Clarke, K. R. and Gorley, R. N. (2001). 'Primer v5. User Manual/Tutorial. Plymouth Routines in Multivariate Ecological Research.' (Primer-E Ltd., Plymouth Marine Laboratories: Plymouth, UK.).
- Crawford, C., Macleod, C. and Mitchell, I. (2002). *Evaluation of techniques for Environmental Monitoring of salmon farms in Tasmania*. Tasmanian Aquaculture and Fisheries Internal Report.
- Crooks, J. A. (1998). Habitat alteration and community-level effects of an exotic mussel, *Musculista senhousia*. *Marine Ecology Progress Series*, **162**:137-152
- Crooks, J. A. and Khim, H. S. (1999). Architectural vs. biological effects of a habitat-altering, exotic mussel, *Musculista senhousia*. *Journal of Experimental Marine Biology and Ecology*, **240**: 53-75.
- Crooks, J. A. (2002). Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. *Oikos*, **97**:153-166.
-

Dame, R. F., Spurrier, J. D., Williams, T. M., Kjerfve and others (1991). Annual material processing by a salt marsh-estuarine basin in South Caroline, USA. *Marine Ecology Progress Series*, **72**: 153-166.

Davies, P. E. (2000). *Use of Fusilade® to Control Rice Grass: Assessment of Environmental Risk*. Internal report to Marine Farming Branch, Department of Primary Industries, Water and Environment: Tasmania.

Davies, P. E. (2001). *Absence of short-term impacts of spraying with Fusilade® (Fluazifop-P) on benthic macroinvertebrates in Spartina and adjacent mudflat habitats in the Montagu River estuary, NW Tasmania*. Internal report to Marine Farming Branch, Department of Primary Industries, Water and Environment: Tasmania.

Doody, J.P. (1990). *Spartina – Friend or Foe? A conservation view point*. In A. J. Gray and P. E. M. Benham (Editors), *Spartina anglica: a research review*. ITE research publication. Natural Environment Research Council, Institute of Terrestrial Ecology, London.

Douglas, M. M., Bunn, S. E. and Davies, P. M. (2005). River and wetland food webs in Australia's wet-dry tropics: general principles and implications for management. *Marine and Freshwater Research*, **56**: 329-42.

DPIWE (2002). *Strategy for the Management of Rice Grass (Spartina anglica) in Tasmania, Australia*. Unpublished internal report.

DPIW (2003). *Spray protocols for the use of Fusilade® and Fusilade Forte®*, Unpublished internal report.

Draper, N. R. and Smith, H. (1981). *Applied Regression Analysis*, Volume 1 Wiley, New York.

Edgar, G. J., Barrett, N. S. and Graddon, D. J. (2000). A classification of Tasmanian Estuaries and Assessment of the Conservation Significance: an analysis using Ecological and Physical Attributes, Population and Land Use. *Tasmanian Aquaculture and Fisheries Institute Technical Series Report 2*.

Evans, P. R. (1986). Use of the herbicide ‘Dalapon’ for control of *Spartina* encroaching on intertidal mudflats: beneficial effects on shorebirds. *Journal of the Colonial Waterbird Society*, **9**: 171-5.

Flynn, M. N., Tararam, A. S. and Wakabara, Y. (1996). Effects of habitat complexity on the structure of macrobenthic association in a *Spartina alterniflora* marsh. *Revista Brasileira de Biologia*, **44** :9-21.

Frid, C. L. J., Chandrasekara, W. U. And Davey, P. (1999). The restoration of mud flats invaded by common cordgrass (*Spartina anglica*, CE Hubbard) using mechanical disturbance and its effects on the macro-benthic fauna. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **9**: 47-61.

Fusilade Forte® 128 EC Selective Herbicide Material Safety Data Sheet (2010). Syngenta Crop Protection Pty Limited.

-
- Gagnaire, B., Thomas-Guyon, H., Burgeot Th. and Renault, T. (2006). Pollutant effects on Pacific oyster, *Crassostrea gigas* (Thunberg), hemocytes: Screening of 23 molecules using flow cytometry. *Cell Biology and Toxicology* **22(1)**: pp. 1-14.
- Gibbs, H. and Phillips, H. (1995). Statement from the Royal Australasian Ornithologists Union. In: *Proceedings of the Australasian Conference on Spartina Control* (Eds J. E. Rash, R. C. Williamson and S. J. Taylor) pp. 72. Victorian Government Publication, Melbourne.
- Gray, A. J., Marshall, D. F. and Raybould, A. F. (1991). A Century of evolution in *Spartina anglica*. *Advances in Ecological Research*, **32(1)**: 63-72.
- Grevstad, F. S. (2005). Simulating control strategies for a spatially structured weed invasion: *Spartina alterniflora* (Loisel) in Pacific Coast estuaries. *Biological Invasions*, **7(4)**: 665-677.
- Griffin, D. J. G. (1971). The ecological distribution of grapsid and ocypodid shore crabs (Crustacea: Brachyura) in Tasmania. *Journal of Animal Ecology*, **40**: 597-621.
- Goss-Custard, J. D. and Moser, M. E. (1990). Changes in the numbers of dunlin (*Calidris alpina*) in British estuaries in relations to changes in abundances of *Spartina*. In: *Spartina anglica: a Research Review* (eds A. J. Gray and P. E. M. Benham) pp 69-71. ITE Research Publication no. 2, London.
-

-
- Guenegou, M., Levasseur, J. E., Bonnot-Curtis, C., Lafond, L. R. and Le Rhun, J. (1991). The geomorphological and botanical changes in Kernic Bay (Brittany, France): Influence on coastal management. *Journal of Coastal Research*, **7**(2): 311-339.
- Hedge, P. (1997). *Ecology, Impact and Control of Rice Grass in Little Swanport Estuary*. Department of Primary Industries and Water, Hobart.
- Hedge, P. (1998). *Ecology, Impact and Control of Rice Grass (Spartina anglica) in Little Swanport Estuary*, Tasmania. Department of Primary Industries, Water and Environment: Tasmania.
- Hedge, P. T., Summers, D., Dittmann, L. and Davies, P. (1999). Toxicological Effects of Fusilade on Pacific Oysters, *Crassostrea gigas*. *Department of Primary Industries, Water and Environment, Tasmania, Australia*.
- Hedge, P. and Kriwoken, L. K. (2000). Evidence for effects of *Spartina anglica* invasion on benthic macrofauna in Little Swanport estuary, Tasmania. *Austral Ecology*, 25: 150-159.
- Hodgkin, E. P., (1994). Estuaries and coastal lagoons. In Hammond, L.S. & Synnot, R.N. (Eds) *Marine Biology*, Longman Cheshire Pty. Ltd.
- Horvath, T. G., Martin, K. M. and Lamberti, G. A. (1999). Effect of Zebra Mussels, *Dreissena polymorpha*, on Macro-invertebrates in a Lake-outlet Stream. *American Midland Naturalist*, **142**:340-347.
-

-
- Hsiao, C., Jacobs, S. W. L., Chaterton, N. J. and Saya, K. H. (1999). A molecular phylogeny of grass family (Poaceae) based on the sequences of nuclear ribosomal DNA (ITS). *Australian Systematic Botany*, **11**: 667-688.
- Humburg, N. E. and Colby, S. R. (1989). In: *Weed Science Society of America. Herbicide Handbook. Degradation of Fluazifop-p (acid)*. 6th Ed.
- ICI internal memo (1982 and 1985). *Fluazifop-butyl, Acute toxicity to larvae of the Pacific Oyster, Crassostrea gigas*.
- Inglis, G. L. (1995). Intertidal muddy shores. In: *Coastal Marine Ecology of Temperate Australia* (Eds A. J. Underwood and M. G. Chapman) pp 171-86. University of New South Wales, Press, Sydney.
- Jackson, D., Mason, C. F. And Long, S. P. (1985). Macro-invertebrate populations and production on a salt-marsh in east England dominated by *Spartina anglica*. *Oecologia (Berlin)*, **56**: 406-411.
- Johnson, C. R. (2007). Seaweed Invasions: conclusions and future directions. *Botanica Marina*, **50**:451 – 457.
- Josefson, A. B. (1998). Resource limitation in marine soft sediments - Differential effects of food and space in the association between the brittle-star *Amphiura filiformis* and the bivalve *Mysella bidentata*? *Hydrobiologia*, **375**:1-3.
-

Kersten, M. and Smedes, F. (2002). Normalization procedures for sediment contaminants in spatial and temporal trend monitoring. *Journal of Environmental Monitoring*, **4**: 109-115.

Kingston, T. (1998). *Impact of the Iron Baron oil spill on flora and fauna within the intertidal region of rocky shores*. Iron Baron Oil Spill 1995, Long Term Environmental Impact and Recovery report.

Krasnicki, T. J. (2002). Environmental Water Requirements for the Rubicon River. *Department of Primary Industries, Water and Environment, Hobart Technical Report No. WRA 02/01*.

Lana, P. C. and Guiss, C. (1991). Influence of *Spartina alterniflora* on structure and temporal variability of macrobenthic associations in a tidal flat of Paranagua Bay (southeastern Brazil). *Marine Ecology Progress Series*, **73**: 231-44.

Lambert, S. J. (2005). *Sediment remobilisation following Spartina control*. Unpublished Masters Thesis, University of Waikato, Hamilton.

Lee, R. W. (2003). Physiological adaptations of the invasive cordgrass *Spartina anglica* to reducing sediments: rhizome metabolic gas fluxes and enhanced O₂ and H₂S transport. *Marine Biology*, **143**(1): 9-15.

Lenihan, H. S. (1999). Physical-biological coupling on oyster reefs: How habitat structure influences individual performance. *Ecological Monographs*, **69**:251-275.

Long, S. P. and Mason, C. F. (1983). *Saltmarsh ecology*. Blackie and Sons, New York.

Luiting, V. T., Cordell, J. R., Olson, A. M. and Simenstad, C. A. (1997). *Does exotic Spartina alterniflora change benthic invertebrate assemblages?* In: K. Pattern (ed) *Proceedings of the Second International Spartina Conference*. Washington State University, Olympia, Washington.

Macleod, C. K., Mitchell, I. M., Crawford, C. M. and Connell R. (2002). *Evaluation of Sediment Recovery after removal of Finfish Cages from Marine Farm Lease No. 76 (Gunpowder Jetty) North West Bay*. Tasmanian Aquaculture and Fisheries Institute Technical Report No 13.

McArdle, B. H. and Anderson, M. J. (2001). Fitting multivariate models to community data: A comment on distance-based redundancy analysis. *Ecology*, **82**: 290-297.

Minchin, D. (2008). *Spartina anglica* Delivering Alien Invasive Species Inventories for Europe.

Osenga, G. A. and Coull, B. C. (1983). *Spartina alterniflora* (Loisel) root structure and meiofaunal abundance *Journal of Experimental Marine Biology and Ecology*, **67**: 221-5.

Palmer, D., Parry, G., Greenshields, P., Crookes, D. and Lockett, M. (1995). *Toxicity of Fusilade to Near-Shore Marine Fauna*. In: J. E. Rash, R. C.

Williamson and S. J. Taylor (Eds), Proceedings of Australasian Conference on Spartina Control, Victorian Government Publication, Australia.

Parker, I. M., Simberloff, D., Lonsdale, W. M., Goodell, K., Wonham, M., Kareiva, P. M., Williamson, M. H., Von Holle, B., Moyle, P. B., Byers, J. E. and Goldwasser, L. (1999). Impact: toward a framework for understanding the ecological effects of invaders. *Biological Invasions*, **1**:3-19.

Partridge, T. R. (1987). *Spartina* in New Zealand. *New Zealand Journal of Botany*, **25**: 567-575.

Patten, K. and O'Casey, C. (2007). Use of Willapa Bay, Washington, by shorebirds and waterfowl after *Spartina* control efforts. *Journal of Field Ornithology*, **78**(4): 395-400.

Pierce, S. M., (1982). What is *Spartina* doing in our estuaries? *South African Journal of Science*, **76**(6): 229-230.

Posey, M. H., Wigand C., and Stevenson, J. C. (1993). Effects of an introduced aquatic plant, *Hydrilla verticillata*, on benthic communities in the upper Chesapeake Bay. *Estuarine, Coastal and Shelf Science*. **37**:539-555.

Pringle, A. W. (1975). The establishment of *Spartina* in the Tamar estuary. *Papers and Proceedings of the Royal Society of Tasmania*, **109**: 65-75

Pringle, A. W. (1988). Condition and distribution of *Spartina anglica* (rice grass) in selected estuaries and inlets along the Bass Strait coast, Tasmania. Unpublished report for Department of Lands, Parks and Wildlife, Tasmania.

Pringle, A. W. (1993). *Spartina anglica* colonisation and physical effects in the Tamar Estuary, Tasmania 1971-91. *Papers and Proceedings of the Royal Society of Tasmania*, **127**: 1-10.

R Version 2.13.0 (2011-04-13) Copyright (C) (2011). The R Foundation for Statistical Computing.

Raffaelli, D. and Hawkins, S. (1996) *Intertidal Ecology*, Chapman & Hall, London.

Reeder, T. G. and Hacker, S. D. (2004). Factors contributing to the removal of a marine grass invader (*Spartina anglica*) and subsequent potential for habitat restoration. *Estuaries*, **27**(2): 244-252.

Reid, A. P. (2010). Impacts of *Maoricolpus roseus* on native assemblages. Unpublished PhD thesis, University of Tasmania.

Reise, K. (1985). *Tidal Flat Ecology: an Experimental Approach to Species Interactions*. Springer-Verlag, Heidelberg.

RGAG (2002). *Strategy for the Management of Rice Grass (Spartina anglica) in Tasmania, Australia*. Department of Primary Industries, Water and Environment: Tasmania.

Ross, D. J., Johnson, C. R. and Hewitt, C. L. (2002). Impact of introduced seastars *Asterias amurensis* on survivorship of juvenile commercial bivalves *Fulvia tenuicostata*. *Marine Ecology Progress Series*, **241**: 99-112.

Ross, D. J., Johnson, C. R. and Hewitt, C. L. (2003a). Assessing the ecological impacts of an introduced seastar: the importance of multiple methods. *Biological Invasions*, **5**:3-21.

Ross, D. J., Johnson, C. R. and Hewitt, C. L. (2003b). Variability in the impact of an introduced predator (*Asterias amurensis*: Asteroidea) on soft-sediment assemblages. *Journal of Experimental Marine Biology and Ecology*, **288**:257-278.

Ruiz, G. M., Fofonoff, P. W., Carlton, J. T. Wonham, M. J. and Hines, A. H. (2000). Invasion of coastal marine communities in North America: Apparent patterns, processes, and biases. *Annual Review of Ecology and Systematics*, **31**: 481-531.

Saintilan, N. (2009). *Australian Saltmarsh Ecology*, CSIRO Publishing, Collingwood, Melbourne, 225 pp.

Shaw, W. B. and Gosling, D. S. (1995). *Spartina* control in New Zealand – an overview. In: J. E. Rash, R. C. Williamson and S. J. Taylor (Editors). *Proceedings of the Australasian conference on Spartina control*. Victorian Government Publication, Melbourne, Australia.

Sheehan, M. R. (2008). Large-scale eradication of *S. anglica*, Tamar Estuary, Tasmania. Unpublished PhD thesis, University of Tasmania.

Simpson, J. (1995). Wading birds of Anderson Inlet and the work of the Victorian Wader Study Group. In: *Proceedings of the Australasian Conference on Spartina Control* (Eds J. E. Rash, R.C. Williamson & S. J. Taylor) pp. 67. Victorian Government Publication, Melbourne.

Stewart, T. W. and Haynes, J. M. (1994). Benthic macro-invertebrate communities of southwestern Lake Ontario following invasion of *Dreissena*. *Journal of Great Lakes Research*, **20**:479-493.

Strong, D.R. and Ayres, D. (2009). *Spartina* introductions and consequences in salt marshes: Arrive, survive, thrive and sometimes hybridize. In *Anthropogenic Modification of North American Salt Marshes*. Eds. Silliman, B. R., Bertness, M. D., and E. D. Grosholz.

Taylor, C. M. and Hastings, A. (2004). Finding optimal control strategies for invasive species: A density-structured model for *Spartina alterniflora*. *Journal of Applied Ecology*, **41**(6): 1049-1057.

Teal, J. M. and Wieser, W. (1966). The distribution and ecology of nematodes in a Georgia saltmarsh. *Limnology and Oceanography*, **11**: 217-22.

The Nature Conservancy (2001). *Weed Control Methods Handbook*.

The Royal Society of Chemistry (1991). *The Agrochemicals Handbook*. The Royal Society of Chemistry, Cambridge, England.

Thompson, R. S. and Comber, M. H. I. (1982). *Fluazifop-Butyl: Acute toxicity to larve of Pacific Oyster (Crassostrea gigas)*, ICI Brixham Laboratory Report No. BL/B/2152.

Thompson, J. D., (1991). The Biology of an invasive plant: What makes *Spartina anglica* so successful. *Bioscience*, **41** (6): 393-401.

Valiela, I. (2006). *Global Coastal Changes*. Blackwell Publishing: Carlton.

Vitousek, P. M. (1990). Biological invasions and ecosystem processes: Towards an integration of population biology and ecosystem studies. *Oikos*, **57**: 7-13.

Wang, H., Huang, S., He, C., Fan, H. and Sheng, L. (2006). Invasive species in wetland ecosystems, China. *Wetland Science*, **4**(1): 7-12.

Wells, A., Melville, W., Harris, S. and Bayly-Stark, J. (1991). Herbicide trials for the control of *Spartina anglica* (rice grass) at Port Sorell, Tasmania, Internal unpublished report, Department of Parks, Wildlife and Heritage, Tasmania.

-
- Williamson, R. C., (1995). *Spartina in Victoria – an overview* In: J. E. Rash, R. C. Williamson and S. J. Taylor (Editors). *Proceedings of the Australasian conference on Spartina control*. Victorian Government Publication, Melbourne, Australia, pp 26-29.
- Wonham, M. J., O'Connor, M. and Harley, C. D. G. (2005). Positive effects of a dominant invader on introduced and native mudflat species. *Marine Ecology Progress Series*, **289**: 109-116.
- Wu, M., Hacker, S., Ayers, D. and Strong, D. R. (1999). Potential of *Prokelisia* spp. as biological control agents of English cordgrass, *Spartina anglica*. *Biological Control*, **16(3)**: 267-273.
- Xie, Y., Li, Z. Y. W., Gregg, P. and Dianmo, L., (2001). Invasive species in China – an overview. *Biodiversity and Conservation*, **10**: 1317-1341.
- Zhang, R. S. et al. (2004). Formation of *Spartina alterniflora* salt marshes on the coast of Jiangsu Province, China. *Ecological Engineering*, **23**: 95-105.