Optimising the management of invasive aquatic plants targeted for extirpation from catchments and waterways: utilising alligator weed (Alternanthera philoxeroides (Mart.) Griseb.) as a target species

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Abstract
Aquatic plants are integral components of freshwater ecosystems and provide essential ecosystem services. However, when invasive species establish in new aquatic environments, there are few natural checks and balances to inhibit their growth and spread. Overabundant aquatic vegetation can harm aquatic systems if left unchecked and negatively impact on agricultural productivity, social amenity and biodiversity values. Prevention and early intervention are recognised as the most cost effective means to manage invasive species that pose a biosecurity risk.

This thesis contributes to the development of effective management strategies for one of the world’s most invasive aquatic plant species, known as alligator weed (*Alternanthera philoxeroides* (Mart.) Griseb.). It focusses on developing management strategies in an early stage of invasion, in order to achieve extirpation of this species from catchments and waterways. Developing effective detection and surveillance strategies are required for invasive aquatic plants, as a key impediment to achieving extirpation is the ability to detect infestations, so that control strategies can be enacted. This thesis investigates the effectiveness of aerial surveillance for detection of alligator weed at different spatial scales, using high altitude aerial imagery (orthophotos) and unmanned aerial vehicle (UAV) technology. An examination of the growth rate of alligator weed in Victoria, Australia, over a five year period, demonstrates the effective use of orthophotos to detect and monitor large infestations of aquatic alligator weed. The efficacy of unmanned aerial vehicle technology, including the use of automated algorithms, to detect patches of alligator weed growing in waterways is evaluated against current detection techniques.

Effective management of invasive aquatic plants targeted for extirpation requires the coupling of effective detection and control efforts to prevent reproduction. To date, development of control strategies for aquatic alligator weed has been limited to evaluating the efficacy of short-term control at a local scale without regard to the effects of management strategies on dispersal of propagules throughout catchments. This thesis determines that viable alligator weed stem fragments are produced following herbicide application, which comprises extirpation efforts. This thesis has gone further than current practice in that it has evaluated the efficacy of current and novel control techniques, in both laboratory and field trials and has developed methods to manage viable fragment production post-herbicide application, to limit dispersal throughout catchments. In this respect, the application of the herbicides glyphosate, metsulfuron-methyl and imazapyr, and their effectiveness when incorporating surfactant systems and plant growth regulators, have been evaluated in field
and laboratory studies to optimise control techniques for aquatic alligator weed. Results have shown that our approaches, when used in an early stage of invasion, are capable of eliminating patches of alligator weed in two to three years. Integral to the research is an experiment to determine the effect of herbicide treatments on the production of alligator weed stem fragments and their subsequent viability. Further investigation to determine the usefulness of commercially available plant growth regulators (PGRs) to reduce the number of viable propagules produced by alligator weed post-herbicide application was found to be ineffective.

This thesis also evaluates the impact of herbicides and surfactant systems, on all key alligator weed response metrics in aquatic environments including; above ground biomass, below ground biomass and viable stem fragmentation. No previous studies have looked simultaneously at these three important measures for determining the efficacy of a particular control regime, and we have determined that this is essential for effective management of aquatic alligator weed in an early stage of invasion.

The thesis has underscored the notion that development of more effective management strategies, based upon experimental trials, will result in an increased likelihood of eradicating invasive aquatic plants that pose a biosecurity risk, and thus move toward the mitigation of the threat that high-risk species pose to aquatic ecosystems.
Declaration

This is to certify that:

i) the thesis comprises only my original work towards the PhD except where indicted in the preface,

ii) due acknowledgement has been made in the text to all other material used,

iii) the thesis is less than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Signed: 

Date: 3/01/2017
Preface

Chapters of thesis published in refereed journals:

Chapter 2 – Clements D, Dugdale TM, Hunt TD (2011) Growth of aquatic alligator weed (Alternanthera philoxeroides) over 5 years in south-east Australia. Aquatic Invasions 6(1): 77-82


Chapter 7 - Clements D, Dugdale TM, Butler KL, Florentine SK, Sillitoe J (Accepted) Herbicide efficacy for aquatic Alternanthera philoxeroides management in an early stage of invasion: integrating above-ground biomass, below-ground biomass and viable stem fragmentation. Weed Research

* Contribution (%) by D. Clements to each published paper.

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My supervisors are; S. Florentine (Associate Professor - Federation University Australia), J. Sillitoe (Associate Professor - Federation University Australia), D. McLaren (Principal Research Scientist - Department of Economic Development, Jobs, Transport and Resources - Agriculture Victoria; Research Fellow - La Trobe University), T.M. Dugdale (Senior Research Scientist - Department of Economic Development, Jobs, Transport and Resources - Agriculture Victoria).

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Figure 1. Generalised invasion curve showing recommended actions appropriate to each stage of invasion (Source: Victorian Government 2015). This thesis focuses on developing effective management strategies for alligator weed (*Alternanthera philoxeroides*) at the ‘ERADICATION’ level of management, to achieve extirpation from catchments and waterways ..........................................................4

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Chapter 1 – Introduction

Management of invasive aquatic plants targeted for extirpation from catchments and waterways: An introduction to eradication, detection, control and dispersal of invasive aquatic plants
1.1 Overview
The availability of high quality freshwater resources is a necessity to sustaining life. Aquatic plants are integral components of freshwater ecosystems and provide essential ecosystem services. However, when invasive species establish in new environments there are few natural checks and balances to inhibit their growth and spread. Overabundant aquatic vegetation can harm aquatic systems if left unchecked, where they can degrade water quality, slow water velocity, exacerbate siltation or flooding, and reduce both flora and fauna species diversity and abundance. Dense infestations impact on recreation, navigation and hydroelectric generation, exacerbate the spread of insect borne diseases, and compromise agricultural productivity by impeding water delivery (Madsen 2005; Dugdale et al. 2013).

Prevention and early intervention are recognised as the most cost effective means to manage invasive species that pose a biosecurity risk (Panetta 2009). The past decade and a half has been an active period for researchers studying the factors that determine the feasibility of weed eradication and evaluating eradication program success (Rejmánek and Pitcairn 2002; Panetta 2009; Gardener et al. 2010; Howell 2012; Pluess et al. 2012; Dodd et al. 2015; Panetta 2015; Panetta 2016). However, research is required to develop and evaluate detection and control techniques for species in an early stage of invasion, particularly for high risk invasive aquatic plants. Ultimately, it is the effectiveness of the detection and control techniques utilised against an individual species that will determine the feasibility of eradication.

This research project aims to develop effective management strategies for one of the world’s most invasive aquatic plant species, alligator weed (*Alternanthera philoxeroides* (Mart.) Griseb.) in an early stage of invasion, in order to achieve extirpation from catchments and waterways. Developing effective detection and surveillance strategies are required, as a key impediment to extirpation of alligator weed in aquatic environments is the ability to detect infestations so that control strategies can be enacted. The effectiveness of aerial surveillance for detection of alligator weed at different spatial scales is elucidated. The use of high altitude aerial imagery (orthophotos) and unmanned aerial vehicle (UAV) technology to detect alligator weed in an early stage of invasion of catchments and waterways is evaluated.

Development of effective control strategies for aquatic alligator weed are required as current research has been limited to evaluating the efficacy of short-term control at a local scale without regard to the effects of management strategies on dispersal of propagules throughout catchments (Dugdale and Champion 2012), limiting extirpation attempts. This research project evaluates the efficacy of current and novel control techniques in both
laboratory and field trials, and develops methods to manage viable fragment production post-herbicide application to limit dispersal throughout catchments. The application of the herbicides glyphosate, metsulfuron-methyl and imazapyr, and the effectiveness of incorporating surfactant systems and plant growth regulators, are evaluated in field and laboratory studies to optimise techniques for control of aquatic alligator weed.

Development of more effective management strategies will result in an increased likelihood of eradicating invasive aquatic plants that pose a biosecurity risk and reduce the impacts on agricultural productivity, social amenity and biodiversity values. Management techniques and principles developed for alligator weed in this thesis provide a model for programs that aim to optimise the management of invasive aquatic plants targeted for extirpation from catchments and waterways. The effective management of invasive aquatic plants is necessary to contribute to the sustainable management of freshwater as a valuable resource which prosperous societies are increasingly dependent upon.

1.2 Biosecurity and invasive species
Since the Age of Exploration in the early 15th century and as the volume of global trade has increased, the introduction of species into new environments has been accelerating (Panetta and Lawes 2005; Hulme 2009; Dodd et al. 2015). When invasive species establish in new environments there are few natural checks and balances to inhibit their growth and spread, and consequently they negatively impact on the ecological integrity of their new environment (Mack et al. 2000). As a result of human activity, introductions of invasive species including weeds, pests and diseases, threaten environmental, social and economic resources worldwide, and these alien species represent a significant management problem to economies and natural environments throughout the world (Vitousek et al. 1996; Mack et al. 2000; Pimentel et al. 2005; Dodd et al. 2015).

Governments are justified, in terms of economic efficiency and providing collective or public good, to protect local environments and social amenity from the negative effects associated with invasive species. A biosecurity approach is commonly undertaken to manage invasive species, involving pre-border preparedness, border protection and post-border management and control. The efficiency argument states that a society’s income and benefit will be maximised by excluding unwanted pests and, if exclusion fails, eliminating the pest on a one-time basis is preferable to long-term control strategies (Dahlsten and Garcia 1989). However, as invasive species incursions increase, the cost of managing those incursions increase exponentially. Figure 1 depicts the recommended actions to implement (prevention,
eradication, containment and asset based protection) for an invasive species, based on the stage of the invasion process. Below the ‘invasion curve’ are the indicative economic returns of management at each stage of invasion, showing declining cost-effectiveness at latter stage of invasion and the benefits of prevention and eradication (Victorian Government 2015). The research presented in this thesis focuses on developing effective management strategies for one of the world’s most invasive aquatic plant species, alligator weed (*Alternanthera philoxeroides*), in an early stage of invasion (‘eradication’ level of management), to achieve extirpation from catchments and waterways.

![Generalised invasion curve showing recommended actions appropriate to each stage of invasion](Source: Victorian Government 2015). This thesis focuses on developing effective management strategies for alligator weed (*Alternanthera philoxeroides*) at the ‘ERADICATION’ level of management, to achieve extirpation from catchments and waterways.

### 1.3 Invasive plants and eradication theory

#### 1.3.1 Invasive plants

Invasive plants, also known as weeds, are plants that require intervention to reduce their impact on environmental, economic, human health or social amenity values. Weeds pose a serious threat to natural environments and primary production worldwide and can have significant impacts on social wellbeing. Weeds displace native species, contribute significantly to land degradation, reduce agricultural productivity and compromise ecosystem functioning. In Australia, weeds have major economic, environmental and social impacts, causing damage to natural landscapes, agricultural lands, waterways and coastal areas (Humphries et al. 1991; Sinden et al. 2005; Australian Government 2014). It has been
estimated that Australia has more than 1900 weedy species that have entered as either accidental contaminants (30%), deliberate introductions via the ornamental plant trade (53%) or for food and produce (17%) (Virtue 2004). Economic statistics show that the impact of weeds on the Australian agricultural sector is significant. The economic loss caused by weeds in Australia was estimated at ca. AU$4 billion per annum in 2010, which was close to 10% of the total gross value of agricultural production (AU$45.9 billion per annum) across a broad range of primary industries. This cost of weeds to the agriculture sector was comparable to the economic production values for each of the largest individual agricultural sectors, including dairy, beef and wheat production (Forster et al. 2013).

The cost of weeds to biodiversity and the natural environment are significant. Weeds are recognised as one of the five most serious factors causing loss of global biodiversity (Vitousek et al. 1996). Of particular concern here is that whilst in Australia it is recognised that weeds degrade many natural ecosystems and threaten nearly all biological communities, quantification of their impact is limited (Adair and Groves 1998).

The Australian Government, including the States and Territories, use Weed Risk Assessment (WRA) systems that determine a species potential risk to Australia based on a species; (1) invasiveness, (2) its impact on social, environmental and agricultural values and (3) its current and potential distribution (Pheloung 2001; Weiss and Iaconis 2002). These decision systems allow governments to direct resources to biosecurity programs, using a ranking system, to determine which exotic species should be considered for management and what level of management should implemented.

1.3.2 Eradication theory
A biosecurity approach is commonly undertaken by governments to manage invasive species, particularly in relation to weeds, where prevention and early intervention (eradication) are recognised as the most cost effective means of management (Humphries et al. 1991; Harris et al. 2001; Wittenberg and Cock 2001; Panetta 2009). Justification of ‘prevention’ and ‘eradication’ programs by governments, rather than through private sector effort, is based on the reasoning that the free market will under-allocate resources to management efforts resulting in an increased likelihood of failure to reach targeted outcomes (Dahlsten and Garcia 1989).

One aspect of the biosecurity approach for weed management, is a goal to eradicate a species from an area in which it has become naturalised, provided it meets certain criteria: (1) it is deemed a species capable of invasion (i.e. it spreads into areas considerable distances
away from parent plants (Richardson et al. 2000)); (2) it is in an early stage of invasion and occupies only a very small part of its potential range; and (3) it poses a significant threat to social, economic or environmental values (Australian Government 2014; Clements et al. 2014a). Eradication can be defined as the elimination of every individual of a species from a geographic area that is sufficiently isolated to prevent reinvasion (Newsom 1978; Myers et al. 1998). However, the definition of eradication is uncertain and variable. That is, both the size of the eradication zone and the period of time over which the population of the pest must be reduced to zero are variable, depending on the political and economic context of a particular eradication program (Dahlsten 1986; Dahlsten and Garcia 1989). Note that extirpation is not synonymous with eradication, as eradication refers to efforts being undertaken on the largest relevant scale, including the prevention or re-invasion (Panetta 2015). For example, extirpation may be feasible for a target aquatic weed species at an individual catchment or waterway scale, however eradication of the target species from a State or Territory may not be deemed feasible. Effective extirpation techniques are essential for achieving eradication, as an eradication program requires co-ordinated extirpation of all infestations.

The probability of eradication is highest, and eradication is achievable at lowest cost, when a weed invasion is detected at a stage when the species is neither abundant nor widely distributed (Panetta 2009). Determining the efficacy of eradication is dependent on: (1) the ‘delimitation criterion’ being the requirement to detect the full extent of an incursion initially and until extirpation occurs over the entire infested area (Panetta 2009; Panetta and Lawes 2005); (2) the ‘extinction criterion’, being the rate of decrease in population numbers of a given species, including seeds and other propagules; (3) the ‘containment criterion’ being the extent to which an eradication program prevents the spread of the target species (Zamora et al. 1989; Panetta and Lawes 2005). It is expected that weed populations will decline at rates according to the biology of the species being targeted (e.g. reproduction time and propagule longevity) and how control tactics interact with the species biology (Panetta and Timmins 2004). If a control regime is not effective in preventing reproduction, new infestations may be created through the dispersal of propagules to other areas, limiting eradication efforts (Panetta and Lawes 2005). Eradication is unlikely to occur in any time period less than the propagule longevity of the target species (Dodd et al. 2015).

To achieve eradication, the effort (including investment) comprises the detection effort required to delimit a weed invasion plus the search and control effort required to prevent reproduction until extirpation occurs over the entire infested area (Panetta 2009). As weed density falls in an eradication program, costs of finding and killing each remaining weed
increases (Zamora et al. 1989). Eradicating the last 1% of a target population can cost more than destroying the first 99% (Myers et al. 1998; Simberloff 2003). The resources required to achieve eradication are far greater than that required for conventional ongoing control and management (Dodd et al. 2015), however over the longer term a society's income will be maximised by eliminating the pest (eradication) compared to employing long-term control strategies (Dahlsten and Garcia 1989).

Factors that influence the feasibility of eradicating invasive plant species can be grouped broadly into ‘organisational’ or ‘site/species’ factors. Organisational factors can be controlled by the management agency. It is recognised that eradication feasibility is influenced not only by biological factors related to a species, but also sociopolitical, economic and operational factors (Panetta et al. 2011; Dodd et al. 2015). Site/species factors are usually beyond the control of the management agency and have been shown to influence extirpation success, including biological factors. Factors including previous eradication success of a species, detectability (annual period of detectability and species search distance) and monitoring rate have been shown to have a positive influence on the rate of extirpation, decreasing the predicted mean time to extirpation, whereas an increase in net infested area, propagule longevity and time to reproductive maturity all negatively influence the probability of success (Panetta and Timmins 2004; Dodd et al. 2015). Lack of success at dispersal, survival or reproduction prevents a species from expanding its range (Cooke et al. 2005). Studies have attempted to estimate extirpation probability and determine if an eradication program should be attempted, this needs to be considered in conjunction with other important variables including the perceived risk of a species, the cost of intervening (Panetta 2009; Dodd et al. 2015) and the long term costs of not intervening (including economic, environmental and social impacts). For invasive species that spread quickly, it has been suggested that eradication attempts should often proceed even with uncertain prospects for success, as the costs of not intervening outweigh the cost of intervening (Simberloff 2003).

1.4 Invasive aquatic plants

1.4.1 Impacts of invasive aquatic plants

Aquatic plants are integral components of freshwater ecosystems and provide essential ecosystem services. However, when invasive species establish in new environments, there are few natural checks and balances to inhibit their growth and spread (Madsen 2005; Dugdale et al. 2013). Once introduced into aquatic environments, invasive aquatic plants commonly form large and dense infestations (monocultures) through rapid growth rates and biomass
accumulation. High reproductive capacity, vigorous growth and the absence of natural enemies (including insects and pathogens) often lead to problematic populations. It is in these situations where invasive aquatic plants harm aquatic systems if left unchecked, causing significant detrimental ecological, economic and social impacts (Lodge et al. 2006). They degrade water quality, slow water velocity, exacerbate siltation or flooding and reduce species diversity and abundance (Madsen 2005; Gettys et al. 2009). Such impacts pose a serious threat to the long-term function of freshwater aquatic ecosystems and, if left unchecked, may result in significant habitat alteration (Barnett and Veitch 2007; Yarrow et al. 2009).

Impacts caused by excess aquatic vegetation can be divided into (1) human and economic impacts and (2) ecological impacts. Human and economic impacts include compromising; human health (by reducing water quality and exacerbating the spread of insect borne diseases (Marsollier et al. 2004; Pimentel et al. 2000)), agricultural productivity (by impeding water delivery and damaging irrigation infrastructure (Bill 1969; Bakry et al. 1992; Dugdale et al. 2013; Clements et al. 2015)), recreation (by posing a risk of entanglement and drowning for waterbody users), fishing (by altering fish and wildlife habitat), navigation, hydropower generation, drainage and flood control, aesthetics and land values (Gettys et al. 2009; CAST 2014).

Nuisance aquatic plants impact on ecological functioning of aquatic communities and habitat in primarily four ways: (1) structurally changing habitat through fast growth rates, greatly increasing populations and biomass; (2) dominating the capture of energy from sunlight; (3) stabilising and limiting water exchange processes; and (4) producing large amounts of dead plant material, or detrital matter. Longer term ecological impacts include; suppression of native plants, a decrease in overall species diversity, potential effects on threatened and endangered species, a shift in animal communities, and an alteration of ecosystem services (Gettys et al. 2009; CAST 2014).

1.4.2 Invasive aquatic plant life-forms
Aquatic plants (macrophytes) can be divided into four main groups that grow partially or completely in water including: (1) emergent plants, which are plants that root in the sediment in shallow water and their leaves extend above the water’s surface (e.g. sagittaria (Sagittaria platyphylla (Engelm.) J.G.Sm.) and cumbungi (Typha spp.)); (2) floating attached plants, including those plants that root into the sediment in shallow water or along the margins of waterbodies and produce floating stems/leaves (e.g. alligator weed (Alternanthera
philoxeroides), parrot’s feather (*Myriophyllum aquaticum* (Vell.) Verdc.) and waterlilies (*Nymphaea* spp.)); (3) free-floating plants, which are those plants which float on or under the water surface and their roots are in the water not in the sediment (e.g. water hyacinth (*Eichhornia crassipes*) and salvinia (*Salvinia molesta*); (4) submersed plants, include those plants that root into the substrate and grow up through the water column. These plants can produce surface-reaching weed beds when infestations are large and dense (e.g. dense waterweed (*Egeria densa* Planch.) and fanwort (*Cabomba caroliniana* A.Gray) (Sainty and Jacobs 2003; Cooke et al. 2005). The growth form of macrophytes have important implications in terms of implementing management strategies. The species, habitat attributes as well as the desired management outcome(s) must be taken into account (Hussner et al. 2017).

1.4.3 Reproduction, dispersal and colonisation of invasive aquatic plants

Reproduction of aquatic plants occurs via sexual (seed) and/or asexual reproduction (vegetatively by stem fragmentation, rhizomes, stolons, tubers, turions) (Haynes 1988; Riis and Sand-Jensen 2006). Reproductive strategies can be divided into three types: (1) annuals, where over-wintering is by seed; (2) perennial herbaceous, where specialised vegetative propagules are formed for overwintering, such as stem fragments, turions, tubers, or winter buds; (3) perennial evergreen, where vegetative, non-reproductive biomass is used for overwintering. Many species are intermediate and reproduce both sexually and vegetatively (Cooke et al. 2005). Species that become aquatic weeds are usually prolific vegetative reproducers (Madsen 1991). Many invasive aquatic plants do not produce viable seed in their introduced range and reproduce solely by vegetative means. Vegetative reproduction usually predominates in most species because vegetative propagules are probably sufficient for overwintering without the high energy investment required for sexual reproduction (Cooke et al. 2005). Unspecialised stem fragment production is a common and efficient reproduction and dispersal mechanism in many aquatic plants (Barrat-Segretain 1996; Bickel 2015), where stem fragments of just a few centimetres can produce a new plant. Some of the world’s most invasive aquatic species reproduce almost exclusively by vegetative reproduction. Floating plants that commonly reproduce vegetatively include; alligator weed (*Alternanthera philoxeroides*), water hyacinth (*Eichhornia crassipes*), parrot’s feather (*Myriophyllum aquaticum*) and salvinia (*Salvinia molesta*). Submersed plants that commonly reproduce vegetatively include; fanwort (*Cabomba caroliniana*), coontail (*Ceratophyllum demersum* L.), dense waterweed (*Egeria densa*) and Eurasian watermilfoil (*Myriophyllum spicatum* L.).
An understanding of a species reproductive biology is important in developing effective methods of control for aggressive species (Haynes 1988).

The importance of vegetative plant dispersal for colonization of catchments and waterways has been established (e.g. Barrat-Segretain et al. 1998; Sand-Jensen et al. 1999; Combroux et al. 2001; Riis 2008). Dispersal can be defined as the movement of diaspores (plant dispersal units) from a source population to a site outside the area occupied by that population (Johansson and Nilsson 1993). The primary mode of dispersal by aquatic plants within catchments is by hydrochory, which is the passive dispersal of organisms by water currents and is an important means of propagule transport, particularly for invasive aquatic plants (Johansson and Nilsson 1993). Hydrochory is a mechanism of long distance dispersal which serves to expand the distributional extent of a species (Cain et al. 2000; Nilsson et al. 2010).

Unspecialised stem fragments can be detached from parent plants facilitated by disturbance caused by changes in water velocity, sediment mobility during high water flow, human activity (e.g. boating) and animal disturbances (e.g. herbivory). The only requirement for dispersal of stem fragments to take place is the presence of plants and a disturbance agent causing detachment (Riis and Sand-Jensen 2006). Hydrochory has been inferred to be an important vector for the spread of many invasive species. In high flow events, propagules can be transported great distances along waterways, and, in addition, the physical forces exerted by water and debris can create new propagules, particularly stem fragments, which are then dispersed downstream. However, even under normal or low flow conditions, some waterborne dispersal of propagules can occur (Truscott et al. 2006). Production of unspecialised stem fragments occurs through autofragmentation or allofragmentation (Madsen et al. 1988; Madsen and Smith 1997) or a combination of these processes. The self-induced abscission of shoots by the breakdown of the cell wall is known as autofragmentation; biochemical processes that occur at precise points of detachment enable organ shedding or abscission to occur (Gonzalez-Carranza et al. 1998; Riis et al. 2009). These points of detachment are predetermined and are called abscission zones (Roberts et al. 2002). Allofragmentation is breakage as a result of disturbance (e.g. flood flows, herbivory, weed cutting or other control activities). Allofragments are usually more important than seeds for dispersal and colonization of aquatic plants (Riis et al. 2009). A thorough understanding of macrophyte biology is the basis for developing effective management approaches for invasive aquatic plants (Cooke et al. 2005).
Within catchments, for effective colonisation to occur, aquatic plants require: (1) upstream production of propagules, where increased propagule pressure can facilitate potential invasion (You et al. 2016); (2) dispersal of propagules; (3) retention of propagules in available habitats; (4) primary colonisation of propagules; (5) net colonisation of propagules; and (6) survival of perennial populations during frequent disturbances or suboptimal growth conditions (e.g. winter conditions) (Riis 2008).

Although hydrochory can explain long-distance downstream dispersal within catchments, it does not explain dispersal across catchments or upstream dispersal within catchments (against the direction of stream flow) (Nilsson et al. 2010). For long distance dispersal between catchments, natural dispersal of whole plants or stem fragments is unlikely. Many aquatic plants (particularly species that produce viable seed) are spread naturally by birds (ornithochory), wind (anemochory) and water currents (Johnstone et al. 1985). Also, human transport, either knowingly or by accident, is recognised as a leading vector of dispersal between catchments (Cooke et al. 2005; Bickel 2015). Human mediated dispersal can be classified into: (1) equipment related dispersal (e.g. through plant fragments attaching onto boats, boat trailers and fishing equipment) and are probably unintentional (i.e. preventing plant fragment uptake on boat trailers is paramount to reduce the risk of further spread of invasive aquatic plants (Bickel 2015)); (2) plant or animal related dispersal (e.g. where exotic plants are introduced from aquarium discards, fish stocking or packaging of ornamental plant stock); and (3) deliberate or intentional dispersal (e.g. for habitat enhancement, aesthetic reasons, agriculture or anti-social behaviour). These introductions can spread plants long distances because of the care given to ensure survival (Johnstone et al. 1985; Les and Mehrhoff 1999; Cooke et al. 2005).

Whilst dispersal of invasive aquatic plants is the initial stage of a multi-layered invasion process and a key stage where management efforts are required, the role of dispersal in the invasion process has been largely overlooked in past research (Puth and Post 2005). Dispersal pathways shape the invasive potential of introduced organisms and influence propagule pressure (Wilson et al. 2009), strongly influencing establishment success (Lockwood et al. 2005). Further, healthy diverse ecosystems previously thought to be relatively immune to invasion by weed species have been shown to be susceptible to invasion (Kohli et al. 2009). Intact vegetation communities provide competition for dispersed vegetative propagules, theoretically reducing the colonisation success of those propagules (Dugdale et al. 2010). However, invasion of intact communities can be associated with increasing propagule
pressure and species lag-time prior to population expansion (Bryson and Carter 2004; You et al. 2016).

Research to limit dispersal mechanisms of invasive aquatic plants is required to minimise the rate of establishment and reduce the impacts caused by damaging species.

1.5 Management of invasive aquatic plants targeted for extirpation

For an invasive species to be targeted for extirpation effective detection (Panetta and Lawes 2005) and control techniques must be available (Myers et al. 2000; Simberloff 2003; Panetta and Timmins 2004; Cacho et al. 2006; Dodd et al. 2015). The full extent of an incursion must be detectable initially and until extirpation occurs over the entire infested area (Panetta 2009; Panetta and Lawes 2005). Therefore, effective detection techniques must be available. As well as effective detection techniques, effective control strategies must be available that (1) decrease population numbers of a given species at a given rate and (2) prevent the spread of the target species (Zamora et al. 1989). Contingency planning for aquatic invasions is similar to planning for other natural disasters; the threat is identified and the resources for dealing with the threat are known (including people, finances, appropriate control measures, equipment and monitoring) and can be deployed rapidly. Rapid action will be hindered if resources, control strategies, permits or legislative approval have not been identified previously (Cooke et al. 2005).

1.5.1 Detection and monitoring of invasive aquatic plants

Early detection of the presence of an invasive and harmful taxon can make the difference between being able to employ feasible offensive strategies (eradication) and the necessity of retreating to a defensive strategy (i.e. containment or asset based protection) that usually means an infinite financial commitment (Rejmánek and Pitcairn 2002). Typically, aquatic plant invasions that go unnoticed or are overlooked become problematic, requiring extensive management intervention (Cooke et al. 2005). Detection of high priority aquatic weeds before they become widespread is critical to achieving their eradication. Early detection and control of weeds improves eradication probability and minimises ecological damage (Timmins and Braithwaite 2002).

The certainty with which a pest’s absence or presence is measured depends on the efficacy of available detection techniques. The conceptual impossibility of measuring zero population with absolute certainty, forces decision makers to balance their need for increased certainty within the context of budgetary constraints (Dahlsten and Garcia 1989). Detection and monitoring are key components of an effective invasive aquatic plant management
strategy. However, an ongoing issue is how to make management decisions when detection methods are less than perfect (Panetta 2015). With advances in technology, detection techniques are required to be developed, evaluated and implemented to increase detection ability, and to enable early detection of invasive species, particularly those targeted for eradication. Detectability and monitoring rate have a large influence on extirpation success. Repeated monitoring of a site (where a species is targeted for eradication) within a season increases the likelihood of finding the last hard-to-detect individuals and has been shown to halve the mean time to extirpation (Dodd et al. 2015). Although the costs of monitoring may increase when pest densities are low, intensive monitoring with effective detection techniques is the only way to determine when to end an eradication campaign (Simberloff 2003).

Detection and monitoring of weeds in aquatic environments is difficult because access on the ground is generally poor, habitat heterogeneity generally high, and weeds can be obscured by the water itself or associated dense vegetation (Inglis et al. 2006; Lang et al. 2015). For species that are targeted for eradication, survey methods must have a high probability of detection at low abundance in the environment and operate at both small (site specific scale) and large spatial scales (landscape scale).

1.5.2 On-ground surveillance
Detection techniques for invasive aquatic plants are based largely on the life-form of the invasive aquatic plant or vegetation community of interest. At a site scale (small spatial scale) for plants that can be observed visually from the water’s surface (emergent, floating attached and free floating plants), traditional sampling methods have included human surveillance involving field surveys. On-ground (or from a boat) field surveys are very resource intensive and are reliant on individual’s ability to detect and document infestations. This detection method is limited by: availability of resources required for extensive on-ground field surveys; experience and training of personnel conducting the surveys; search effort required and timing of surveys; and accessibility of sites (Clements et al. 2014b). These on-ground monitoring techniques at the landscape scale are usually cost prohibitive (Lang et al. 2015), and thus at the landscape scale, detection techniques often involve public and/or industry reporting of infestations. Investment by governments in training the community and industry to identify targeted species is a developed surveillance technique and is a relative effective way to identify and report incursions of species targeted for eradication, allowing control to be enacted (e.g. Victorian Government Weed Spotter program). However, if an invasive
species has reached large-scale awareness, typically it is already widespread and costs of eradication are high and chances of achieving eradication are low (Cooke et al. 2005).

For submersed plants which grow beneath the water’s surface and cannot be viewed from the water’s surface (unless dense surface reaching weed beds are present and generally if this is the case achieving eradication is difficult), a variety of detection techniques are utilised at a site scale. These involve on-ground/boat field surveys including: visual observation from a boat or utilising divers; destructive harvest (e.g. grapnel anchor/rake throws from boat or diver-quadrat sampling), underwater surveillance using submersible cameras and hydroacoustic (echosounder) technology. However, with this latter approach, discriminating between species is currently not feasible (Madsen and Wersal 2012). Public and/or industry reporting of submersed plant infestations is employed at a landscape scale, as described above for emergent and floating species, however is generally less effective because these species cannot be observed at low abundance when beneath the water’s surface, particularly in turbid water.

Complementary methods are required to detect invasive aquatic plants targeted for extirpation. Detection ability is currently lacking at a landscape scale, i.e. detecting completely new infestations across the landscape in an early stage of invasion, and at an individual plant or patch scale at a known infested site targeted for eradication (Clements et al. 2014b). Novel tools are required to be developed, evaluated and implemented to ensure that new incursions are detected at an early stage, while the window of opportunity to achieve site eradication remains an achievable goal, to mitigate the threat that high-risk species pose (Dahlsten and Garcia 1989). With advances in technology, detection techniques used for aquatic plants should continue to be developed and adapted, to increase detection ability and rates and enable early detection of high priority invasive aquatic plant species, particularly those targeted for eradication.

1.5.3 Remote Sensing
Remote sensing has the potential to add to our ability to detect invasive aquatic plant species and provide natural resource managers with accurate and timely information to inform eradication programs. Different types of remotely sensed data are currently being utilised for a range of surveillance operations including; aerial photographs, multispectral images, hyperspectral images, synthetic-aperture radar (SAR) and LiDAR (high resolution maps). A range of platforms are available to collect remotely sensed data including: low altitude aircraft (unmanned aerial vehicles, UAVs); high altitude aircraft (fixed wing aircraft or
helicopters); and spacecraft (satellites). Each of these datasets and data capture techniques have advantages and disadvantages and selecting an appropriate remote sensing method is determined by scale (being the resolution required to detect the target organism or environment of interest) and the resources available to collect the desired dataset. Scale is an issue with any mapping project as it determines the targeted map unit (Lang et al. 2015). Generally, there is a trade-off between scale and resolution when utilising remote sensing for detection of invasive plant species. Usually, large scale techniques have low resolution and therefore are only effective at detecting larger infestations, whereas smaller scale techniques provide greater resolution to detect small infestations but are only effective for monitoring small areas. The advantages and disadvantages of remotely sensed datasets and data capture techniques have been reviewed by Lang et al. (2015).

Utilising aerial imagery is an effective tool for weed surveillance. Aerial photography was the first remote sensing method to be employed for studying and mapping vegetation, with early studies dating back to the 1960s and 1970s (Silva et al. 2008). Aerial photography interpretation (API) or manual interpretation, has been used extensively around the world to detect changes in species composition and distribution. The limitations of API are varied; the process requires suitable aerial photography relevant to the question of interest, and an analyst to identify key elements of images (including tone, colour, pattern, shape, shadow, texture, contrast) for the species or habitat of interest (Jensen 2007). Most analyses of aerial imagery rely on visual interpretation, where plant species can be discriminated when using high spatial resolution images (Silva et al. 2008). Notwithstanding the contribution of these approaches, identifying boundaries between vegetation community types is a recognised problem (Adam et al. 2010) since API depends on the subjective judgement of the interpreter and the quality of images used (Finkbeiner et al. 2001 and McGlone 2004 cited in Zhu et al. 2007). While interpretation of aerial photography has many limitations, many of these limitations and others are also present when analysing other (higher resolution) remotely sensed datasets (Lang et al. 2015). For example, although identifying boundaries between vegetation communities may be difficult with aerial photography, it may still be impossible to distinguish between some vegetation types when using hyperspectral datasets (Yang and Artigas 2010). Utilising multispectral and hyperspectral images also come with their own set of limitations including requiring larger amounts of available computer memory (storage) and processing power than utilising aerial imagery, as image resolution increases (Lang et al. 2015), which ultimately means more resources required to implement the technology. All
forms of data face challenges posed by the targeted organism and the environment of interest to achieve accurate detection and classification.

It is important not only to differentiate between different types of imagery, but also between the different platforms used to collect those images because there are advantages and disadvantages inherent to both aspects (Lang et al. 2015). For example the advantages of utilising satellite imagery include large spatial coverage, timeliness, repeatability and often low costs. Whilst collection of sub-metre satellite data is now possible (e.g. GeoEye-1; 0.41 m spatial resolution), poor resolution when utilising this technology has been a key limitation in the past that is still continuing to be overcome (Lang et al. 2015). Disadvantages of using satellite imagery include interference from weather and the atmosphere during data collection and the inability to collect data at key time periods. These aspects can be overcome utilising low altitude aircraft (unmanned aerial vehicles (UAVs)) or high altitude aircraft (fixed wing aircraft or helicopters), but these platforms have limited spatial coverage. Acquisition of imagery must be timed to facilitate identification or collected at multiple times to cover a range of conditions that will aid the image analyst (Lang et al. 2015). Repeated monitoring of a site within a season also increases the likelihood of finding the last hard-to-detect individuals and has been shown to halve the mean time to extirpation of invasive plant species (Dodd et al. 2015), and this is a benefit of using low altitude aircraft that can be deployed readily.

A considerable amount of research has been conducted on various machine vision techniques for automatic detection and identification of features from remotely sensed data (Hung 2013; Lang et al. 2015). Computer based image analysis is largely an automated classification process compared to manual interpretation of aerial photography and may offer considerable time savings, and datasets may be updated more frequently. However, the upfront costs can be prohibitive, requiring expensive software, substantial computer processing power, large amounts of available memory (storage), as well as a higher learning curve for analysts. Classification systems may have to be simplified and the level of classification adjusted to match the capability of remotely sensed data to accurately and consistently categorise aspects of an image (Lang et al. 2015). For example, one challenge with aerial photography is that spectral signatures of neighbouring plant species may have similar reflectance values and even with hyperspectral images it may not be possible to distinguish between some vegetation types (Yang and Artigas 2010). Different datasets may require modified classification techniques, and in addition, scale and resolution determine the targeted map unit and the degree of mixing of classes (Lang et al. 2015).
Research on various machine vision techniques for automatic weed detection and identification for individual species classification have shown accuracies in the range of 65–95% under ideal conditions. However, most of the machine vision techniques investigated have yet to be adapted to real world conditions (Slaughter et al. 2008).

The use of remote sensing has been employed on both floating and submersed aquatic weed species. Aerial detection technology has shown promise for invasive floating species (e.g. alligator weed (*Alternanthera philoxeroides*), water hyacinth (*Eichhornia crassipes*), and salvinia (*Salvinia molesta*) (Sukkarieh 2009; Robles et al. 2010; Hung and Sukkarieh 2013). Aerial detection of submersed aquatic weeds has proved difficult, with limited success as plants are obscured by the water’s surface or high water turbidity. Water absorbs or reflects most wavelengths of electromagnetic energy. Only visible wavelengths penetrate water, and the depth of penetration is influenced by the turbidity of the water. Whilst penetration is essentially restricted to the visible region (Hosny 2005), more recent studies have indicated that optical remote sensing with UAVs holds promise for completing spatially precise, and multi-temporal measurements of algae or submerged aquatic vegetation in shallow rivers with low turbidity and good optical transmission (Flynn and Chapra 2014). Dense surface-reaching weed beds of invasive submersed species have been detected (Everitt et al. 2011), however achieving eradication when infestations are at this stage is difficult, as infestations are generally large monocultures. The most common source of error cited when utilising remote sensing is occlusion between species, followed by poor plant segmentation caused by variables including weather and wind, water, poor illumination and shadows (Slaughter et al. 2008). Although much research and development has been conducted on aerial surveillance, including the development of airborne platforms and image interpretation (Slaughter et al. 2008; Göktoğan et al. 2010), the effectiveness of aerial surveillance as a detection technique has largely gone unquantified (quantitatively compared to current detection methods), which has limited its uptake in real world situations.

Additional methods are also being advanced for the detection of invasive species, including utilising DNA in environmental samples (eDNA) (Bohmann et al. 2014). A recent study has demonstrated the concept for the early detection of Eurasian watermilfoil (*Myriophyllum spicatum*) (Newton et al. 2016). However, research in this field is in an early stage of development and it is not well understood how these methods can be employed effectively in real world situations.
1.5.4 Control techniques for invasive aquatic plants

A large variety of management techniques are available to control problematic aquatic weed populations, and techniques are often species-specific (Cooke et al. 2005; Hussner et al. 2017). Management techniques can be broadly grouped into four main categories including: (1) physical; (2) habitat manipulation; (3) biological; and (4) chemical control (Sainty and Jacobs 2003; Australian Government 2014). Within each of these categories there are numerous specific control techniques and often a combination of techniques (integrated management) are required for effective control of invasive aquatic plant species (Petty 2005; Hussner et al. 2017). All aquatic plant management techniques have positive and negative attributes and none are without potential adverse environmental impact. Selection of management techniques are based on economic, environmental, and technical constraints (Petty 2005; Madsen 2006; Gettys et al. 2009). Defining management goals or outcomes is of primary importance when determining which control techniques to utilise. For example, a particular control technique may be adequate to achieve containment or biomass reduction for asset based protection (Figure 1), but could not achieve eradication (Hussner et al. 2017).

Physical control techniques can be effective in eradicating invasive aquatic plants. Physical control techniques are often a useful tool for either small infestations (particularly the initial invasion of a catchment or after a long period of chemical control has suppressed formerly high levels of biomass to low levels (van Oosterhout 2007)), or in locations where chemical use may be deemed inappropriate (Clements et al. 2014a). Physical methods include manual removal and mechanical harvesting (Madsen 2006). These methods are often resource intensive and can have detrimental environmental impacts. However, they are often employed with the perceived belief that they are ‘better’ than using chemicals (Madsen 2006) and often considered to be environmentally benign (Cooke et al. 2005). These methods commonly require the use of physical barriers, such as floating booms, to reduce or eliminate floating plant propagules (that are produced during control works) or free floating species from spreading to downstream locations (Deutsch 1974, Cooke et al. 2005).

Control techniques utilising habitat manipulation reduce or eliminate plant growth through altering the environment, rather than directly controlling plants. Habitat manipulation control techniques include; benthic barriers, water level manipulation or drawdown, dredging, light attenuation or shading, and nutrient inactivation (Gettys et al. 2009; Hussner et al. 2017).

Biological control of invasive aquatic plants involves the introduction of parasites, predators, or pathogens into the environment (from the target plants native range) for the
suppression of the target plant. The aim of biological control is not to eradicate a weed from a specific area (this would also eliminate the introduced biological control agent), but reduce the spread and density of infestations once established (Petty 2005). Biological control is therefore not employed as an eradication control technique, but as a long-term control strategy (Grodowitz 1998) in the ‘containment’ and ‘asset based protection’ stage of invasion (Figure 1; McLaren et al. 2016). Biological control can be very expensive to implement initially as it requires (1) declaration of the target weed as a target for biological control; (2) surveys for potential agents (insects and/or pathogens) in the country of origin; (3) host specificity testing of the agent(s); (4) an application to import and release the agent, if host specificity studies are positive; (5) mass rearing and release of the agent(s) and (6) monitoring of establishment and impacts of the agent(s). This process can cost several $millions to implement and successful results are not guaranteed. However, on average in Australia, biological control has returned $23:1 on investment (Page and Lacey 2006), making it a viable control option for widespread weeds.

Management of invasive aquatic plants using chemicals, known as herbicides, is recognised as one of the most effective and widely used options for controlling invasive aquatic plants worldwide. Chemicals are effective, relatively predictable and costs are competitive when compared to other control techniques (Cooke et al. 2005; Petty 2005; Madsen 2006; Gettys et al. 2009). However, effective and appropriate chemical use is essential to maximise control and minimise off-target impacts. Although applying any chemical to the aquatic environment is undesirable, the effective use of chemicals to eliminate problematic invasive aquatic plants can have significant long term benefits and considerably outweigh potential short-term impacts caused by chemical use or ineffective control using other techniques. Selecting the appropriate herbicide for a given species and situation is critical. Different products or formulations of the same herbicide vary in their efficacy on targeted plants, and use restrictions of herbicides apply (Madsen 2006). Use restrictions are particularly important in aquatic environments and are generally based on water use and toxicological data. Efficacy and use restrictions are approved by government registration authorities, based on significant research programs, to protect the health and safety of humans, animals, and crops utilising the treated water, as well as providing effective control of the target species (Madsen 2006). Further, herbicides are often applied with surfactants or other adjuvants to improve herbicide efficacy. Adjuvants are chemicals added to herbicides, and they include: wetting agents and emulsifiers, that allow herbicides to mix effectively; spreaders, that allow herbicides to spread effectively over surfaces; stickers,
thickeners, invert emulsifiers and foaming agents that increase herbicide adherence to treated surfaces and limit herbicide drift; and penetrants that enhance herbicide absorption by decreasing surface tension or by penetrating through waxy coatings on vegetative surfaces (Cooke et al. 2005). Many herbicide labels instruct that an adjuvant be used when applied to aquatic plants, however many adjuvants are toxic to aquatic biota and have higher environmental toxicity than the herbicide itself (Brausch et al. 2007; Siemering et al. 2008; Tatum et al. 2012). The selection of appropriate chemicals is critical to maximise efficacy of control while minimising off-target impacts.

For effective management of invasive aquatic plants, control of above ground and below ground biomass, as well as control of viable propagule production (sexual and asexual propagules), is required. For the management of invasive aquatic species that have established in catchments and waterways, an effective control mechanism is to limit viable propagule production, before dispersal occurs, so that viable propagules are limited at their source. Eradication efforts will be limited if dispersal of viable propagules is not accounted for, as new infestations are likely to be created downstream. Understanding dispersal pathways of aquatic plants enables effective management strategies to be developed (Bickel 2015). The unintentional spread of invasive aquatic plants is a function of the regeneration ability of plant propagules (including stem fragments) and the suitability of habitat conditions for successful invasion (Johnstone et al. 1985; Jacobs and Macisaac 2009). The rate of arrival of viable fragments (propagule pressure) is directly linked to potential invasion success (Lockwood et al. 2009; You et al. 2016). Therefore, limiting infestation expansion and viable propagule production at their source, reduces the impact of factors that cannot be controlled (e.g. high water flow events), ultimately limiting dispersal of invasive aquatic plants throughout catchments and waterways. Climate change may also alter the role of hydrochory by modifying the hydrology of water-bodies as well as conditions for propagule release and plant colonization, potentially exacerbating the plant invasion processes (Nilsson et al. 2010).

1.6 Introducing the invasive aquatic plant alligator weed (*Alternanthera philoxeroides*)

In frequently disturbed habitats, such as aquatic environments, plants have developed strategies to cope with disturbance. As noted earlier, many invasive aquatic plants reproduce solely by vegetative means, where disturbance induces destruction of above ground plant parts and fragmentation of clones allows species to proliferate (Dong et al. 2010). This reproduction and dispersal mechanism therefore makes controlling invasive aquatic plants
difficult, particularly when targeting eradication. In Victoria, Australia, four highly invasive aquatic plant species are subjected to eradication programs administered by the State Government; alligator weed (*Alternanthera philoxeroides*), lagarosiphon (*Lagarosiphon major* (Ridl.) Moss), salvinia (*Salvinia molesta*) and water hyacinth (*Eichhornia crassipes*). Of these four species that predominately reproduce asexually, alligator weed is the most widespread within the State’s catchments and waterways. Salvinia and lagarosiphon are not naturalised and water hyacinth is in an early stage of invasion of a single catchment. Management techniques for invasive aquatic plants are currently underdeveloped, including detection and control techniques, allowing alligator weed to proliferate. The research presented in this thesis uses alligator weed as a target species to develop a research program to optimise the management of invasive aquatic plants targeted for extirpation from catchments and waterways. Note, as described previously, that extirpation is not synonymous with eradication, as eradication refers to efforts being undertaken on the largest relevant scale (Panetta 2015), in this case the state of Victoria. Here we are examining the localised extirpation of alligator weed from individual catchments and waterways.

Management techniques and principles developed for alligator weed in this thesis provide a model for programs that aim to optimise the management of other invasive aquatic plants targeted for extirpation from catchments and waterways.

1.6.1 A description of alligator weed

Alligator weed (*Alternanthera philoxeroides*), is a perennial stoloniferous herbaceous plant that belongs to the family Amaranthaceae. It originates from the Parana River area of South America, including southern Brazil, Paraguay and northern areas of South America (Julien et al. 1995; Sainty et al. 1998). Subsequently it has spread to and invaded aquatic and terrestrial environments in over 30 countries across Asia, Europe, North America, South America and Oceania (EPPO 2016). It has invaded many countries around the world including; southern USA (first detected in 1897), New Zealand (1906), China (1930s), Australia (1946), India, Burma and Indonesia (by the 1960s). More recently it has been detected in Puerto Rico, Singapore, Vietnam, Thailand, Sri Lanka, Italy and France (Julien et al. 1995; Gunasekera and Adair 1999; Dugdale and Champion 2012).

This weed species is an aggressive invader of both aquatic and terrestrial environments (Sainty et al. 1998). It produces masses of vegetative hollow stems that can grow flat (prostrate growth) along the ground or water surface and form dense mats with vertical growth (Julien et al. 1995; Clements et al. 2011). It can grow under a wider range of soil and
water conditions than any other aquatic plant (Durden et al. 1975), being capable of growing on substrata from sand to heavy clay and can tolerate fresh to slightly saline conditions (30% sea strength salinity). It has been found growing above the high tide zone on beaches in NSW, Australia (Sainty et al. 1998), and whilst preferring a warm growing season, it can tolerate winter frosts (Julien et al. 1995). It is particularly successful in aquatic and semi-aquatic environments where it is capable of extremely rapid growth (Clements et al. 2011). In aquatic environments, alligator weed roots into the soil near the water’s edge or in the substrate beneath shallow water and produces mats of entangled stems that float and extend over the water surface. Adventitious roots and taproots may be attached to the substrate or the bank and in deeper water adventitious roots are free floating (Julien 1995). With each consecutive season of growth, new stems are produced from prostrate mats from the previous season’s growth. Overwater biomass can double in 40 days during the growing season (Julien et al. 1992) and a few stems at a site can grow into a patch 1-3 m wide in one growing season through stoloniferous growth (Sainty et al. 1998). Julien et al. (1992) report mats of aquatic alligator weed growing to 70 m wide and Spencer and Coulson (1976) report that mats can extend ca. 100 m over the water surface. However, there have been very few published studies reporting the growth rate of aquatic alligator weed, particularly over consecutive years, most likely due to control programs being enacted soon after detection (Julien et al. 1992).

Chapter 2 of this thesis reports on the growth rate of alligator weed in Victoria, Australia, over a five year period. The growth rate of alligator weed has not previously been recorded in southern Australia.

It is well documented that alligator weed can choke waterbodies by forming a blanket of floating vegetation that can cover the surface of the water. Reviews of the biology and impacts of alligator weed have been described extensively (Julien and Broadbent 1980; Julien 1995; van Oosterhout 2007; Dugdale and Champion 2012), and it is known that alligator weed poses a significant threat to waterways, wetlands, floodplains, water storages and irrigation systems (van Oosterhout 2007). It has the potential to occupy and seriously affect most Australian freshwater bodies (Julien et al. 1995; Gunasekera and Adair 1999). In aquatic environments, dense infestations of alligator weed restrict human use, exclude desirable plant species, interfere with aquatic ecology and restrict water flows (Spencer and Coulson 1976; Gangstad 1978; Julien et al. 1979; Julien and Broadbent 1980; Julien et al. 1992; Parsons and Cuthbertson 1992; Clements et al. 2011). Human and economic impacts related to excessive alligator weed growth in aquatic environments include; restricting the
growth of fish, impeding access and navigation (inhibits boat movement), restricting water flows and drainage, exacerbating sedimentation or flooding, reducing water quality and providing a breeding ground for mosquitoes (Dong et al. 2010; van Oosterhout 2007). In agricultural irrigation networks, alligator weed impedes water delivery (by acting as a barrier to water flow) and can damage irrigation infrastructure directly or by accumulation of debris associated with blockages (van Oosterhout 2007).

Alligator weed impacts on the ecological functioning of aquatic communities and habitats by; restricting light penetration into the water, increasing sedimentation, preventing gas exchange between the water and atmosphere, creating anaerobic conditions, reducing native invertebrate densities and altering community composition (Gangstad 1978; Julien and Broadbent 1980; Julien and Bourne 1988; Parsons and Cuthbertson 1992; Shen et al. 2005; Pan et al. 2010). Dense infestations increase evapotranspiration and water loss from standing waterbodies and alter the timing and amount of litter inputs into the water. The amount and timing of organic matter entering water bodies influences dissolved oxygen concentrations, impacting on aquatic organisms and nutrient cycling. In addition, alligator weed has a faster decomposition rate than native riparian communities (Boyd 1987; Cooke et al. 2005; Bassett et al. 2010). As a consequence, ecological impacts include; suppression of native plants, a decrease in overall species diversity, potential effects on threatened and endangered species, a shift in animal communities, and an alteration of ecosystem services (Gettys et al. 2009; CAST 2014). These impacts pose a serious threat to the long-term function of freshwater aquatic ecosystems and may result in significant habitat alteration (Barnett and Veitch 2007; Yarrow et al. 2009).

An important issue, and quite a unique feature of alligator weed (compared to other aquatic species), is that it can invade terrestrial as well as aquatic ecosystems. In terrestrial situations alligator weed produces an extensive underground root system, with dense mats of stolons, taproots and adventitious roots beneath the soil. Morphology, anatomy and growth of alligator weed in aquatic conditions differ significantly from those in dry, terrestrial habitats but these differences are most likely plastic responses rather than genetically-based changes (Dong et al. 2010). Terrestrial growth is highly competitive and able to displace other plants (Julien and Bourne 1988). It has been widely reported that alligator weed has invaded a range of terrestrial environments including pasture (Julien and Broadbent 1980; Coffey and Clayton 1988), arable crops (Shen et al. 2005) and urban areas (Gunasekera and Bonilla 2001). It displaces and aggressively competes with native plant communities, pasture and cropping species in moist agricultural areas. Any irrigated or floodplain-based agricultural production
is at risk where alligator weed is present (van Oosterhout 2007). Crops can be impacted by alligator weed, and yield losses have been reported in rice, cotton, wheat, soybean, peanut and other vegetables. Orchards, tea plantations, turf farms, berry fields and herb crops have also been impacted (Shen et al. 2005; van Oosterhout 2007). It is palatable to livestock, but the grazing of alligator weed has been associated with photosensitivity and resultant skin lesions, liver damage and death in cattle, calves and lambs. As a result, farming practices for cattle have been modified in New Zealand where this species occurs (Bourke and Rayward 2003; Dugdale and Champion 2012). Alligator weed has the ability to accumulate heavy metal ions and calcium oxalate which may be toxic at certain levels (Parsons and Cuthbertson 1992). These impacts and the severity of invasion, have led to alligator weed being regarded as one of the world’s worst weed species, several countries list alligator weed as one of their most troublesome pests (Holm et al. 1997; Gunasekera and Bonilla 2001, Schooler 2012; You et al. 2016).

In its introduced range, alligator weed reproduces solely by clonal growth, as viable seeds are not produced (Julien 1995). It efficiently disperses within catchments and waterways via stem fragmentation, where hollow stem fragments or floating mats break off and disperse with water currents to surrounding areas, creating new infestations (Eggler 1953, Julien and Broadbent 1980, Julien et al. 1992; Dugdale et al. 2010). Increased propagule pressure greatly facilitates the growth and invasion potential of alligator weed and other invasive species (Lockwood et al. 2005; Simberloff 2009; You et al. 2016). It subsequently reproduces effectively because each node on a stem or taproot is capable of forming a new plant (Hofstra and Champion 2010; Dugdale and Champion 2012). Under high flow events (flooding), floating mats of alligator weed can be swept downstream (Gunasekera and Adair 1999), before becoming lodged (Sainty et al. 1998; Gunasekera and Adair 1999) either within a waterbody or on an adjacent floodplain. This is one potential pathway of invasion to agricultural areas. Therefore, it is critical to enact control strategies in an early stage of invasion before large infestations are formed, to restrict propagule pressure and potential invasion success.

Alligator weed has been spread both deliberately and accidently by human mediated dispersal (Julien and Bourne 1988). It can be spread by physical transport of stem or root fragments on machinery, boats, fishing nets or animals (e.g. trapped within the hoofs of livestock) (Dugdale and Champion 2012). The inadvertent movement by machinery (e.g. earthmoving, watercraft, slashing and mowing) and transportation of contaminated materials (e.g. turf, mulch, hay, soil, gravel and sand) from infested to un-infested locations are
strongly implicated (Sainty et al. 1998; van Oosterhout 2007). It has also been dispersed deliberately by humans through propagation as a garden vegetable (Gunasekera and Bonilla 2001) and the ornamental/aquarium plant trade (van Oosterhout 2007).

Historically, alligator weed was first reported in Australia in 1946 near Newcastle, New South Wales, in heaps of ballast dumped by post-World War II cargo ships (Hockley 1974). It is declared a noxious weed in all Australian states and territories and is a prohibited weed in Victoria and Tasmania (Gunasekera 1999). Early invasion of alligator weed occurs in and around the metropolitan area of Melbourne, the capital city of Victoria, Australia, where it was first detected in 1996 (Gunasekera and Bonilla 2001). It is anticipated that, if left unchecked, these infestations will act as source populations for dispersal to other areas of Victoria, where it will significantly compromise agricultural productivity, block irrigation and drainage infrastructure and reduce biodiversity and social amenity of aquatic environments. Thus, in addition to being a weed of national significance in Australia (Australian Government 2014), alligator weed is declared a State Prohibited Weed (SPW) species, and is targeted for eradication in Victoria (Victorian Government 2014). This situation is similar to New Zealand, where it is designated as an unwanted organism (New Zealand Government 2010). In the USA, China and the Australian state of New South Wales (where it has been a problem weed for more than 65 years), it is in a later stage of invasion, where it is much more widespread and abundant, so eradication is not deemed feasible. In these locations, suppression programs exist based on herbicide (Dugdale and Champion 2012) and biological control (Sainty et al. 1998), which aim to contain infestations and reduce the spread and impact of the weed. Whilst biological control with insects specific to alligator weed has been successful in the United States and some parts of Australia (Coulson 1977; Johnson and Brooke 1989; Cook et al. 2005), in New Zealand, northern latitudes in the USA and areas of southern Australia (Victoria), biological control agents (alligator weed beetle (Agasicles hygrophila Selman and Vogt) and moth (Arcola mallow (Pastrana))) are not considered suitable for widespread control (Julien et al. 1995; Stewart et al. 1999; Cooke et al. 2005; Hayes 2007; Winks 2007; Hofstra and Champion 2010). This is because temperature significantly impacts the success of A. hygrophila, as there is no winter diapause and is therefore eliminated in cool environments. It has been estimated that its effectiveness roughly corresponds with a mean winter temperature of 12°C (Cooke et al. 2005). Further, differences in susceptibility of the terrestrial and aquatic forms of alligator weed to biological control have also been shown, where effective control of the aquatic form is achieved but not for the terrestrial form (Spencer and Coulson 1976; Julien and Broadbent 1980; Kay and
Haller 1982; Julien and Bourne 1988; Li and Ye 2006). Biological control is employed as a long-term control strategy (Grodowitz 1998) and is not utilised in eradication programs (van Oosterhout 2007). Of wider interest is that in China, US$72 million is spent each year to manage alligator weed (Liu and Diamond 2005), and in the USA, there has been considerable effort to control it since the 1950s (Gangstad 1978). Figures for Australia and New Zealand are not available, however it has been the subject of concerted eradication and suppression programs for over 20 years.

1.6.2 Invasion history of alligator weed in Victoria, Australia.

In Victoria, Australia, alligator weed has been targeted for eradication (under the Victorian Catchment and Land Protection Act 1994 (Victorian Government 2014)) since 1996, when it was first detected growing in urban backyards (~800 backyards) (Gunasekera et al. 2006). Alligator weed was largely being cultivated as a food plant in home gardens by members of the Sri Lankan community, in the mistaken belief that it was mukunuwenna or sessile joy weed (Alternanthera sessilis (L.) R.Br. ex DC.), which is a popular vegetable in Sri Lanka (Gunasekera 2008). Deliberate cultivation in Victoria has been reduced because of a successful government education campaign and distribution of a native plant as a substitute food plant (Alternanthera denticulata R.Br.) (Gunasekera and Bonilla 2001). These backyard infestations act as source populations for potential dispersal and naturalisations in catchments and waterways. Potential dispersal pathways from backyard infestations include vegetative spread under fences and disposal of garden waste along stream corridors (Gunasekera et al. 2006; McLaren et al. 2016). Approximately 65–75% of backyard infestations were successfully eradicated (to 2006) by the state government using single herbicide treatments, usually of dichlobenil granules (a herbicide not permitted for use in most aquatic environments) (Gunasekera et al. 2006). Around 50 backyard sites remained in 2006. However, alligator weed subsequently spread and become naturalised in urban waterways as well as a few peri-urban outlier sites (Gunasekera 1999; Gunasekera and Adair 1999; Gunasekera and Bonilla 2001; Gunasekera et al. 2006; Gunasekera and Bonilla 2008; Clements et al. 2011; McLaren et al. 2016). Five naturalised infestations were detected in 1997 and increased to 11 in 1999, 17 sites in 2002, 34 naturalised in 2005 (estimated total area of 200 m² in December 2005) (Gunasekera et al. 2006). Although the increase in detection of new naturalised infestations was reported of some concern, most were found when still in the very early stages of invasion. The herbicide glyphosate was utilised solely in aquatic environments up until 2005, where repeat applications (two-monthly intervals during
the growing season) over several years were reported as being required to eradicate an average size (10 m$^2$) infestation using glyphosate (Gunasekera et al. 2006). However, it was also reported that the registered rate of glyphosate for alligator weed in waterways was not very effective and is one reason why progress in eradicating naturalised infestations was slow. There was also concern that after glyphosate treatment, viable stem fragments may be washed down watercourses. The program was reported as progressing towards eradication in 2006, however because the slow progress towards eradication, a more effective herbicide treatment was sought to hasten the eradication of naturalised infestations (Gunasekera et al. 2006). Metsulfuron-methyl was recognised to be effective for alligator weed control in terrestrial environments, with a low toxicity to aquatic organisms, but it was not (and is still not) registered for use in aquatic situations in Australia. The Victorian state government obtained a permit from the Australian Pesticides and Veterinary Medicines Authority (APVMA, Australian Government) in 2005 to use small amounts of metsulfuron-methyl for alligator weed in specified aquatic situations. Treatments were made with metsulfuron in 2005 and 2006 and initial indications were reported as promising (Gunasekera et al. 2006). Over the next five years, control works were carried out by government personnel as well as the private industry (on behalf of the government) utilising glyphosate and metsulfuron-methyl herbicides for control of alligator weed. However, in 2009 the largest known infestation of alligator weed was reported growing in an urban pond (7300 m$^2$), that had gone undetected for over five years (Clements et al. 2011). By 2010, 615 individual patches and two core infestations (large infested areas) were reported growing across Victorian catchments and waterways (Eames 2010). These infestations in Victoria are concentrated heavily within the Melbourne metropolitan region, although there are a few peri-urban outlier infestations (Clements et al. 2011).

Obviously, between 2005 and 2010, alligator weed expanded its range significantly. However, this expansion occurred within multiple but specific catchments and waterways where previous detections have occurred, and dispersal to new catchments did not occur within this time frame. The reasoning for the proliferation within catchments is not entirely clear; whether it was due to ‘site/species’ or ‘organisational’ factors, or the combination of these factors (Dodd et al. 2015) has not been determined. ‘Site/species’ factors that are expected to have hindered successful extirpation include; the biology of the species (including rapid reproduction rates and propagule longevity) and how employed control tactics have interacted with the species biology (including the production of viable fragment production post-herbicide application and the rate of decrease in population numbers of each
utilised control tactic) (Panetta and Timmins 2004). The control regime may have been ineffective in preventing reproduction and new infestations may have been created through the dispersal of viable propagules within catchments, limiting eradication efforts (Panetta and Lawes 2005). However, none of these aspects relating to the biology of the species have been quantified, particularly over the longer term (greater than one year) (Dugdale and Champion 2012) prior to this research project. The research presented in this thesis develops methods to manage the spread of alligator weed within catchments and waterways and optimises control and detection techniques, with the continued aim of eradication from the state of Victoria, to minimise the long-term significant impacts posed by this invasive species.

Organisational factors may have also hindered successful extirpation, including economic and operational factors (Panetta et al. 2011; Dodd et al. 2015). As described earlier (section 1.3.2), to achieve eradication, the effort (including investment) comprises the detection effort required to delimit a weed invasion plus the search and control effort required to prevent reproduction until extirpation occurs over the entire infested area (Panetta 2009). Detectability, being the requirement to detect the full extent of an incursion (Panetta and Lawes 2005) over an annual period may have not been effective or possible with current detection techniques. Repeated monitoring within a season, which has been shown to have a positive influence on the rate of extirpation (Dodd et al. 2015), may have not been realised. The development and implementation of effective detection methods at both small and large spatial scales (‘site scale’ and ‘landscape scale’, respectively) is required to improve detection rates and provide the deployment of timely and effective control tactics, improving eradication likelihood. The research presented in this thesis develops methods to improve surveillance and detection techniques for alligator weed within catchments and waterways.

1.6.3 Optimising detection techniques for alligator weed
As mentioned earlier, a key impediment to the successful eradication of alligator weed is the ability to detect infestations in an early stage of invasion so that control can be enacted before the weed can spread further. Detection ability is currently lacking at a ‘landscape scale’, i.e. detecting completely new infestations at an early stage of invasion, and at a ‘patch scale’ at a known infested site, i.e. detecting patches of alligator weed for herbicide treatment in a reach of creek known to contain alligator weed. For example, in the past decade, there have been several accounts of large infestations of alligator weed found within the Melbourne metropolitan region of Victoria, Australia, but these have not been detected at an early stage
resulting in long term costs and associated problems caused by uncontrolled infestations (Clements et al. 2011).

Currently, the sole method used to detect alligator weed is on-ground human surveillance, involving either on-ground field surveys, or public and/or industry reporting of infestations. As described earlier (section 1.5.1), these methods are limited and can mean detecting infestations in an early stage of invasion is difficult which can result in insufficient monitoring for high probability of early detection (Mack et al. 2000). For these reasons, additional methods are required to supplement existing activities to enable improved detection of alligator weed as well as other high priority aquatic weeds.

Remote sensing has the potential to add to our ability to detect alligator weed and provide accurate and timely information to inform eradication programs. Utilising aerial imagery has the ability to overcome many of the limitations recognised by current on-ground human surveillance methods. At a ‘landscape scale’, the use of high resolution aerial imagery (orthophotos) may provide an effective technique to detect and monitor infestations improving eradication likelihood. This technology has been previously used in weed management practices, but it’s efficacy for detecting alligator weed in waterways has not been demonstrated and subsequently has not been incorporated into eradication programs.

Chapter 2 of this thesis demonstrates the effective use of high altitude aerial imagery (orthophotos) to detect and document large infestations of alligator weed at a ‘landscape scale’.

At a ‘site scale’, detection of alligator weed using unmanned aerial vehicle (UAV) technology has shown promise (Slaughter et al. 2008; Sukkarieh 2009; Göktoğan et al. 2010). However, the effectiveness of the detection technique for alligator weed (and invasive aquatic plants in general) has gone unquantified (quantitatively comparing the technology to current detection methods), which has limited it’s uptake in eradication programs. The efficacy of UAV technology for detection of alligator weed (and other high risk invasive aquatic plants) is required to be evaluated against current on-ground detection techniques. Another hurdle for this technology to be incorporated into eradication programs is its cost-effectiveness compared to current detection techniques, this has subsequently been attempted in a recent report (Apps and Deer 2015). However, because the efficacy of UAV technology for detection of aquatic weeds is largely unknown compared to current detection methods, the cost-benefit analysis assumes each detection method has the same level of detection ability, which is highly unlikely.
Chapter 3 of this thesis reports on the current efficacy of unmanned aerial vehicle technology, including the use of automated algorithms, to detect patches of alligator weed at the ‘site scale’ that are growing in waterways and compares results to current detection techniques.

The development and implementation of this technology will likely enable eradication programs to detect new infestations earlier and provide the deployment of control measures in a timely response, improving eradication likelihood and potentially at a reduced cost.

1.6.4 Optimising control techniques for alligator weed targeted for extirpation

Alligator weed has been proven difficult to control worldwide using physical, biological and chemical control techniques, which all have their benefits and shortcomings in specific situations (van Oosterhout 2007). Current control techniques utilised for alligator weed targeted for extirpation include (1) herbicide control techniques and (2) physical (manual and mechanical removal) control techniques (van Oosterhout 2007).

The effectiveness of herbicides currently utilised for management of alligator weed has been comprehensively reviewed by Dugdale and Champion (2012), and more recently further information for herbicide control of alligator weed in rice has been documented (Willingham et al. 2015). It is recognised that multiple herbicide applications over multiple years are required to kill any above ground alligator weed and deplete underground root storages to eventually exhaust the plant (Bowmer et al. 1991; van Oosterhout 2007; Hofstra and Champion 2010). However, there is limited field information on the long term (greater than one year) effectiveness of any herbicides in eliminating alligator weed in an early stage of invasion of catchments and waterways (Dugdale and Champion 2012). In Australia, New Zealand and the USA, the herbicides glyphosate, metsulfuron-methyl and more recently imazapyr are commonly utilised for control of alligator weed in aquatic situations. Glyphosate is considered to be less effective in controlling above and below ground alligator weed than metsulfuron-methyl and imazapyr (Hofstra and Champion 2010; Dugdale and Champion 2012). Control of rooted alligator weed has been difficult to achieve requiring multiple applications of herbicide per year, with the most effective herbicides reported as being imazapyr (Allen et al. 2007; Hofstra and Champion 2010; Langeland 1986; Tucker et al. 1994) and metsulfuron-methyl (Hofstra and Champion 2010; Schooler et al. 2008; Schooler et al. 2010). However, effective control has also been achieved with glyphosate (Schooler et al. 2008; Clements et al. 2014a). Floating aquatic alligator weed can be controlled effectively with glyphosate (Langeland 1986; Sainty et al. 1998; Chandrasena and
Although, management of subsequent fragmentation following herbicide application may be required (Gunasekera et al. 2006; Dugdale et al. 2010; Clements et al. 2012). Single or infrequent herbicide applications per season provide only short term control. Above ground or above water biomass is reduced following herbicide application, however rapid regeneration occurs from plants parts that are protected from herbicide application. Underwater and underground stems, roots and rhizomes that are protected from aerially applied herbicide and poor translocation through the plant contributes to regeneration success of alligator weed (Dugdale and Champion 2012). It is acknowledged that translocation of herbicide to underground portions of the plant is poor and regrowth will occur. Several repeat applications are usually required to achieve adequate control, even at maximum label rates (Sainty et al. 1998; Hofstra and Champion 2010). The eradication approach is to damage above ground plant material by repeated and frequent herbicide applications. After each herbicide application it is expected that the plant will respond by establishing new shoot growth, maintained by underground root and rhizome reserves. If each successive application is applied before there is significant regrowth, then the plant will eventually exhaust its reserves and be eliminated (a resource depletion strategy). However, if the frequency of herbicide application is such that it allows replenishment of the underground parts, eradication will not be achieved (van Oosterhout 2007; Dugdale and Champion 2012). Although this strategy is utilised in eradication programs in Australia and New Zealand it is unknown how many applications are required over a given period to eliminate infestations in aquatic environments (Dugdale and Champion 2012).

Chapter 4 of this thesis addresses this research gap and reports on the effectiveness of herbicides in eliminating patches of aquatic alligator weed in an early stage of invasion.

Eradiation remains difficult to achieve with herbicides and physical removal of the last remaining plants is sometimes required (Dugdale and Champion 2012). Physical removal techniques for control of alligator weed targeted for extirpation have been described (van Oosterhout 2007). Physical control techniques require the complete excavation of all above and below ground alligator weed to prevent regrowth (Sainty et al. 1998). In aquatic situations alligator weed generally lacks a deep penetrating root system compared to the terrestrial form, most probably due to the roots obtaining the required water and nutrients directly from the water column and sediment (Geng et al. 2007; Julien et al. 1992). This growth habit therefore lends itself to physical removal. Physical removal is initially much more labour intensive than herbicide application, however due to the difficulty of controlling alligator weed with herbicide (multiple applications, over multiple years), it provides a
method that can remove most, if not all of the weed and eliminate regrowth in one instance. Physical removal should not result in dispersal of plant propagules, provided appropriate hygiene measures are adopted. However, if appropriate hygiene measures are not implemented, stem fragments generated from physical removal only encourage its spread (Johnson and Brooke 1989; Sainty et al. 1998; van Oosterhout 2007; Hofstra and Champion 2010). Physical removal provides an alternative to herbicide use in areas where herbicide may be deemed inappropriate (Clements et al. 2014a) and it is recommended for small infestations; particularly for the initial invasion of a catchment or after a period of chemical control has suppressed formerly high levels of biomass to low levels (van Oosterhout 2007). Manual excavation has been shown to be effective for eradication of small patches of terrestrial alligator weed (Sainty et al. 1998), but no studies have determined the effectiveness of physical removal as an eradication technique for aquatic alligator weed.

Chapter 4 of this thesis addresses this research gap and reports on the effectiveness of physical removal in eliminating patches of aquatic alligator weed in an early stage of invasion.

Following herbicide applications in aquatic situations, a substantial decrease in biomass can be observed at treatment sites, but regrowth nearly always occurs, either at the treatment site or at downstream locations within a catchment (Dugdale et al. 2010). Anecdotal observations suggest that herbicide application results in the production of many alligator weed stem fragments and that a proportion of these are viable and capable of colonisation, potentially contributing to the spread of alligator weed within catchments and waterways (Gunasekera et al. 2006; Prichard 2002). However, fragmentation rates and the viability of stem fragments derived from particular control techniques have not been determined and need elucidating to inform eradication programs.

Chapter 5 of this thesis addresses this research gap and reports on an experiment to determine the effect of herbicide treatments on the production of stem fragments in aquatic alligator weed and their subsequent viability.

If eradication programs based on herbicide application inadvertently increase viable stem fragment production, then eradication programs will be compromised (Dugdale et al. 2010). The number of viable propagules entering the water is required to be evaluated under different control regimes and subsequently restricted. The use of barriers to prevent stem fragment dispersal following herbicide application is recommended, as well as the continued development of techniques to limit viable stem fragment production (Prichard 2002; van Oosterhout 2007; Dugdale et al. 2010). For example, incorporating commercially available
plant hormones into herbicide treatments may prevent disintegration of patches (Chandrasena and Pinto 2007). This potential technique to reduce fragmentation and the production of viable stem fragments has not been attempted.

Chapter 6 of this thesis addresses this research gap and reports on an experiment to investigate the usefulness of commercially available plant growth regulators (PGRs) in reducing the number of viable propagules produced by alligator weed post-herbicide application.

It is difficult to determine which herbicide is most effective in aquatic environments because the impacts of herbicides on all key alligator weed response metrics (above ground biomass, below ground biomass and stem and root viability) are unknown for most herbicides (Dugdale and Champion 2012). Further, herbicides are often applied with surfactants or other adjuvants to improve herbicide efficacy, however their efficacy for control of alligator weed has largely gone unquantified in aquatic environments. Given many adjuvants are toxic to aquatic biota and have higher environmental toxicity than the herbicide itself (Brausch et al. 2007; Siemering et al. 2008; Tatum et al. 2012), determining their efficacy is required to enhance eradication programs, as well as minimising off-target impacts. Although there is a lot of data available in the literature regarding the efficacy of different herbicides on short term control of above and below ground alligator weed, none have looked at all aspects of control that need to be studied for effective management of alligator weed in aquatic environments. No study has simultaneously looked at the impact of herbicide control on above and below ground biomass as well as viable stem fragment production. These are the three important measures when determining the efficacy of a particular control regime for effective alligator weed management in aquatic environments, particularly when incorporated into eradication programs.

Chapter 7 of this thesis addresses this research gap and reports on an experiment that evaluates these three important response metrics, for the control of alligator weed in an early stage of invasion of waterways, utilising multiple herbicides and a range of surfactant systems.

Chapter 8 provides a concluding discussion which summarises the major findings of the body of published work, implications for aquatic plant management and directions for future research.
1.7 Aims and outline of thesis
The overall aim of this PhD research was to examine and optimise the management of the invasive aquatic plant, alligator weed (*Alternanthera philoxeroides*), to increase the probability of extirpation from catchments and waterways. Two specific aspects are recognised for optimising the management of invasive aquatic plants, including alligator weed: (1) improving detection and surveillance strategies to enable early detection so that effective control measures can be employed and (2) optimising control techniques (being existing or novel techniques). This research project outlines and answers key research questions within each of these distinct overarching categories. Chapters 2 – 3 address improving detection and surveillance strategies, and Chapters 4 – 7 address optimising control techniques. The research conducted in this thesis and how each study interrelates is depicted in Figure 2. The key research questions evaluated in each of the publications / chapters are described below (section 1.7.1).
Figure 2. The structure, organisation and research into optimising the management of invasive aquatic plants targeted for extirpation from catchments and waterways: utilising alligator weed (*Alternanthera philoxeroides*) as a target species.
1.7.1 Key research questions

(1) Can orthophotos (high altitude aerial imagery) be utilised to detect large infestations of floating aquatic alligator weed infestations at a ‘landscape scale’?
(2) Can retrospective orthophotos be utilised to determine how long an infestation of alligator weed has been present to provide guidance to eradication programs?
(3) What is the potential growth rate of aquatic alligator weed if left uncontrolled in southern Australia?
(4) What is the annual lateral rate of expansion of floating alligator weed if left uncontrolled in southern Australia?
(5) What is the biomass accumulation of alligator weed over a five year period in southern Australia?


(1) Can Unmanned Aerial Vehicle (UAV) technology (low altitude aircraft) be utilised to detect patches of alligator weed invading catchments and waterways targeted for extirpation?
(2) Is UAV technology as effective at detecting patches of alligator weed growing along urban creeks and wetlands as conventional on-ground detection methods?
(3) What size of alligator weed infestations growing along urban creeks and wetlands can be detected using conventional on-ground detection methods?
(4) What size of alligator weed infestations growing along urban creeks and wetlands can be detected through visual assessment of standard Red Green Blue (RGB) aerial images collected by a UAV?
(5) What size of alligator weed infestations growing along urban creeks and wetlands can be detected using an automated algorithm to scan RGB images for the spectral signature of alligator weed?
(6) Can alligator weed be distinguished from other vegetation types growing along urban creeks and wetlands by visual assessment of RGB images collected by a UAV?

(7) Can automated algorithm technology scan RGB images for the spectral signature of alligator weed and distinguish it from other vegetation types growing along urban creeks and wetlands?

(8) Is UAV technology as effective at detecting patches of alligator weed growing over water and along the margins of waterbodies (in amongst dense marginal vegetation) as conventional on-ground detection methods?

(9) How effective are on-ground field control staff at detecting patches of alligator weed growing along urban creeks and wetlands?


(1) Are herbicides and physical removal effective control techniques to eliminate patches of aquatic alligator weed in an early stage of invasion of catchments and waterways?

(2) Does herbicide rate impact the efficacy of single applications of the herbicides glyphosate, metsulfuron-methyl and dichlobenil to control aquatic alligator weed?

(3) Does the use of a surfactant in combination with the herbicide, metsulfuron-methyl, increase efficacy of control of aquatic alligator weed?

(4) Is repeated physical removal an effective control regime to eliminate patches of aquatic alligator weed in an early stage of invasion of catchments and waterways?

(5) Can patches of aquatic alligator weed in an early stage of invasion of catchments and waterways be eliminated by repeated applications of herbicides?

(6) How many years of repeated herbicide application or physical removal are required to eliminate aquatic alligator weed in an early stage of invasion?

(7) Does herbicide rate impact the efficacy of the herbicide glyphosate when applied multiple times over multiple years to eliminate patches of aquatic alligator weed from catchments and waterways?

(1) Does herbicide application result in the production of *viable* alligator weed stem fragments capable of regeneration, potentially increasing the rate of dispersal and establishment throughout catchments?

(2) Do herbicides currently utilised in eradication programs for alligator weed produce differing amounts of viable alligator weed stem fragments?

(3) Does herbicide rate impact on viable alligator weed stem fragment production?

(4) Does the time of stem fragmentation and viable stem fragment production differ between different herbicides?

(5) Does the use of a surfactant in combination with the herbicide metsulfuron-methyl impact on viable fragment production?


(1) Can plant growth regulators (PGRs) be used in association with herbicides to improve control regimes for alligator weed?

(2) Do PGRs reduce the number of *viable* alligator weed stem fragments produced post-herbicide application?

(3) Do PGRs delay the time of stem fragmentation compared to herbicide alone?

(4) Do PGRs alter the efficacy of herbicides i.e. improve control of above and below ground biomass?

(5) Can the production of alligator weed stem fragments produced post-herbicide application be associated with an abscission (i.e., autofragmentation) process?

Chapter 7 - Clements D, Dugdale TM, Butler KL, Florentine SK, Sillitoe J (Accepted) Herbicide efficacy for aquatic *Alternanthera philoxeroides* management in an early stage of invasion: integrating above-ground biomass, below-ground biomass and viable stem fragmentation. *Weed Research*.

(1) Do the herbicides not currently utilised in Australia that are employed abroad improve the probability of extirpating alligator weed from catchments and waterways?
(2) Does the size of alligator weed infestations impact on the efficacy of herbicides to control above and below ground biomass of alligator weed and viable fragment production?

(3) Does the herbicide imazapyr produce fewer viable alligator weed stem fragments compared to glyphosate and metsulfuron-methyl?

(4) Does the time to viability of stem fragments differ between the herbicides imazapyr, glyphosate and metsulfuron-methyl?

(5) Is the herbicide imazapyr more effective at controlling above ground biomass of alligator weed than glyphosate and metsulfuron-methyl?

(6) Is the herbicide imazapyr more effective at controlling below ground biomass of alligator weed than glyphosate and metsulfuron-methyl?

(7) Do different formulations of the herbicide glyphosate produce the same amount of viable alligator weed stem fragments and achieve the same level of control of above and below ground biomass of alligator weed.

(8) Do surfactant systems in combination with the herbicides imazapyr, glyphosate and metsulfuron-methyl enhance herbicide efficacy on above and below ground biomass of alligator weed and reduce the amount of viable alligator weed stem fragments produced.

The development of more effective management strategies for alligator weed will result in an increased likelihood of extirpating this invasive aquatic plant from catchments and waterways and reduce the impacts on agricultural productivity, social amenity and biodiversity values. Chapter 8 provides a synthesis of the major research findings, implications for aquatic plant management and directions for future research. The structure and organisation of research, as well as the management techniques and principles developed for alligator weed in this thesis, provide a model for programs that aim to optimise the management of invasive aquatic plants that pose a biosecurity risk and those targeted for extirpation from catchments and waterways.


**Bridge between Chapters 1 and 2**

Chapter 1 described the scope of the research conducted in this thesis, in context with the key relevant literature, to optimise the management strategies for the invasive aquatic plant, alligator weed (*Alternanthera philoxeroides*) in an early stage of invasion in order to achieve extirpation from catchments and waterways. Chapter 2 demonstrates the extent of the problem and the potential impact that alligator weed can have on waterways by utilising high altitude aerial imagery (orthophotos) to detect large infestations of aquatic alligator weed and provides an insight into the use of retrospective aerial imagery to guide subsequent management actions. Effective management of invasive aquatic plants targeted for extirpation from catchments and waterways requires coupling effective detection and control strategies to prevent reproduction.
Chapter 2 – Extent of the problem: alligator weed an invasive aquatic plant

Citation:
Growth of aquatic alligator weed (*Alternanthera philoxeroides*) over 5 years in south-east Australia

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Abstract

The largest known infestation of alligator weed *Alternanthera philoxeroides* (Mart.) Griseb. in Victoria, Australia, was reported in January 2009 in an urban pond. To determine how long the infestation had been present, high quality digital aerial images (orthophotos) were gathered for the site. Since all infestations are subject to ongoing eradication programs in Victoria, historical orthophoto records provide a unique opportunity for retrospective analysis, to calculate and report on the uncontrolled growth of aquatic alligator weed. Using geographic information system software (GIS), orthophotos were visually assessed to delineate the area of infestation for each year, from which annual increases in area were calculated. The infestation increased in area from ca. 0.029 ha in December 2004 to ca. 0.73 ha in December 2009, to cover 33% of the 2.2 ha water body. The annual area expansion was 200% for the first year of record. This reduced each year, to 22% at the end of the five year period. The mean lateral rate of expansion for floating alligator weed over the five years was 4.3 m (SD 2.2) annually. The average biomass of alligator weed at the site in summer 2010 was 4.9 kg dry weight m$^{-2}$. Using the area of infestation from the December 2009 orthophoto the total estimated biomass in the pond equated to 35.6 tonnes dry weight.

Key words: *Alternanthera philoxeroides*, alligator weed, growth, expansion, biomass

Alligator weed *Alternanthera philoxeroides* (Mart.) Griseb. is a serious weed that has invaded a wide range of habitats in Australia and other regions of the world including the USA, New Zealand, China and other parts of Asia (Sainty et al. 1998). It poses a significant threat to Australia's waterways, wetlands, floodplains and irrigation systems and has the potential to become far more widespread. Modelling has shown that most of eastern and southern areas of continental Australia are suitable for its growth (Julien et al. 1995). Alligator weed is a stoloniferous and rhizomatous perennial that grows rapidly in both terrestrial and aquatic habitats (Sainty et al. 1998). In aquatic situations floating mats can cover waterbodies, restricting human use, excluding desirable plant species, interfering with aquatic ecology and restricting water flow (Julien et al. 1992). Rooted in the soil near the water's edge or in the substrate beneath shallow water, mats of interwoven horizontal stems, from which upright stems arise, float and extend over the surface of deeper water (Julien 1995). Alligator weed does not reproduce sexually in its introduced range, its primary means of dispersal is through stem fragmentation where stem fragments break off or floating mats dislodge and drift, lodging elsewhere and creating new infestations (Julien et al. 1992 and Dugdale et al. in press).

Julien et al. (1995) reported that alligator weed grows best and forms dense monospecific stands in sub-tropical to cool, but not cold, temperature climates. In cooler, higher latitude regions the shorter growing season and occurrence of frosts kills top growth and restricts biomass accumulation. Southern Victoria, Australia, 37°S, provides warm summer growth periods (December-March) and cool winters with infrequent frosts, where growth of alligator weed ceases.

Alligator weed was first recorded in Victoria, Australia, in 1995, being grown as a vegetable (Gunasekera and Bonilla 2001), and naturalised populations were subsequently found in 1997 (Gunasekera and Adair 1999). It is declared a high priority weed being targeted for eradication from the state. Currently, alligator weed
Infestations in Victoria are concentrated within the Melbourne metropolitan region, although there are a few outlier infestations. The largest known infestation of alligator weed in Victoria was reported to authorities in January 2009 in an urban pond in Springvale (37°57'18.65"S; 145°10'10.06"E, Figure 1). The site consists of a 2.2 ha standing water body that is used for stormwater retarding and irrigation purposes. The pond receives high nutrient, urban stormwater runoff from Mile Creek (total nitrogen and phosphorus averaged 1.48 mg L⁻¹, and 0.073 mg L⁻¹, respectively, from monthly low flow sampling during the five year period (Melbourne Water 2010)).

There have been very few published studies reporting the growth rate of aquatic alligator weed, particularly over consecutive years (Julien et al. 1992), most likely due to control programs being enacted soon after detection. High resolution (15–35 cm) digital aerial images (orthophotos) dating back five consecutive years were available for the study site, providing an opportunity to document the uncontrolled growth and expansion of aquatic alligator weed. Aerial photography was the first remote sensing method to be employed for studying and mapping vegetation, with early studies dating back to the 1960s and 1970s (Silva et al. 2008). Aerial photography interpretation (API) has been used extensively around the world to detect changes in species composition and distribution and to evaluate estimates of habitat features when accompanied with complementary field investigations. API allows for comparison of aerial images taken over time, providing a cost effective, time efficient tool to detect and analyse change, while providing information from the past that has not previously been recorded (Fitzgerald et al. 2006). The limitations of API are varied; the process requires suitable aerial photography relevant to the question of interest, and an analyst to identify key elements of the image (including tone, colour, pattern, shape, shadow, texture, contrast) for the species or habitat of interest. Most analyses of aerial imagery rely on visual interpretation where plant species can be discriminated when using high spatial resolution images (Silva et al. 2008), although identifying boundaries between vegetation community types is a recognised problem (Adam et al. 2010).

Orthophotos were available during the summer growth period for February 2001, December 2004, December 2005, November 2006, December 2007, January 2009 and December 2009. Using the orthophoto from January 2009, infestations within the pond were identified and then compared to infestations recorded during a site visit in May 2009. This was repeated in February 2010 using the December 2009 orthophoto. Both activities confirmed that all patches of alligator weed present in the pond were visible and comparable to the orthophotos. A thorough search of the inlet stream and associated water bodies upstream of the site was also conducted, which confirmed that this was the most upstream infestation of alligator weed in the catchment.

Using ArcView® GIS software, orthophotos were visually assessed to delineate the area of infestation for each year. A polygon layer was created defining the outer boundary of the pond, using the February 2001 orthophoto. Using orthophotos from December 2004 to December 2009, polygons were then created outlining the extent of each infestation for each subsequent year (Figure 2). All polygon layers were created using a viewing scale between 1:50 to 1:125, dependent on image quality. API depends on the subjective judgement of the interpreter and the quality of photographs used (Finkbeiner et al. 2001 and McGlone 2004 cited in Zhu et al. 2007), and variability was minimised by using the same interpreter for all orthophoto analysis. Infestation polygons were clipped with the February 2001 outline polygon so that only the alligator weed present within the pond was included in the assessment. Using the outline of the pond as a boundary means any alligator weed growing on the margins was ignored and therefore the area of alligator weed present was underestimated, restricting reporting to the increase of infested area within the pond alone. The area of each patch of alligator weed was calculated, giving an estimated total area of the infestation for each consecutive year. The annual area expansion was determined by calculating the difference between the areas occupied in two consecutive years and dividing this value by the area occupied in the first of those years. These values were normalised to reflect “annual periods” associated with the differing number of days and months between available orthophotos.

No alligator weed was detected in the February 2001 orthophoto. Orthophotos were not available thereafter until December 2004, when two small infestations could be detected (Figure 2). It has been observed (Sainty et al. 1998) that a few stems at a site can grow into a patch 1-3 m
Growth of aquatic alligator weed in south-east Australia

**Figure 1.** The authors sampling an infestation of floating alligator weed at the study site.

**Figure 2.** Orthophotos of alligator weed growing in an urban stormwater pond. Infestations outlined in white. Image: A) 3 December 2004; B) 11 December 2005; C) 17 November 2006; D) 25 December 2007; E) 3 January 2009; F) 2 December 2009.
Figure 3. Area of alligator weed present and annual increase in area. Bars represent the area of alligator weed present at the study site for each year of growth (bottom x axis). Line represents the percentage annual increase in area (top x axis) over the 5 year growth period. Values have been normalised to reflect annual periods.

Table 1. Annual lateral expansion (m) of alligator weed over a five year period. Values have been normalised to reflect annual periods.

<table>
<thead>
<tr>
<th>Annual Period</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
<th>Mean</th>
<th>SD</th>
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<tr>
<td>Dec 2004 – Dec 2005</td>
<td>4.4</td>
<td>4.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.6</td>
<td>0.2</td>
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<tr>
<td>Dec 2005 – Nov 2006</td>
<td>3.2</td>
<td>3.9</td>
<td>4.9</td>
<td></td>
<td></td>
<td></td>
<td>4.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Nov 2006 – Dec 2007</td>
<td>10.1</td>
<td>2.4</td>
<td>3.5</td>
<td>10.4</td>
<td></td>
<td></td>
<td>6.6</td>
<td>4.3</td>
</tr>
<tr>
<td>Dec 2007 – Jan 2009</td>
<td>4.7</td>
<td>5.5</td>
<td>5.5</td>
<td>3.5</td>
<td>2.4</td>
<td>4.8</td>
<td>4.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Jan 2009 – Dec 2009</td>
<td>2.2</td>
<td>2.2</td>
<td>2.6</td>
<td>2.6</td>
<td>3.5</td>
<td>3.3</td>
<td>2.7</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Table 2. Means and ranges for water depth and alligator weed infestation characteristics at Springvale in February 2010.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>Mean</th>
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</thead>
<tbody>
<tr>
<td>Water depth (m)</td>
<td>1.9–3.95</td>
<td>3.04</td>
</tr>
<tr>
<td>Overall vertical extent of weed mat (m)</td>
<td>1.4–1.95</td>
<td>1.64</td>
</tr>
<tr>
<td>Depth of submerged weed mat (m)</td>
<td>0.6–1.05</td>
<td>0.82</td>
</tr>
<tr>
<td>Height of emergent weed mat (m)</td>
<td>0.7–1.0</td>
<td>0.82</td>
</tr>
<tr>
<td>Dry biomass (g m⁻²)</td>
<td>3,730–6,960</td>
<td>4,867</td>
</tr>
</tbody>
</table>

wide in one growing season through stoloniferous growth. In December 2004 the infestation covered ca. 290 m² and increased to ca. 880 m² by December 2005, an annual area increase of 200%. In comparison Julien et al. (1992) report that over-water biomass can double, or increase by 100%, in 41 days during the growing season. Over the next four years the infestation increased and by December 2009 was calculated to be ca. 7,300 m². Although the annual area expansion had decreased to 22%, there was still an increase in area of ca. 1,200 m² during the last annual period of record (Figure 3). This represents an increase from 1.3% of the 2.2 ha pond covered by alligator weed to 32.7% in a five year period. As can be seen from the data, floating alligator weed mats are capable of extremely rapid growth in Victoria.

The annual rate of lateral expansion for alligator weed at the site was also determined. Infestations present were measured from a standard shoreline point along a perpendicular transect to the outermost edge of the weed bed for each year of orthophoto. The mean annual lateral expansion during the five year period was 4.3 m (SD 2.2) (Table 1). Mean values for the December 2004 to December 2005 annual period are based on the two infestations present in December 2004, and when new infestations appeared throughout the next five years data were added. By the January 2009 to December
2009 annual period, mean values were based on six individual infestations (Table 1). In December 2009 the interwoven mats of plant material extended laterally out to a maximum of ca. 31 m from the embankment. Julien et al. (1992) report mats growing to 70 m wide and Spencer and Coulson (1976) report that mats can extend ca. 100 m over the water surface.

Sampling was conducted in February 2010 to determine the biomass of alligator weed present. Six transects spread evenly around the pond were selected and transects traversed infestations from the shore to the outward edge of the floating mats. Twenty samples in total were taken at 5 m intervals spread evenly across the six transects. Biomass samples were harvested by wading out on top of the floating alligator weed mats and cutting through the entire depth of the weed bed within a square-sectioned tube (0.0572 m², 1.0 m in length). All plant material within the core was sorted into above- and below-water portions (emergent and submersed), placed into bags, cleaned later the same day, weighed wet and placed into drying ovens at 80°C until constant dry weight was achieved. At each sampling point, water depth, depth of the submersed alligator weed and height of the emergent alligator weed was recorded (Table 2).

Biomass averaged 4,867 g dry weight m⁻² (SD 899) (Table 2) or 43.03 kg wet weight m⁻² (SD 7.90). In December 2009 the infestation measured ca. 7,300 m², and using the average biomass from February 2010 the total estimated biomass in the pond was 35.6 tonnes dry weight (Table 2). Biomass values reported here are much higher than previously reported in the literature for aquatic alligator weed. In Sydney, Australia, peak biomass was attained in late summer and was 3,214 g m⁻² for a freshwater site and 3,252 g m⁻² for an estuarine site (Julien et al. 1992), while in South Carolina biomass peaked at 392 g m⁻² in late summer for a stream infestation (Davis et al. 1983 cited in Julien et al. 1992). Pesacreta (1999) reported mean biomass of ca. 600 g m⁻² in control plots in North Carolina between June and July 1998. On the margins of a lake in northern New Zealand, above ground biomass in patches of alligator weed ranged from ca. 700 to 1,700 g m⁻² (Bassett et al. 2010). A study in Oksiopat Lake (Bishnupur), Manipur, India reported biomass between 19.94 g m⁻² and 139.41 g m⁻² over a two year study period (Devi and Sharma 2010). Other studies of biomass taken from lakes in India have reported biomass of less than 250 g m⁻² (reviewed by Devi and Sharma 2010). From the results of this study we have shown that biomass accumulation of alligator weed can be high in Victoria if left uncontrolled.

The aquatic ecotype of alligator weed forms mats of entangled stems and has adventitious roots that may be attached to the substrate or the bank, or in deeper water may be free floating. With each consecutive season of growth new stems are produced from prostrate mats from the previous season’s growth (Julien et al. 1992). Dense aquatic alligator weed infestations are reported to consist of mats of older stems up to 0.3 m thick, supporting erect stems up to 0.8 m tall (Julien, 1995), and may extend 1 m or more down into the water (Spencer and Coulson 1976). In our study the submersed alligator weed (including root material) accounted for an average of 60.4% of the biomass at each sampling site, while the overall vertical extent of the alligator weed mat averaged 1.64 m (Table 2). This very dense growth was robust enough to support two people wading over the weed bed without sinking more than ca. 0.25 m into the water and is probably a result of good growing conditions provided by stable and slow moving water conditions, low interspecific competition and abundant nutrient input. The water depth at the sampling points ranged from 2–4 m and all alligator weed was free floating (Table 2). There were weak positive linear correlations between water depth and biomass (R² = 0.115), and distance from shore and biomass (R² = 0.1573), indicating there was no net accumulation of alligator weed biomass in the areas of the mat that have been intact the longest, i.e. those nearest the embankment.

This study demonstrates the potential growth rate of alligator weed if left unchecked in Victoria and provides an example of the insight that retrospective use of aerial photos can provide. A continued emphasis on the implementation and development of early detection programs and containment of new outbreaks when infestations are small should remain a priority in Victoria, minimising long term costs and problems caused by uncontrolled infestations.

Acknowledgements

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References


Bridge between Chapters 2 and 3

Chapter 2 investigated the growth rate of alligator weed (*Alternanthera philoxeroides*) in Victoria, Australia, over a five year period (which has not previously been recorded in southern Australia) and demonstrates the potential impact that alligator weed can have on catchments and waterways. Further, this study demonstrated the use of high altitude aerial imagery (orthophotos) to detect large infestations of aquatic alligator weed, and thus provides an insight into the use of retrospective aerial imagery to guide subsequent management actions. However, as discussed in Chapter 1 (Section 1.3.2), a key impediment to eradication of high priority aquatic weeds is the ability to detect infestations so that control measures can be enacted. Detection and surveillance techniques for alligator weed are lacking at both a ‘landscape scale’ and at a ‘site scale’ (large and small spatial scales, respectively). As demonstrated in Chapter 2, large infestations of aquatic alligator weed are able to be detected and monitored utilising high altitude aerial imagery (orthophotos), and this surveillance technique, after being demonstrated in this paper, has been incorporated into the eradication program in Victoria for detection of alligator weed and other high priority aquatic weeds, including water hyacinth (*Eichhornia crassipes*) and salvinia (*Salvinia molesta*). However, this detection technique is limited to larger spatial scales (large infestations; >5 m$^2$ in this study). Effective detection capability is required at smaller spatial scales, for example, detecting completely new infestations of alligator weed in an early stage of invasion or detecting individual patches in a reach of creek known to contain alligator weed, so that control measures can be enacted. Consequently, Chapter 3 determines the current efficacy of unmanned aerial vehicle (UAV) technology to gain low altitude aerial imagery at a small spatial scale, and investigates the use of post processing and algorithms to automatically detect smaller patches of alligator weed, such as individual patches along urban creeks and wetlands. The efficacy of this technology is evaluated against conventional on-ground detection methods, which is required to be evaluated if the technology is to be utilised by agencies responsible for the management of high risk invasive species.
Chapter 3 – Detection and surveillance of alligator weed

Citation:


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Effective strategies for detection and surveillance of alligator weed (*Alternanthera philoxeroides*) have been developed and demonstrated in Chapters 2 and 3 of this thesis. The ability to detect infestations in an early stage of invasion, so that control measures can be enacted, is critical to achieving extirpation of high risk invasive aquatic plant species. However, the ability to detect infestations is not the only effort required to achieve extirpation; effective management requires coupling detection effort with control effort to prevent reproduction. As discussed in Chapter 1 (Section 1.3.2), as well as effective detection techniques (Chapters 2 and 3), effective control measures must be available that (1) decrease population numbers of a given species at a given rate and (2) prevent the spread of the target species. Chapter 4 has been developed to address the rate of decrease in population numbers of a given species, by developing effective management strategies for the control of aquatic alligator weed in an early stage of invasion of catchments and waterways, to optimise our ability to control one of the world’s most invasive aquatic plant species. There is limited information in the literature on the long term (greater than one year) effectiveness of any herbicides in eliminating patches of alligator weed in an early stage of invasion of catchments and waterways, and it is unknown how many applications are required over a given period to eliminate infestations in aquatic environments. Further, no studies have determined the effectiveness of physical removal as an extirpation technique for aquatic alligator weed. Chapter 4 evaluates the efficacy of herbicides (including surfactant systems) and physical removal, in both laboratory and field studies, to determine the ability of employed control strategies to extirpate infestations of aquatic alligator weed at the site scale.
Chapter 4 – Control of alligator weed at the site scale

Citation:

Management of aquatic alligator weed (*Alternanthera philoxeroides*) in an early stage of invasion

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Abstract

Alligator weed *Alternanthera philoxeroides* (Mart.) Griseb. is an amphibious plant that aggressively invades aquatic and terrestrial environments. It has invaded at least 14 countries and is difficult to control. The present study investigates the effectiveness of herbicides and physical removal in eliminating patches of aquatic alligator weed in an early stage of invasion. This paper firstly describes a screening trial to determine the relative efficacy of single application of three herbicides used in Australia (glyphosate, metsulfuron-methyl ± surfactant, and dichlobenil), each applied at three rates to containers of alligator weed. Control was greatest for all herbicides at rates higher than the manufacturer’s recommendation (label rate). Glyphosate at 3 × label rate (3.6 kg a.i. ha⁻¹; 10.8 g a.i. L⁻¹) and dichlobenil at 2 × label rate (31 kg a.i. ha⁻¹) provided the greatest level of control at 48 and 91 weeks after treatment. The presence of surfactant did not improve metsulfuron-methyl efficacy. Field studies were then carried out to evaluate the effectiveness of repeated physical removal and repeated applications of chosen herbicides to eliminate patches of aquatic alligator weed in an early stage of invasion of two urban streams in Melbourne, Australia. Glyphosate and metsulfuron-methyl (without a surfactant) were applied to patches of aquatic alligator weed in a best practice regime, consisting of up to three applications per year for up to five consecutive years. Glyphosate was applied at 3 × label rate, as well as at label rate. No alligator weed remained after two years of the herbicide application regime for patches treated with metsulfuron-methyl, while for glyphosate alligator weed remained in only one of 18 patches after three years. Physical removal eliminated 75% of patches after initial treatment and minimal follow up treatments were required where regrowth occurred. This study demonstrates that the management methods utilised are capable of eliminating patches of aquatic alligator weed in an early stage of invasion in two to three years.

Key words: *Alternanthera philoxeroides*, alligator weed, aquatic weed management, eradication, herbicide, physical removal

Introduction

Alligator weed *Alternanthera philoxeroides* (Mart.) Griseb. is a perennial stoloniferous herb in the Amaranthaceae family, originating from the Parana River area of South America (Julien et al. 1995). It has subsequently spread to and increased its range within many countries including; southern USA (first detected in 1897), New Zealand (1906), China (1930s), Australia (1946), India, Burma and Indonesia (by the 1960s). More recently it has been detected in Puerto Rico, Singapore, Vietnam, Thailand, Sri Lanka, Italy and France (Dugdale and Champion 2012).

Alligator weed is an aggressive invader of both aquatic and terrestrial environments (Sainty et al. 1998). It is particularly successful in aquatic and semi-aquatic environments where it is capable of extremely rapid growth (Clements et al. 2011). In aquatic environments alligator weed roots into the soil near the water’s edge or in the substrate beneath shallow water and produces mats of entangled stems that float and extend over the water surface. Floating mats of alligator weed can choke waterbodies, restricting human use, excluding desirable plant species, interfering with aquatic ecology and restricting water flow (Julien et al. 1992). Alligator weed poses a significant threat
to waterways, wetlands, floodplains and irrigation systems (van Oosterhout 2007). It can also invade terrestrial situations, such as pasture (Julien and Broadbent 1980), arable crops (Shen et al. 2005) and urban areas (Gunasekera and Bonilla 2001).

In its introduced range, alligator weed reproduces solely by clonal growth, as viable seeds are not produced. It efficiently disperses via stem fragmentation, where stem fragments or floating mats break off and disperse to surrounding areas, creating new infestations (Dugdale et al. 2010 and Julien et al. 1992). Although it prefers a warm growing season, it can tolerate winter frosts (Julien et al. 1995).

A biosecurity approach is commonly undertaken to manage invasive species, particularly weeds. One aspect of this approach for weed management is a goal to eradicate a species from an area in which it has become naturalised, provided it meets certain criteria: 1) it is deemed a species capable of invasion (i.e. it spreads into areas considerable distances away from parent plants (Richardson et al. 2000)); 2) it is in an early stage of invasion and occupies only a very small part of its potential range; and 3) it poses a significant threat to social, economic or environmental values.

Early invasion of alligator weed occurs in and around the metropolitan area of Melbourne, the capital city of Victoria, Australia, where it was first detected in 1996 (Gunasekera and Bonilla 2001). If left unchecked, it is anticipated that these infestations will act as a source population for dispersal to other areas of Victoria, where it will significantly compromise agricultural productivity, block irrigation and drainage infrastructure and reduce biodiversity and social amenity of aquatic environments. Thus, in addition to being a weed of national significance in Australia (Australian Government 2012), alligator weed has been declared a state prohibited species and targeted for eradication in Victoria (Victorian Government 2014). This situation is similar to New Zealand, where it is designated as an unwanted organism (New Zealand Government 2010). In the USA, China and the Australian state of New South Wales (where it has been a problem weed for more than 65 years, first detected in 1946), it is in a later stage of invasion, where it is much more widespread and abundant, so eradication is not feasible. In these locations suppression programs exist based on herbicide (Dugdale and Champion 2012) and biological control (Sainty et al. 1998), which aim to contain infestations and reduce the spread and impact of the weed. In China US$72 million is spent each year to manage alligator weed (Liu and Diamond 2005).

In Victoria, the herbicides glyphosate or metsulfuron-methyl have been the preferred methods of alligator weed management in early invasion aquatic situations. Physical removal has also been employed depending on site characteristics, environmental sensitivity and resources available. Glyphosate is labelled for use in aquatic areas but is considered to be less effective against alligator weed than metsulfuron-methyl (Dugdale and Champion 2012). The herbicide metsulfuron-methyl is commonly used in terrestrial situations, however because of its apparent success in controlling alligator weed, a permit was obtained to use it in some aquatic situations in Victoria. There are however questions of its use in aquatic environments because of toxicity, and the propensity for alligator weed to fragment and disperse after herbicide application (Clements et al. 2012; Dugdale et al. 2010). Dichlobenil is also registered for use on alligator weed in static water aquatic systems that are not used for irrigation purposes (van Oosterhout 2007).

The effectiveness of herbicides for management of alligator weed has been reviewed by Dugdale and Champion (2012). It is recognised that multiple herbicide applications over multiple years are required to kill any emergent alligator weed and deplete underground root storages to eventually exhaust the plant (Bowmer et al. 1991; van Oosterhout 2007). However, there is limited field information on the long term (greater than one year) effectiveness of any of these herbicides in eliminating alligator weed in an early stage of invasion.

Alligator weed is also managed by either manual or mechanical removal methods. These physical approaches require the complete excavation of all above and below ground alligator weed to prevent regrowth (Sainty et al. 1998). In aquatic situations alligator weed generally lacks a deep penetrating root system compared to the terrestrial form, most probably due to the roots obtaining the required water and nutrients directly from the water column and sediment (Geng et al. 2007; van Oosterhout 2007). This growth habit therefore lends itself to physical removal. Physical removal is initially much more labour intensive than herbicide application, however due to the difficulty of controlling alligator weed with herbicide (multiple applications, over multiple years), it provides a method that can remove most, if not all alligator weed and eliminate regrowth in one instance.
Table 1. Herbicides and rates applied to containers of alligator weed. Five replicates per treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Product</th>
<th>Rate</th>
<th>Tank rate (herbicide a.i./L)</th>
<th>a.i. (g / ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metsulfuron, 0.5 × label</td>
<td>Esteem® 600 g / kg</td>
<td>5 g / 100 L</td>
<td>0.03</td>
<td>10</td>
</tr>
<tr>
<td>Metsulfuron, 1 × label</td>
<td>Esteem® 600 g / kg</td>
<td>10 g / 100 L</td>
<td>0.06</td>
<td>20</td>
</tr>
<tr>
<td>Metsulfuron, 2 × label</td>
<td>Esteem® 600 g / kg</td>
<td>20 g / 100 L</td>
<td>0.12</td>
<td>40</td>
</tr>
<tr>
<td>Metsulfuron, 0.5 × label + surfactant</td>
<td>Esteem® 600 g / kg + Pulse®</td>
<td>5 g / 100 L + 200 mL / 100 L</td>
<td>0.03</td>
<td>10</td>
</tr>
<tr>
<td>Metsulfuron, 1 × label + surfactant</td>
<td>Esteem® 600 g / kg + Pulse®</td>
<td>10 g / 100 L + 200 mL / 100 L</td>
<td>0.06</td>
<td>20</td>
</tr>
<tr>
<td>Metsulfuron, 2 × label + surfactant</td>
<td>Esteem® 600 g / kg + Pulse®</td>
<td>20 g / 100 L + 200 mL / 100 L</td>
<td>0.12</td>
<td>40</td>
</tr>
<tr>
<td>Glyphosate®, 1 × label</td>
<td>Roundup Biactive® 360 g / L</td>
<td>10 mL / L</td>
<td>3.6</td>
<td>1,206</td>
</tr>
<tr>
<td>Glyphosate®, 3 × label</td>
<td>Roundup Biactive® 360 g / L</td>
<td>30 mL / L</td>
<td>10.8</td>
<td>3,618</td>
</tr>
<tr>
<td>Glyphosate®, 6 × label</td>
<td>Roundup Biactive® 360 g / L</td>
<td>60 mL / L</td>
<td>21.6</td>
<td>7,236</td>
</tr>
<tr>
<td>Dichlobenil, 0.5 × label</td>
<td>Sierraron® G, 67.5 g / kg</td>
<td>1,150 g / 100 m²</td>
<td>N/A</td>
<td>7,763</td>
</tr>
<tr>
<td>Dichlobenil, 1 × label</td>
<td>Sierraron® G, 67.5 g / kg</td>
<td>2,300 g / 100 m²</td>
<td>N/A</td>
<td>15,525</td>
</tr>
<tr>
<td>Dichlobenil, 2 × label</td>
<td>Sierraron® G, 67.5 g / kg</td>
<td>4,600 g / 100 m²</td>
<td>N/A</td>
<td>31,050</td>
</tr>
<tr>
<td>No Herbicide</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: a.i. = active ingredient, metsulfuron = metsulfuron-methyl

* All glyphosate present as the isopropylamine salt formulation

Further, physical removal provides an alternative to herbicide use in areas where herbicide may be deemed inappropriate. Physical removal is recommended for small infestations; particularly the initial invasion of a catchment or after a long period of chemical control has suppressed formerly high levels of biomass to low levels (van Oosterhout 2007).

The present study investigates the effectiveness of herbicides and physical removal in eliminating patches of aquatic alligator weed in an early stage of invasion. It includes a screening trial, in containers, to study the relative effectiveness of single applications of herbicides (herbicide type, herbicide rate and presence of surfactant). Field studies were then carried out to evaluate the effectiveness of repeated physical removal and chosen herbicide strategies, over multiple years, to eliminate alligator weed in an early stage of invasion of two urban streams in Melbourne, Australia.

Materials and methods

Screening trial

Alligator weed stem cuttings consisting of four nodes with apical shoot tips, without roots, were collected from a single patch at Patterson River (38°24’59.98”S; 145°10’11.78”E) in November 2007. Sixty-five containers (0.58 m diameter by 0.45 m tall) were half filled with topsoil that was augmented with 4 kg m⁻² Osmocote® general purpose fertiliser (9 month slow release). A layer of washed sand was then added, before being filled with municipal water (10 to 15 cm above soil height). Five alligator weed stems were planted into each container and left to establish for 15 weeks in a shade house. Water levels were maintained during the study period with fresh water extracted from a pond. The herbicides metsulfuron-methyl (2-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoyl sulfamoyl) benzoic acid), glyphosate (N-(phosphonomethyl) glycine, present as the isopropylamine salt) and dichlobenil (2, 6-dichlorobenzonitrile) were applied to each of the alligator weed containers in March 2008 (see Table 1 for herbicides, rates of application and surfactants used). Treatments were assigned in a random manner to containers so that the trial has a completely randomised design (Cochran and Cox 1957). Although wind speed was low during treatment, a temporary barrier (tent) was erected over each container to prevent herbicide drift contaminating adjacent containers. Liquid herbicide was applied from above with a pneumatic sprayer fitted with a calibrated Even Flat Spray Tip (TP8002E). The sprayer was operated in the range of 2.8 to 3.0 bar and each treatment was sprayed for 10 seconds, when runoff was observed on aerial foliage, delivering a spray volume of 335 L ha⁻¹ of spray solution. A control treatment did not include any herbicide application.

To assess herbicide efficacy the number of apical and lateral shoot tips >2 mm in length (hereafter referred to as shoot tips) were counted for each container prior to herbicide application and at 3, 5, 7, 9, 11, 48 and 91 weeks after treatment (WAT). To assess the efficacy on the parent plant only,
all stem fragments produced in each container were removed at the same WAT intervals above. The viability of these alligator weed fragments was assessed and has been reported in a previous paper (Dugdale et al. 2010).

**Screening trial statistics**

After square root transformation, the number of shoot tips at each sampling was analysed using an analysis of variance appropriate for the design (Table 2). In all analyses a container was the experimental unit. The square root of the number of shoot tips just prior to herbicide application was used as a covariate for the number of shoot tips at 48 and 91 weeks after treatment. This covariate was not used at other times because it was not an effective tool for improving the precision of the results. Some reported tests are calculated using t-statistics derived from the tables of means (with standard errors) obtained from the analyses of variance outputs described in Table 2. At 91 WAT one container was removed from the analysis as an outlier. Permutation tests were also carried out for a number of shoot tips analyses, but the P values obtained were similar to those used assuming an F-distribution and hence are not reported. All these analyses were carried out using the ANOVA directive and APERMUTE procedure within GenStat 16 (Payne 2013).

**Field study**

Study sites were established along the margins of two urban lowland streams; Merri Creek (37°46'3.68"S 144°59'4.02"E) and Patterson River (38°3'2.16"S 145°9'45.48"E) in Melbourne, Australia, between 2008 and 2010. Each study site consisted of 4 to 18 (depending on stream and year) disjoint patches of alligator weed, within a defined stream reach (Table 3). The patches were rooted into the embankment and growing out into the water body as a floating mat, typical of aquatic alligator weed (Figure 1). Each eradication technique was applied to several entire patches of alligator weed.

In summary, thirty-three patches of alligator weed were treated with the herbicides metsulfuron-methyl applied at $1 \times$ label rate or glyphosate applied at $1 \times$ label rate or $3 \times$ label rate. Twelve patches were subjected to physical removal (Table 3). In particular, a reach of the Merri Creek was selected in 2008 that contained 17 patches of alligator weed. Three patches were selected *(ad hoc)* to be treated with metsulfuron-methyl applied at $1 \times$ label rate (0.06 g a.i. L$^{-1}$) in 2008, two patches were selected to be treated with metsulfuron-methyl in 2009, 4 patches were selected for physical removal in 2008 and 8 patches were selected for physical removal in 2009 (Table 3). In 2010 a downstream reach of

![Figure 1. Alligator weed in an early stage of invasion of two urban lowland streams in Melbourne, Australia, used in the field study. Patch of alligator weed at: (A) Patterson River prior to herbicide application. (B) Merri Creek prior to physical removal.](image-url)
Management of aquatic alligator weed

Table 2. Analysis of variance for the container trial.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbicide (control v dichlobenil v glyphosate v metsulfuron-methyl)</td>
<td>3</td>
</tr>
<tr>
<td>Herbicide rate within dichlobenil</td>
<td>2</td>
</tr>
<tr>
<td>Herbicide rate within glyphosate</td>
<td>2</td>
</tr>
<tr>
<td>Herbicide rate within metsulfuron-methyl</td>
<td>2</td>
</tr>
<tr>
<td>Presence of surfactant within metsulfuron-methyl</td>
<td>1</td>
</tr>
<tr>
<td>Interaction of herbicide rate and presence of surfactant within metsulfuron-methyl</td>
<td>2</td>
</tr>
<tr>
<td>Residual</td>
<td>52</td>
</tr>
</tbody>
</table>

Table 3. Treatment and site location details for patches of alligator weed in the field study.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of patches</th>
<th>Site</th>
<th>Mean initial patch size, m² (SD)</th>
<th>Initial spring application</th>
<th>Years of application and assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metsulfuron-methyl¹, 1 × label rate (0.06 g a.i. L⁻¹) Brushoff²</td>
<td>3</td>
<td>Merri Creek</td>
<td>5.6 (3.9)</td>
<td>2008</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>Merri Creek</td>
<td>10.1 (8.0)</td>
<td>2009</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Patterson River</td>
<td>21.6 (0.3)</td>
<td>2008</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Patterson River</td>
<td>17.0 (1.0)</td>
<td>2009</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Glyphosate, 1 × label rate (3.6 g a.i. L⁻¹) present as isopropylamine salt Roundup Biactive³</td>
<td>9</td>
<td>Merri Creek</td>
<td>1.2 (2.6)</td>
<td>2010</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Patterson River</td>
<td>13.9 (6.9)</td>
<td>2008</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Patterson River</td>
<td>15.3 (4.5)</td>
<td>2009</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Glyphosate, 3 × label rate (10.8 g a.i. L⁻¹) present as isopropylamine salt Roundup Biactive³</td>
<td>9</td>
<td>Merri Creek</td>
<td>0.8 (0.9)</td>
<td>2010</td>
<td>3</td>
</tr>
<tr>
<td>Physical Removal</td>
<td>4</td>
<td>Merri Creek</td>
<td>6.9 (4.4)</td>
<td>2008</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>Merri Creek</td>
<td>14.5 (9.0)</td>
<td>2009</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: SD = standard deviation ¹ Surfactant not used

the Merri Creek containing 18 patches of alligator weed were selected to be treated with glyphosate. Nine of these eighteen patches were selected (using random numbers) for glyphosate applied at 1 × label rate (3.6 g a.i. L⁻¹) and the other nine patches were selected for glyphosate applied at 3 × label rate (10.8 g a.i. L⁻¹). At Patterson River four patches were selected in 2008, two of these four patches were selected (using random numbers) for glyphosate applied at 1 × label and the other two patches were selected for metsulfuron-methyl applied at 1 × label. At Patterson River six patches, on another stretch of river, were selected in 2009. Three of these six patches were selected (using random numbers) for glyphosate applied at 1 × label and the other three patches were selected for metsulfuron-methyl applied at 1 × label (Table 3).

The application of alligator weed treatments was undertaken in a staged approach from 2008 to 2010 due to the constraints of the active alligator weed eradication program. No control patches (untreated) were used as this would have compromised the eradication program. Our methodology assumes that patches in our study area would remain at a similar size or expand if left untreated. High flow events can dislodge aquatic plants growing along steam banks, particularly in urban stream settings. At the sites used in this study it is unlikely that elimination of alligator weed patches can be attributed to dislodgement by high flow events. Author observational data from the study sites suggest that patches of alligator weed remain without substantial size reduction after high flow events. Further, during the period of the present study, the rapid expansion of alligator weed has been demonstrated if left uncontrolled in Victoria (Clements et al. 2011).

Herbicide application

Herbicide was applied to patches of alligator weed based on the annual treatment program described by van Oosterhout (2007). Specifically, herbicide was applied whenever there was any foliar alligator weed present in spring (November), summer (January) and autumn (March) for up to five consecutive years (Table 3). All herbicide was applied with a pneumatic single nozzle hand wand applicator to aerial foliage, until runoff
occurred. Prior to herbicide application a netting barrier consisting of polyethylene netting (15 mm diamond mesh) attached to steel stakes was constructed around each patch of alligator weed to prevent any alligator weed stem fragments from entering or exiting the treatment areas.

**Physical removal**

Physical removal was conducted by experienced contractors (Thiess Services Pty Ltd). All above ground alligator weed was removed, followed by stems and roots that were traced back into the substrate and removed by hand or with mattocks. A floating boom with a netting skirt hanging from it was positioned to encircle each patch against the bank to catch any alligator weed fragments produced during excavation. Once removed, all alligator weed and associated soil was placed into bags and transported to a deep burial site for safe disposal.

**Efficacy of herbicide and physical removal treatments**

To assess the efficacy of all herbicide and physical removal treatments, the area occupied by each alligator weed patch was measured at three month intervals (November, January and March) each year, for up to five years post initial treatment (Table 3). For the assessments, the presence or absence of alligator weed was recorded and, when present, the area occupied was determined by measuring the maximum length and width of the patch (including all stem material visible both above the water surface and above the sediment for the portions that were growing on the embankments) and approximating it to the shape of an ellipse, from which an area was calculated. A visual estimate of alligator weed percent coverage, defined as the vertical projection of all plant material on the ground surface, within the ellipse was made. The area and cover values were then multiplied to give an area metric calculation, termed ‘area occupied’ by alligator weed. The effectiveness of physical removal and herbicide treatments were examined for up to five years (Table 3).

**Field study statistics**

At Merri Creek in 2010, glyphosate rate treatments were applied randomly to the 18 available patches. Thus a cause and effect hypothesis test can be constructed to examine the effect of glyphosate rate on the time until alligator weed was absent (first recording occasion after the final time alligator weed was observed). However, hypothesis tests based on the normal distribution are not appropriate and standard non-parametric tests are ineffective due to the ordinal form of the data. In this case, a standard approach is to use proportional odds models (McCullagh and Nelder 1989), which are commonly referred to as ordinal logistic regression models. More specifically, the effect of glyphosate rate on the efficacy of control at Merri Creek was tested by fitting an ordinal logistic model, with an estimated over-dispersion parameter, for the number of days until alligator weed was absent from each patch to the logarithm of the initial area of infestation and the rate of glyphosate application, and then using an analysis of deviance F test for testing the glyphosate effect adjusted for the logarithm of the initial area of infestation. The initial area of infestation is included as a covariate to improve the power of the hypothesis test. Prior to fitting the ordinal logistic model, the number of days until alligator weed was absent from each patch is pooled into 4 groups, namely (i) week 10, (ii) week 39, (iii) week 50, 59 or 93 and (iv) week 103. This allows several observations in each group, so that the model numerically converges and so that the F approximation is more reasonable.

To determine any relationship between patch size and the efficacy of physical removal at Merri Creek, the effect of initial patch size and year of removal was determined by fitting a generalised linear model with Poisson errors, logarithmic link and over-dispersion parameter that includes effects for the logarithm of the initial area of infestation and the number of regrowth occasions. Permutation tests are calculated using analysis of deviance F statistics. All modelling and testing was carried out using the generalised linear model facilities in GenStat 16 (Payne 2013).

**Results**

**Screening trial**

Prior to any herbicide application, alligator weed plants growing in the containers had a moderately dense growth habit (62 of the 65 containers had >75% cover). The stem material extended over the water surface and as a tangled mat beneath it, typical of the aquatic form of alligator weed. The plants were prostrate (<0.1 m), and had an average of 65 (SD 15) shoot tips. Over the duration of the experiment, treatments without herbicide showed
Management of aquatic alligator weed

Table 4. Effect of herbicide rate on the number of shoot tips in the container trial for (A) Glyphosate, (B) Metsulfuron-methyl and (C) Dichlobenil. P values are bolded when P < 0.05; values are square-root transformed, except back transformed means in parentheses. WAT = Weeks after treatment. SED = standard error of difference between square-root transformed means. Values for control are the same in parts A. B. and C. of table.

A. Glyphosate

<table>
<thead>
<tr>
<th>WAT</th>
<th>Residual degrees of freedom</th>
<th>Control (n=5)</th>
<th>1 × label (n=5)</th>
<th>3 × label (n=5)</th>
<th>6 × label (n=5)</th>
<th>SED</th>
<th>P value</th>
<th>Control vs 1 × label</th>
<th>Rate 1 vs 3 vs 6 × label</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>52</td>
<td>9.24 (85)</td>
<td>0.8 (1)</td>
<td>0.0 (0)</td>
<td>0.4 (0)</td>
<td>0.63</td>
<td>1.1 × 10⁻¹⁴</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>52</td>
<td>7.9 (63)</td>
<td>0.3 (0)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
<td>0.36</td>
<td>3.5 × 10⁻²⁷</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>52</td>
<td>7.7 (59)</td>
<td>0.5 (0)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
<td>0.40</td>
<td>6.2 × 10⁻²⁴</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>52</td>
<td>7.0 (49)</td>
<td>0.2 (0)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
<td>0.23</td>
<td>4.4 × 10⁻³⁴</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>52</td>
<td>7.5 (56)</td>
<td>2.5 (6)</td>
<td>0.2 (0)</td>
<td>0.2 (0)</td>
<td>0.47</td>
<td>1.7 × 10⁻¹⁴</td>
<td>2.6 × 10⁻⁶</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>51</td>
<td>12.3 (152)</td>
<td>6.8 (47)</td>
<td>0.3 (0)</td>
<td>2.3 (5)</td>
<td>1.53</td>
<td>0.000067</td>
<td>0.00025</td>
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</tr>
<tr>
<td>91</td>
<td>50</td>
<td>14.22 (202)</td>
<td>3.1 (10)</td>
<td>-0.6 (0)</td>
<td>1.7 (3)</td>
<td>1.50</td>
<td>1.3 × 10⁻⁸</td>
<td>0.052</td>
<td></td>
</tr>
</tbody>
</table>

* This value becomes 5.1 (26) if outlier was not removed from the analysis.

B. Metsulfuron-methyl

<table>
<thead>
<tr>
<th>WAT</th>
<th>Residual degrees of freedom</th>
<th>Control (n=10)</th>
<th>0.5 × label (n=10)</th>
<th>1 × label (n=10)</th>
<th>2 × label (n=10)</th>
<th>SED</th>
<th>P value</th>
<th>Control vs 0.5 × label</th>
<th>Rate 0.5 vs 1 vs 2 × label</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>52</td>
<td>9.24 (85)</td>
<td>2.7 (7)</td>
<td>2.8 (8)</td>
<td>2.5 (6)</td>
<td>0.44</td>
<td>9.2 × 10⁻²⁷</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>52</td>
<td>7.9 (63)</td>
<td>1.4 (2)</td>
<td>1.4 (2)</td>
<td>1.1 (1)</td>
<td>0.26</td>
<td>8.4 × 10⁻²⁷</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>52</td>
<td>7.7 (59)</td>
<td>1.0 (1)</td>
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<td>0.1 (0)</td>
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<td>1.8 × 10⁻²⁵</td>
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<td>52</td>
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<td>1.8 (3)</td>
<td>2.4 (6)</td>
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<td>0.028</td>
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<tr>
<td>48</td>
<td>51</td>
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<td>10.6 (112)</td>
<td>9.3 (86)</td>
<td>3.1 (10)</td>
<td>1.08</td>
<td>0.19</td>
<td>7.2 × 10⁻⁸</td>
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</tr>
<tr>
<td>91</td>
<td>51</td>
<td>14.22 (202)</td>
<td>8.9 (80)</td>
<td>10.0 (99)</td>
<td>7.9 (62)</td>
<td>1.06</td>
<td>0.000114</td>
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</table>

C. Dichlobenil

<table>
<thead>
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<th>WAT</th>
<th>Residual degrees of freedom</th>
<th>Control (n=10)</th>
<th>0.5 × label (n=10)</th>
<th>1 × label (n=10)</th>
<th>2 × label (n=10)</th>
<th>SED</th>
<th>P value</th>
<th>Control vs 0.5 × label</th>
<th>Rate 0.5 vs 1 vs 2 × label</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
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<td>9.24 (85)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
<td>0.63</td>
<td>2.3 × 10⁻²⁵</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>52</td>
<td>7.9 (63)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
<td>0.36</td>
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<td>1.00</td>
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<td>0.0 (0)</td>
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<td>2.5 × 10⁻²⁵</td>
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<td>0.0 (0)</td>
<td>0.0 (0)</td>
<td>0.23</td>
<td>1.1 × 10⁻²⁴</td>
<td>1.00</td>
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<td>0.0 (0)</td>
<td>0.0 (0)</td>
<td>0.47</td>
<td>1.1 × 10⁻²¹</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>48</td>
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<td>6.0 (36)</td>
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<td></td>
</tr>
<tr>
<td>91</td>
<td>50</td>
<td>14.22 (202)</td>
<td>5.6 (31)</td>
<td>4.0 (16)</td>
<td>0.1 (0)</td>
<td>1.50</td>
<td>5.1 × 10⁻⁷</td>
<td>0.0018</td>
<td></td>
</tr>
</tbody>
</table>

an increase in the production of shoot tips (Table 4). At the conclusion of the trial, observations of the root mass of the plants showed that stems had produced multiple adventitious roots that extended into the top ~ 10 cm of the substrate, but true tap roots were rare (probably because of the anoxic nature of the sediment below this depth and roots obtaining the required water and nutrients directly from the water column and sediment).

All of the herbicide treatments considerably reduced alligator weed abundance relative to controls, which was still notable 91 WAT (Table 4). For each herbicide there was an effect of herbicide rate, with higher rates resulting in fewer shoot tips, although this was not apparent until 48 WAT for dichlobenil (Table 4).

Within glyphosate treatments, the 3 × label rate treatment provided the greatest level of control and was considerably more effective than 1 × label rate at 11 and 48 WAT (P = <0.01; Table 4). By 91 WAT no difference in herbicide rate was observed between glyphosate treatments (P = 0.052), and abundance remained considerably less than controls (Table 4A). The 6 × label rate treatment did not provide any further control than the 3 × label rate treatment and was less effective at 48 and 91 WAT (Table 4A).
Figure 2. Temporal reduction in alligator weed (% area occupied), compared to area occupied at time of initial herbicide application, for (A) Metsulfuron-methyl (0.06 g a.i. L\(^{-1}\)) at Merri Creek, n=5; and (B) Metsulfuron-methyl (0.06 g a.i. L\(^{-1}\)) at Patterson River, n=5. Each point in the figure represents the result from a single patch at a sampling occasion. Values between 98-100% are expressed as 98% for clarity. X axis represents intervals of herbicide application and measurement at each site. Note different scales on X axis. WAT = weeks after treatment.

Table 5. Effect of surfactant (Pulse\textsuperscript{®}) in metsulfuron-methyl treatments on the number of shoot tips in the container trial. P values are bolded when P < 0.05; values are square-root transformed, except back transformed means in parentheses. WAT = Weeks after treatment. SED = standard error of difference between square-root transformed means.

<table>
<thead>
<tr>
<th>WAT</th>
<th>No surfactant</th>
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<th>SED</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2.7 (7)</td>
<td>2.6 (7)</td>
<td>0.36</td>
<td>0.89</td>
</tr>
<tr>
<td>5</td>
<td>1.4 (2)</td>
<td>1.3 (2)</td>
<td>0.21</td>
<td>0.65</td>
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<tr>
<td>7</td>
<td>0.6 (0)</td>
<td>0.4 (0)</td>
<td>0.23</td>
<td>0.37</td>
</tr>
<tr>
<td>9</td>
<td>0.4 (0)</td>
<td>0.0 (0)</td>
<td>0.13</td>
<td>0.37</td>
</tr>
<tr>
<td>11</td>
<td>2.1 (4)</td>
<td>1.8 (3)</td>
<td>0.27</td>
<td>0.27</td>
</tr>
<tr>
<td>48</td>
<td>7.8 (61)</td>
<td>7.5 (56)</td>
<td>0.88</td>
<td>0.25</td>
</tr>
<tr>
<td>91</td>
<td>9.9 (97)</td>
<td>8.0 (64)</td>
<td>0.87</td>
<td>0.11</td>
</tr>
</tbody>
</table>

All metsulfuron-methyl treatments reduced alligator weed abundance to near zero by 7 to 9 WAT, however, by 48 WAT regrowth had occurred. By 91 WAT no difference in herbicide rate was observed between metsulfuron-methyl treatments (P = 0.16), and abundance remained considerably less than controls (Table 4B). The addition of a surfactant to metsulfuron-methyl treatments had no effect on control efficacy at all intervals (P >0.1), except at 9 WAT (P = 0.0074; Table 5).

Dichlobenil provided excellent control, reducing alligator weed abundance by 100% for all rates up to 11 WAT, which was maintained at 48 and 91 WAT for 2 × label rate (Table 4C). The dichlobenil treatment at 2 × label rate was more effective than the 1 × and 0.5 × label rate treatments at these times (P = <0.01; Table 4C).

No viable plant material was present at 48 and 91 WAT for glyphosate at 3 × label rate and dichlobenil at 2 × label rate. Metsulfuron-methyl provided less control than glyphosate and dichlobenil irrespective of herbicide rate. The rate of decline of shoot tips was slower for metsulfuron-methyl treatments compared to glyphosate and dichlobenil. The presence of a surfactant did not improve metsulfuron-methyl efficacy. To reduce abundance of shoot tips to near zero, metsulfuron-methyl treatments took between 7 and 9 WAT, glyphosate and dichlobenil treatments responded much earlier, within 3 and 5 WAT (Table 4).

Field study

Metsulfuron-methyl

At the time of initial herbicide application the five patches of alligator weed subjected to metsulfuron-methyl at Merri Creek ranged in size from 1.4 to 15.7 m\(^2\). All patches recorded regrowth, which
Management of aquatic alligator weed

Figure 3. Temporal reduction in alligator weed (% area occupied), compared to area occupied at time of initial herbicide application, for (A) Glyphosate 1 × label rate (3.6 g a.i. L⁻¹) at Merri Creek, n=9; (B) Glyphosate 3 × label rate (10.8 g a.i. L⁻¹) at Merri Creek, n=9; and (C) Glyphosate 1 × label rate (3.6 g a.i. L⁻¹) at Patterson River, n=5. Each point in the figure represents the result from a single patch at a sampling occasion. Values between 98-100% are expressed as 98% for clarity. X axis represents intervals of herbicide application and measurement at each site. Note different scales on X axis. WAT = weeks after treatment.

occurred following one to four herbicide applications. After two years of three applications per year, no regrowth was recorded out to four or five years of monitoring (Figure 2A). This shows that applications of metsulfuron-methyl three times per year for two years can reduce the area occupied by alligator weed to near zero (99.7% reduction, SD 0.05) by the end of the second year of application and that no regrowth occurred in subsequent years. This is supported by data obtained at Patterson River where metsulfuron-methyl achieved a 99.9% reduction (n=2) over two seasons of treatment, and 97.1% reduction in one year of treatment, (n=5) (Figure 2B). Further monitoring and treatment at Patterson River was abandoned, as stem fragments from nearby patches of alligator weed (outside of the trial patches) overtopped the mesh barriers during a flood making it impossible to determine if regrowth was derived from within the trial patches or reinvasion from fragments entering into the trial patches.

Glyphosate
The 18 patches subjected to glyphosate application along Merri Creek ranged in initial patch size from 0.02 to 7.9 m² (89% of patches were <2.5 m²), prior to treatment. No differences (P = 0.60) in efficacy were detected between glyphosate at 1 × label rate (3.6 g a.i. L⁻¹) and 3 × label rate (10.8 g a.i. L⁻¹), based on the number of days until alligator weed was absent from each patch. The rate at which alligator weed declined is shown in Figures 3(A) and 3(B). All patches recorded regrowth, which occurred following one to seven applications. By the end of the third year of treatment and monitoring (112 weeks), alligator weed was still present in only one patch (glyphosate at 1 × label rate); this was the largest patch at the start of the trial (7.9 m²) and was reduced by 99% (0.08 m²). No other patches remained active irrespective of herbicide rate. At Patterson River a similar result was achieved where glyphosate at 1 × label rate achieved an average of 99.95% reduction (n=2) after two seasons of treatment, and an average of 92.9% reduction after one year of treatment (n=5) (Figure 3C). These patches at Patterson River were abandoned after two years of monitoring as described above.

Physical removal
The alligator weed patches subjected to physical removal along Merri Creek varied in size, ranging from 3.5 to 30.5 m² prior to treatment in 2008 and 2009. Following physical removal, regrowth was recorded from three out of the 12 patches (25%), out to four-five years of monitoring. The patches that recorded regrowth ranged in initial patch size from 11.2 to 23.6 m². Two patches needed one instance of follow up removal in the first year after initial treatment, with no subsequent regrowth. One patch needed follow up removal three times over two consecutive years. There was no effect of year (P = 0.77) or initial patch area (P = 0.19) in determining whether or not regrowth occurred. However, the power of this test was lacking because only three out of 12 patches had any regrowth. It is reasonable to assume that larger patches of alligator weed are
more likely to produce regrowth following physical 
removal as the abundance of viable propagules 
and root material increases with patch size.

**Discussion**

**Screening trial**

Improved control was achieved for all herbicides 
at rates greater than the manufacturers recommended 
rate (label rate) 48 WAT (Table 4). This suggests 
that there is scope to revise herbicide labels or 
for users to apply to the statutory authority for 
minor use permits to allow for improved 
management of alligator weed. However, the use 
of herbicides in natural environments must 
consider more than just the sensitivity of the 
target weed to the active ingredient and additives 
in the chemical product.

Only two previous studies have reported 
excellent (90–100% reduction in abundance) long-
term (> 52 weeks) control of alligator weed after 
a single herbicide application. These studies used 
dichlobenil (Blackburn and Durden 1974) and 
metsulfuron-methyl (Hofstra and Champion 2010). 
Excellent long-term control (under the above 
definition) was achieved with single applications 
of dichlobenil (rates above 15.5 kg a.i. ha⁻¹) and 
metsulfuron-methyl (40 g a.i. ha⁻¹) in the current 
trial, 48 WAT (however regrowth had occurred 
by 91 WAT for metsulfuron-methyl treatments). 
Our results validate those of Hofstra and Champion 
(2010), who used 36 g a.i. ha⁻¹ of metsulfuron-
methyl on plants of similar age to those used in 
our study. However, they also report control was 
much less effective for plants that had been 
cultured for multiple growing seasons prior to 
metsulfuron-methyl application (the plants in the 
current study were cultured for 15 weeks).

Alligator weed abundance was reduced by >90% 
in the current trial with a single application of 
glyphosate at 10.8 g a.i. L⁻¹ (3 × label; 3.6 kg a.i. 
ha⁻¹). Dugdale and Champion (2012) report that 
in four separate studies using a single application 
of glyphosate, less than 60% control was achieved 
after ~ 52 weeks using rates up to 7.2 g a.i. L⁻¹ 
(6.4 kg a.i. ha⁻¹). It is unlikely that we achieved 
greater control than the other studies simply because 
of the high rate we used; Hofstra and Champion 
(2010) used glyphosate at 6.4 kg a.i. ha⁻¹ and 
achieved ~60% control in outdoor containers very 
similar to ours. It is possible that our excellent 
control with a single application of glyphosate 
was achieved because we removed all of the stem 
fragments that were generated from the herbicide 
application. However, this is unlikely given we 
showed that only ~ 2% of these were viable for 
glyphosate (Dugdale et al. 2010). It is more 
likely that excellent control was achieved because 
the alligator weed very rarely formed tap roots in 
our containers; instead it produced many adventi-
titious roots. Given a key mode of regeneration 
after herbicide application is from roots, this is a 
likely source of difference. Observations from 
past field management programs support this, as 
extensive areas of floating aquatic alligator weed 
were effectively controlled with a single glyphosate 
application (Sainty et al. 1998). This suggests that 
the results presented in the screening trial may 
only be representative of newly colonising plants, 
in an early invasion stage, established from floating 
asexual fragments.

The excellent control achieved with 3 × 
glyphosate and 2 × dichlobenil in the container 
trial prompted us to test the former in the field 
study. Although dichlobenil was not tested in the 
field trial (because its use is limited to standing 
water situations, of which there are currently too 
many sites containing alligator weed in Victoria to 
use as experimental sites), these results suggest 
dichlobenil is likely to present a viable option 
for alligator weed management.

Metsulfuron-methyl performance against alligator 
weed was not reduced when used without a 
surfactant in the container trial, further, patches 
were eliminated when metsulfuron-methyl was 
used in the field without a surfactant. The Australian 
product label instructs that a surfactant be used, 
so this result was unexpected. Although we have 
not found any publications that report on the 
effect of surfactants on metsulfuron-methyl efficacy 
against alligator weed, control of the woody weed 
*Diodia ocimifolia* and weeds of wheat (*Aegilops 
yulax* L. and *Bromus secalinus* L.) was not improved by addition of 
non-ionic surfactants (Olson et al. 2000, Ooi 1999). 
Given many surfactants are toxic to aquatic biota 
(Brausch et al. 2007; Siemering et al. 2008), 
using metsulfuron-methyl without a surfactant 
may reduce the risk of off-target impacts without 
compromising control efficacy.

**Herbicide field study**

All herbicides (glyphosate at 3.6 and 10.8 g a.i. L⁻¹ 
and metsulfuron-methyl at 0.06 g a.i. L⁻¹) applied 
up to three times per year were very effective in 
reducing the amount of alligator weed present in 
the field. The area occupied by alligator weed 
was reduced by >99% (e.g. <0.35 m² patch size) 
using either herbicide within two years.
Following this period of treatment a reduced number of applications were required as the area occupied by alligator weed was at very low levels or absent. Regular monitoring during this period (three to five years following initial treatment) is crucial, even when alligator weed is absent, to enable early detection and treatment of any regrowth before it can regenerate below ground reserves or stems capable of dispersal. The improved control with glyphosate at 10.8 g a.i. L\(^{-1}\) compared to 3.6 g a.i. L\(^{-1}\) that we recorded in the container trial was not apparent in the field. There are at least two possible explanations for this: Firstly, the alligator weed in the field is likely to have had a more developed root system at the time of initial treatment, which would have provided a source of regeneration after each herbicide application; secondly, the alligator weed patches were monitored and resprayed at three-month intervals between spring and autumn, so any regrowth was destroyed before it could grow enough for differences in patch size between rates to become apparent. Therefore, we do not recommend using elevated rates of glyphosate on alligator weed when applied three times per year. However, this result suggests a more efficient management program can be developed by using higher rates of herbicide. For example, it may be possible to achieve equivalent levels of control of alligator weed with elevated rates of either glyphosate or metsulfuron-methyl with one or two applications per year, compared to applying these herbicides three times per year at label rate. Further research is required to test this approach and determine if improved herbicide regimes can be established for alligator weed management.

Results from our field trial support previous findings where multiple applications per season of glyphosate (Schooler et al. 2008) or metsulfuron-methyl (Hofstra and Champion 2010, Schooler et al. 2008, Schooler et al. 2010) provide good to excellent (80–100%) long-term control (~ 52 WAT) of above or belowground alligator weed (Dugdale and Champion 2012). The literature suggests that multiple annual applications of metsulfuron-methyl is the preferred herbicide treatment (Hofstra and Champion 2010; Schooler et al. 2008), and is usually preferred over glyphosate for alligator weed management programs (Bowmer et al. 1991; Champion 2008; Dugdale and Champion 2012; Sainty et al. 1998; van Oosterhout 2007). However, recent container studies have shown that glyphosate application results in fewer viable stem fragments than metsulfuron-methyl post herbicide application, indicating a reduced risk of dispersal and likelihood of new infestations establishing in aquatic situations (Clements et al. 2012; Dugdale et al. 2010).

Physical removal field study

Physical removal provides a method to control alligator weed that should not result in dispersal of fragments, or rely on multiple applications over multiple years. We have demonstrated that manual removal is effective at eliminating individual patches of aquatic alligator weed, although regular follow-up assessments are crucial so that repeat control can occur before the plant can replenish its underground reserves. Manual excavation has been shown to be effective for eradication of small patches of terrestrial alligator weed (Sainty et al. 1998), but as far as we are aware this is the first study to report on the effectiveness of manual removal of aquatic alligator weed. One key driver of successful physical removal of alligator weed is the proficiency of the personnel conducting the management works. Although most of the biomass of aquatic alligator weed is in the mats that float over the water, most of the effort in manual removal is required on below ground parts of the plant (roots, particularly tap roots and stem material) in the sediment near the water’s edge. If all of the below ground plant material is not removed, rapid regrowth will occur. Further, Wilson et al. (2007) has shown that alligator weed develops a different morphology after physical removal, where plants that were subjected to shoot removal just above the soil level (to mimic mowing) had a higher below ground root biomass, a higher ratio of root to stem biomass and positioned its leaves closer to the ground, consequently making subsequent management efforts more difficult in aquatic and riparian environments. A disadvantage of manual removal is that it is very labour intensive. To remove each patch of alligator weed in this study took between 4.5 to 10.5 person hours per square metre. This means manual removal is costly in the initial year of treatment but if conducted properly, few resources are required in subsequent years.

Management implications

The results of the herbicide field studies demonstrate that we can eliminate patches of alligator weed with three applications per year of glyphosate or metsulfuron-methyl (at label rate) in Victoria, and thus validates the best practice guidelines of van Oosterhout (2007). A notable departure of the control method we used compared
to that recommended, is that we sprayed patches with herbicide whenever there were any alligator weed shoots present. van Oosterhout (2007) recommends skipping herbicide application when stems are <10 cm long and have <5–6 sets of leaves, or patches are <30 cm diameter (in the case of prostrate regrowth). Our data shows that very effective suppression can be achieved when alligator weed is treated without regard to ensuring that it is of a minimum size. Further, we do not recommend skipping applications when the stems are <10 cm long as alligator weed regrowth can be very rapid in aquatic environments creating a large number of stems for potential dispersal, and allowing below ground reserves to be replenished, both of which will impair an eradication program. The container trial suggests it may be possible to develop a more efficient herbicide control program using elevated rates of either glyphosate or metsulfuron-methyl to reduce the number of applications required each year, but we did not verify this in the field. Further research is required to evaluate this approach.

The results also demonstrate that physical removal is effective at eliminating patches of alligator weed. Because alligator weed has been known to regrow for up to 10 years after last being recorded (van Oosterhout 2007), we have not declared that any of the patches in this study have been eradicated. Despite this, the results can be used to guide ongoing suppression of alligator weed leading to eradication.

This study demonstrates that the methods used in Victoria’s alligator weed eradication program are capable of eliminating patches of alligator weed in two to three years and indicates the eradication program has the tools required to succeed.

Acknowledgements

This research was funded by Department of Environment and Primary Industries and Melbourne Water. Fiona Ede and Rhys Colemen provided valuable review of an earlier version of this manuscript. Thank you to the anonymous reviewers for their constructive input.

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Management of aquatic alligator weed


Bridge between Chapters 4 and 5

Chapter 4 determined the efficacy of control regimes utilised in eradication programs in Australia, to extirpate individual patches of aquatic alligator weed (*Alternanthera philoxeroides*) in an early stage of invasion. This study assessed the effectiveness of herbicide and physical removal control techniques and evaluated their ability to eliminate patches of alligator weed over a five year period in aquatic environments, which has not previously been evaluated. It showed that effective management strategies are available, but a consistent approach is required involving multiple applications over multiple years to achieve extirpation of patches of aquatic alligator weed within two to three years. However, previous research has been limited to evaluating the efficacy of control at a site scale (i.e. the ‘extinction criterion’ being the rate of decrease in population numbers or individual patches of alligator weed) with disregard to the effects of management strategies on dispersal throughout catchments (i.e. the ‘containment criterion’, which is the extent to which an eradication program prevents the spread of the target species), limiting extirpation attempts.

Following herbicide application a substantial decrease in alligator weed biomass can be observed at treatment sites (demonstrated in Chapter 4), however, anecdotal evidence from the field suggests that herbicide application, which is the most efficient method of control, may result in the production of alligator weed stem fragments and it is possible that some of these fragments may be viable post-herbicide application, potentially increasing the rate of spread of the weed throughout catchments. If control regimes are not effective in preventing reproduction, new infestations may be created through the dispersal of propagules to other areas, limiting extirpation efforts. This critical aspect to achieving effective control of aquatic alligator weed at a catchment scale is evaluated in Chapter 5. This is the first study of its kind designed to determine the viability of stem fragments (for any invasive aquatic plant) produced post-herbicide application.
Chapter 5 – Control of alligator weed dispersal

Citation:


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Bridge between Chapters 5 and 6

Chapter 5 assessed the impact of herbicide treatments on the production of alligator weed stem fragments and determined the viability of fragments produced post-herbicide application. It showed that herbicide application dramatically increases stem fragmentation of alligator weed in aquatic environments and that a proportion of stem fragments produced are viable. It also found that the actual number of fragments, viability rates of those fragments, and number of viable fragments differ greatly with herbicide. These viable fragments, when lodged in a suitable habitat, are likely able to colonise adjacent areas, making eradication programs using herbicides ineffective unless the problem of viable fragmentation is addressed. As discussed in Chapter 1 (Section 1.3.2), one aspect to achieving successful eradication is to prevent the spread of the target species (i.e. the ‘containment criterion’), and if a control regime is not effective in preventing reproduction, new infestations may be created through the dispersal of propagules to other areas. The investigation in Chapter 6 is aimed at limiting the amount of viable fragments produced post-herbicide application, utilising plant growth regulators (PGRs). These chemicals are used in the horticultural industry to reduce pre-harvest fruit drop of stone fruits and apples, by manipulating the abscission process that occurs within plants. This novel approach to utilise PGRs to reduce abscission and subsequent dispersal has not been attempted previously for aquatic plant management.
Chapter 6 – Control of alligator weed dispersal continued

Citation:


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Bridge between Chapters 6 and 7

Chapter 6 attempted to incorporate commercially available plant growth regulators (PGRs) into herbicide treatments (glyphosate and metsulfuron-methyl) for alligator weed control, to limit the amount of viable fragments being produced post-herbicide application and therefore limit dispersal of alligator weed throughout catchments and waterways. Laboratory trials showed there was no significant effect detected between treatments, suggesting that PGRs do not have any effect on the total number of viable fragments produced post-herbicide application. Whilst PGRs did alter the timing and extent of stem fragmentation, at the application rates and timings tested, there appears to be no benefit in incorporating PGRs into herbicide control programs for aquatic alligator weed. Further, an antagonistic effect was detected where the plant produced greater below-ground biomass in response to the plant growth regulators. This line of research was therefore discontinued because this method was not effective in reducing the number of viable fragments produced post-herbicide application. The herbicides glyphosate and metsulfuron-methyl (under permit) are currently utilised for control of alligator weed in aquatic situations in Australia, whilst the herbicide imazapyr has more recently been registered for the control of alligator weed in the USA and New Zealand. However, glyphosate is considered to be less effective in controlling above and below ground alligator weed than metsulfuron-methyl and imazapyr. Although there is much data available in the literature regarding the efficacy of different herbicides on control of above and below ground alligator weed, none have looked at all aspects of control that need to be studied for effective management of alligator weed in aquatic environments. No study has simultaneously looked at the impact of herbicide control on above and below ground biomass as well as viable stem fragment production. These are the three critical measures when determining the efficacy of a particular control regime for effective alligator weed management in aquatic environments, particularly when incorporated into eradication programs. Chapter 7 evaluates these three important factors that have been overlooked in previous studies for the control of aquatic alligator weed using herbicides. In addition, a potential new herbicide to be utilised in eradication programs in Australia is evaluated against currently employed herbicide strategies, based on these three important efficacy measures. This study brings together the research developed in Chapters 4 - 6 of this thesis to optimise control strategies for alligator weed in aquatic environments.
Chapter 7 – Effective control of aquatic alligator weed requires integrating key response metrics

Citation:

Clements D, Dugdale TM, Butler KL, Florentine SK, Sillitoe J (Accepted) Herbicide efficacy for aquatic Alternanthera philoxeroides management in an early stage of invasion: integrating above-ground biomass, below-ground biomass and viable stem fragmentation. *Weed Research*

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Bridge between Chapters 7 and 8

Effective management of invasive aquatic plants targeted for extirpation from catchments and waterways requires coupling detection effort with control effort to prevent reproduction. Research conducted in Chapters 2 – 3 of this thesis addressed improving detection and surveillance strategies for alligator weed in aquatic environments, to enable early detection so that effective control measures can be employed. Chapters 4 – 7 addressed optimising control techniques for aquatic alligator weed in an early stage of invasion, in order to achieve extirpation from catchments and waterways. Chapter 8 provides a concluding discussion which summarises the major findings of the body of published work, implications for aquatic plant management and directions for future research.
Chapter 8 – Research synthesis

Summary of major findings, research outcomes, management implications and future research
8.1 Summary of major findings

Research in recent years on the feasibility of weed eradication and evaluating eradication program success (Rejmánek and Pitcairn 2002; Panetta 2009; Gardener et al. 2010; Howell 2012; Pluess et al. 2012; Dodd et al. 2015; Panetta 2015) has determined the factors required to achieve eradication of high risk invasive plant species (Panetta 2016). Effective management of invasive aquatic plants targeted for extirpation from catchments and waterways requires that three criteria are met: (1) the ‘delimitation criterion’ being the requirement to detect the full extent of an incursion (Panetta 2009; Panetta and Lawes 2005); (2) the ‘extinction criterion’, being the rate of decrease in population numbers of a given species, as the ability to extirpate new infestations is crucial for controlling the spread of invasive organisms; and (3) the ‘containment criterion’ being the extent to which a management program prevents the spread of the target species (Zamora et al. 1989; Panetta and Lawes 2005; Schooler 2012). Dispersal and subsequent establishment of propagules will allow an invasive species to persist (Fletcher and Westcott 2013). To achieve these criterion effective detection and control techniques must be available. Ultimately, it is the effectiveness of the detection and control techniques utilised against an invasive species that will determine the feasibility of eradication. To enable the effective management of invasive aquatic plants targeted for extirpation from catchments and waterways, effective detection and control techniques are required to be developed, evaluated and implemented.

The research presented in this thesis has developed management strategies for one of the world’s most invasive plant species, alligator weed (Alternanthera philoxeroides) in an early stage of invasion of catchments and waterways by: (1) optimising detection and surveillance strategies to enable early detection so that effective control measures can be employed and (2) optimising control techniques (being existing or novel techniques) to enhance extirpation likelihood. Prior to this research, management techniques were underdeveloped allowing alligator weed to proliferate, compromising extirpation efforts at a catchment scale.

A summary of the major findings of the published work presented in this thesis is presented in Table 1. The management techniques and principles developed for alligator weed in this thesis provide a model for programs that aim to optimise management techniques for other invasive aquatic plants targeted for extirpation.
Table 1. Summary of the major findings of the published work presented in this thesis for optimising detection and control strategies for alligator weed (*Alternanthera philoxeroides* (Mart.) Griseb.).

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Key research findings</th>
<th>Source</th>
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<tr>
<td><strong>Optimising detection and surveillance strategies</strong></td>
<td>1. Orthophotos (high altitude aerial imagery) can be utilised to detect large infestations of floating aquatic alligator weed at a landscape scale (infestations &gt;5 m$^2$ were detected in this study). Further, orthophotos can be utilised to determine how long large infestations of alligator weed have been present and provide information to inform eradication programs. Research presented reveals for the first time; the rapid growth rate, expansion and biomass accumulation of alligator weed if left uncontrolled, over a five year period in southern Australia, which can be utilised to inform risk management procedures.</td>
<td>Chapter 2 (Clements et al. 2011)</td>
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<td></td>
<td>2. Unmanned Aerial Vehicle (UAV) technology (low altitude aircraft) can be utilised to detect patches of alligator weed at a site scale (i.e. invading catchments and waterways targeted for extirpation), however limitations apply. (a) Current automated detection algorithms utilising standard Red, Green, Blue (RGB) aerial images delineated patches of alligator weed growing between &gt;2.5 to 4 m$^2$ (area cover metric). Refinement of the algorithm is required before it is useful for detection of alligator weed patches for use in eradication programs, where a high proportion of patches must be detected. (b) Visual assessment of images collected with the UAV could detect patches of alligator weed &gt;0.06 m$^2$. Manually searching aerial imagery collected by the UAV can be employed at present without further technological advancement. However, alligator weed could not confidently be visually detected from these images when mixed in amongst other dense vegetation so intensive on-ground surveys need to be incorporated. (c) Intensive on-ground surveys detected patches &gt;0.002 m$^2$, and on-ground surveys must be incorporated into eradication programs where a high proportion of patches must be detected.</td>
<td>Chapter 3 (Clements et al. 2014b)</td>
</tr>
<tr>
<td>Criteria</td>
<td>Key research findings</td>
<td>Source</td>
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<td><strong>Optimising control strategies</strong></td>
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<tr>
<td>(3) Addressing the ‘extinction criterion’ to eliminate individual patches of aquatic alligator weed.</td>
<td>3. Effective management strategies are available to eliminate aquatic alligator weed at the site scale, involving a consistent approach with multiple applications over multiple years to achieve extirpation of individual patches of alligator weed. (a) The herbicides glyphosate and metsulfuron-methyl (three applications per year at label rate) and physical removal eliminated patches of aquatic alligator weed within two to three years.</td>
<td>Chapter 4 (Clements et al. 2014a)</td>
</tr>
<tr>
<td>(4) Addressing the ‘containment criterion’ to prevent the spread of aquatic alligator weed.</td>
<td>4. Alligator weed produces viable stem fragments in response to herbicide treatment, compromising the ‘containment criterion’ and limiting extirpation efficacy at a catchment scale. (a) The herbicide metsulfuron-methyl produced significantly more viable fragments than glyphosate in container trials, 66 m$^2$ and 9 m$^2$ respectively. However, in field trials a high proportion of stem fragments were viable (60-80%) for patches &gt;5 m$^2$, regardless of herbicide used. For patches &lt;5 m$^2$ viability was low. Further investigation to understand this result was required (Chapter 6 and 7). (b) Plant growth regulators did not reduce the number of viable fragments produced post-herbicide application and increased below ground biomass of aquatic alligator weed. This line of research was subsequently abandoned.</td>
<td>Chapter 5 (Dugdale et al. 2010)</td>
</tr>
<tr>
<td>(5) Addressing both the ‘extinction criterion’ and ‘containment criterion’ simultaneously.</td>
<td>5. Development of effective management strategies for alligator weed in aquatic environments requires control of above and below ground biomass and viable stem fragment production. (a) The herbicide glyphosate provides more effective control of overwater alligator weed than either imazapry or metsulfuron-methyl (which are similar) and minimises the relative frequency of viable stem fragment production, therefore reducing potential for dispersal throughout catchments and waterways. In contrast, imazapry and metsulfuron-methyl provide more effective control than glyphosate for alligator weed growing on exposed embankments.</td>
<td>Chapter 7 (Clements et al. accepted)</td>
</tr>
</tbody>
</table>
8.2 Research outcomes, management implications and future research

8.2.1 Optimising detection and surveillance strategies
Detection of high risk invasive aquatic plants before they become widespread is critical to achieving their extirpation (Timmins and Braithwaite 2002). Although current aquatic weed detection methods provide some success (including on-ground field surveys and public and industry reporting of infestations for emergent aquatic species), it is imperative that any new incursions are detected as early in the invasion process as possible, while the window of opportunity to achieve extirpation remains an achievable goal (Dahlsten and Garcia 1989).

Tools are required to be developed and implemented to detect infestations in an early stage of invasion to mitigate the threat that these high risk species pose. A further risk for aquatic weeds is that infestations that are not detected provide a source of propagules that can rapidly and easily spread via water flow to connected water bodies, and during floods to disconnected water bodies, creating new infestations. An example of this is the detection of alligator weed presented in Chapter 2 (Clements et al. 2011), where an analysis utilising historic aerial images revealed that alligator weed could be detected utilising high altitude aerial imagery (orthophotos) and had been present for at least five years at the study site before being detected (despite an active eradication program), allowing the species to proliferate and making subsequent control efforts problematic. This study provides an example of the invasiveness and potential impact alligator weed can have on waterbodies (particularly in south-east Australia). In this study, overwater alligator weed growth increased by ~7300 m² over a five year period in an urban pond in Victoria, Australia. Infestations >5 m² were detected utilising orthophotos. The information gained from this study can be utilised to inform risk management procedures targeting alligator weed. There appears to have been little attempt in the literature to estimate the invasiveness or potential impact of new weed incursions (being invasive terrestrial or aquatic species) based on real-time field observations (Panetta 2016). This study provides an example for aquatic alligator weed. Enacting control at an earlier stage of invasion could have reduced the potential for downstream dispersal within the catchment of this study site and limited control costs. This surveillance technique, after being demonstrated in this paper, has been incorporated into the eradication program in Victoria for detection of alligator weed, as well as other high priority aquatic weeds being targeted for eradication, including water hyacinth (*Eichhornia crassipes*) and salvinia (*Salvinia molesta*).
However, this detection technique is limited to larger spatial scales (i.e. large infestations >5 m$^2$) and effective detection capability is required at smaller spatial scales, for example, detecting completely new infestations of alligator weed in an early stage of invasion or detecting individual patches in a reach of creek known to contain alligator weed, so that control measures can be enacted.

Subsequently, Chapter 3 (Clements et al. 2014b) compared the current efficacy of unmanned aerial vehicle (UAV) technology, including the use automated algorithms, to detect patches of alligator weed growing in catchments and waterways and compared results to current detection techniques. The automated algorithm was able to detect and delineate patches of alligator weed growing between >2.5 to 4 m$^2$ (area cover metric) along the urban creek, while visual assessment of the images collected with the UAV could detect patches of alligator weed >0.06 m$^2$. Intensive on-ground surveys detected patches >0.002 m$^2$. Refinement of the algorithm is required before it is useful for detection of alligator weed patches for use in eradication programs, where a high proportion of patches must be detected. Despite these problems, Chapter 3 demonstrated that the UAV combined with the algorithm system can be used to detect alligator weed patches at least 4 m$^2$ in size when they are growing overwater. This size is small enough to be useful for detecting patches before they are too large to eradicate. It is anticipated that further research can refine the algorithm so that improved automated detection levels will be achieved for alligator weed. Further, the UAV was able to collect standard Red, Green, Blue (RGB) images of high quality that allowed patches >0.06 m$^2$ to be visually detected, from overwater and marginal situations. This study demonstrated that visual assessment (manually searching) of aerial imagery collected by UAV technology is an effective detection method for alligator weed targeted for extirpation from catchments and waterways that can be employed at present without further technological advancement. However, alligator weed could not confidently be visually detected from these images when mixed in amongst other dense vegetation and incorporating intensive on-ground surveys are required where a high proportion of patches must be detected in eradication programs. This method would likely be effective for other high priority aquatic weeds, including water hyacinth and salvinia, that are free floating species that do not inhabit terrestrial situations, although overhanging and adjacent vegetation will also likely limit aerial detection capability. No studies have quantitatively determined the effectiveness of aerial surveillance compared to current detection techniques (on-ground human surveillance) for water hyacinth and salvinia, as presented for alligator weed in Chapter 3, which has
limited the uptake of aerial surveillance as a valid detection technique in real world situations.

Although Chapter 2 (Clements et al. 2011) demonstrated the effective use of high altitude aerial imagery (orthophotos) to detect aquatic alligator weed infestations across the landscape, it is extremely time consuming to manually search images, because they can cover large areas of the landscape. Developing a tool that couples an automated detection algorithm (similar to that developed in Chapter 3) with such orthophotos (or other applicable forms of remote sensing data), to scan these images as they become available and highlight patches of suspected invasive aquatic plants, offers a way to proactively search for new infestations. Detecting infestations earlier would allow the early deployment of control measures, reducing the chance of downstream dispersal, reduce control costs and improve the likelihood of extirpation. This technique has the potential capability to be utilised for alligator weed as well as other high risk invasive aquatic plants, including water hyacinth and salvinia. These three species all form dense monotypic infestations that float over the water surface and displace other species, dense infestations are therefore visible from high altitude aerial images. Further, these invasive species grow in areas where on-ground access is difficult, with swampy ground or tall emergent vegetation that obscures the weed when viewed from the margins of waterbodies. Further research is required to determine if such a tool can be developed with a low enough false positive rate (i.e. the rate at which the algorithm falsely delineates vegetation that is not a target species) to be useful in an operational sense (i.e. it provides waterway managers with a time-efficient tool to determine if there are any undiscovered patches of the target species).

Further advancement of aerial surveillance detection techniques will need to be quantified (quantitatively compared to current detection methods) for these methods to be utilised by agencies responsible for the eradication of high risk invasive aquatic plants. Evaluation of search effort and timing of surveillance strategies needs further elucidation to optimise detection capability of high risk invasive aquatic plants. Further, additional methods are also being advanced for the detection of invasive species, including utilising DNA in environmental samples (eDNA) (Bohmann et al. 2014). A recent study has demonstrated the concept for the early detection of the submersed aquatic weed Eurasian watermilfoil (*Myriophyllum spicatum*) (Newton et al. 2016). However, research in this field is in a very early stage of development and further research is required to understand how to incorporate these methods effectively into surveillance programs to enable early detection of invasive aquatic plant species.
The ability to detect infestations is not the only effort required to achieve extirpation; effective management requires coupling detection effort with control effort to prevent reproduction (Panetta 2009).

8.2.2 Optimising control strategies for invasive aquatic plants
Effective control strategies must be available for invasive aquatic plants targeted for extirpation from catchments and waterways that: (1) decrease population numbers of a given species at a given rate and (2) prevent the spread of the target species until extirpation occurs over the entire infested area (Zamora et al. 1989; Panetta and Lawes 2005). For alligator weed (where reproduction is solely by clonal growth and viable seeds are not produced in its introduced range), control regimes that are ineffective in preventing reproduction at the site of infestation and that create new infestations through the dispersal of viable propagules in response to control efforts, limit extirpation efforts within catchments.

Prior to the research conducted in this thesis, previous studies have been limited to evaluating the efficacy of alligator weed control at a site scale (i.e. the ‘extinction criterion’) with disregard to the effects of management strategies on dispersal throughout catchments (i.e. the ‘containment criterion’ (Zamora et al. 1989; Panetta and Lawes 2005)), limiting extirpation attempts. Further, there was limited information in the literature on the long term (greater than one year) effectiveness of any herbicides or physical removal in eliminating patches of alligator weed in an early stage of invasion of waterways (Dugdale and Champion 2012). It was unknown how many applications of a given control regime are required over a given period to eliminate infestations in aquatic environments. Chapter 4 (Clements et al. 2014a) determined the efficacy of control regimes utilised in eradication programs in Australia, to extirpate individual patches of aquatic alligator weed in an early stage of invasion. This study determined that effective management strategies are available, but a consistent approach is required involving multiple applications over multiple years to achieve extirpation of individual patches of aquatic alligator weed. The herbicides glyphosate and metsulfuron-methyl and physical removal were capable of eliminating patches of alligator weed in field trials in two to three years. Further, either herbicide regime reduced the area occupied by ≥99% within two years. Mesocosm screening trials showed that improved control of aquatic alligator weed was achieved for all herbicides at rates greater than the manufactures recommended rates (with one application). This result prompted a subsequent field study, whereby the improved control with glyphosate at 3 times label rate that was recorded in the mesocosm trial, was not apparent in the field. Therefore, we do not
recommend using elevated rates of glyphosate on aquatic alligator weed when applied three times per year in eradication programs. The mesocosm study also showed that using a surfactant in combination with the herbicide metsulfuron-methyl did not increase efficacy of control of aquatic alligator weed (further evaluation of surfactants is included in Chapter 7). This study demonstrated, for the first time, that employed control strategies are capable of extirpating infestations of aquatic alligator weed at the site scale and indicates that programs targeting extirpation from catchments and waterways have the tools required to succeed.

Previous research has been limited to evaluating the efficacy of control at a site scale (including Chapter 4 of this thesis) with disregard to the effects of management strategies on dispersal throughout catchments, which has limited extirpation attempts. Following herbicide application a substantial decrease in alligator weed biomass is observed at treatment sites, as demonstrated in Chapter 4 (Clements et al. 2014a). However, herbicide application, which is the most efficient method of control, results in the production of alligator weed stem fragments and a proportion of these fragments are viable post-herbicide application, increasing the rate of spread of the weed throughout catchments. This critical aspect to achieving extirpation of aquatic alligator weed was evaluated in Chapter 5 (Dugdale et al. 2010), which is the first study of its kind designed to determine the viability of stem fragments (for any invasive aquatic plant) produced post-herbicide application. This study showed that herbicide application results in the production of viable alligator weed stem fragments, with differing rates recorded for different herbicide active ingredients. The herbicide metsulfuron-methyl produced significantly more viable fragments than glyphosate in container trials, 66 m$^{-2}$ and 9 m$^{-2}$ respectively. However, in field trials a high proportion of stem fragments were viable (60 to 80%), regardless of herbicide used for patches >5 m$^2$, for patches <5 m$^2$ viability was low. Further, there was no evidence that increasing herbicide rate (higher than label rate) within glyphosate or metsulfuron-methyl (with or without a surfactant) reduced the amount (or timing) of viable fragment production post-herbicide application. These viable fragments are capable of regeneration and increase the rate of dispersal throughout catchments, making eradication programs using herbicides ineffective unless the problem of viable fragmentation is addressed. If a control regime is not effective in preventing reproduction, new infestations may be created through the dispersal of propagules to other areas (Panetta and Lawes 2005).

Subsequently, the investigation in Chapter 6 (Clements et al. 2012) aimed to limit the amount of viable fragments produced post-herbicide application (glyphosate and metsulfuron-methyl), utilising plant growth regulators (PGRs). These chemicals are used in
the horticultural industry to reduce pre-harvest fruit drop of stone fruits and apples, by manipulating the abscission process that occurs within plants. This novel approach to utilise PGRs to reduce abscission and subsequent dispersal had not previously been attempted for aquatic plant management. This laboratory trial showed there was no significant effect detected between treatments regarding viable fragment production (at the rates and timings that were tested), and there appears to be no benefit in incorporating PGRs into herbicide control programs for aquatic alligator weed. The addition of PGRs did delay fragmentation marginally, however, this effect was insufficient to warrant incorporating PGRs into control programs. Further, an antagonistic effect was detected where the plant produced greater below ground biomass in response to the plant growth regulators. Therefore, this line of research was abandoned. Although a null result was attained in this study, because of the novel approach, the methodology that was developed and the results achieved, this investigation was published by the Weed Science Society of America (Clements et al. 2012). Rarely do we see published accounts of ineffective treatments in weed research and reporting ineffective treatments is important, as they provide direction for future research. Further, information was advanced in this study (in combination with Chapter 5; Dugdale et al. 2010) that suggests that autofragmentation (the self-induced abscission of shoots by the breakdown of the cell wall) drives stem fragment production of alligator weed post-herbicide application, indicating the possibility that further research could investigate other techniques to limit stem fragment production post-herbicide application.

The herbicides glyphosate and metsulfuron-methyl (under permit) were (prior to the research conducted in this thesis) utilised for control of alligator weed in aquatic situations in Australia, whilst the herbicide imazapyr has more recently been registered for the control of alligator weed in the USA and New Zealand (Hofstra and Champion 2010). Although there is much data available in the literature regarding the efficacy of these herbicides on control of above and below ground alligator weed, none had previously looked at all aspects of control that need to be studied for effective management of alligator weed in aquatic environments. No study had looked at the impact of herbicide control on above and below ground biomass as well as viable stem fragment production. These are the three critical measures when determining the efficacy of a particular control regime for effective alligator weed management in aquatic environments, particularly when incorporated into eradication programs. Chapter 7 (Clements et al. accepted) evaluated these three important factors that have been overlooked in previous studies for the control of aquatic alligator weed using herbicides and compared currently employed control techniques (glyphosate and
metsulfuron-methyl), to a potential new herbicide (imazapyr) to be utilised in eradication programs in Australia. This study synthesises the research developed in Chapters 4-6 of this thesis to optimise control strategies for alligator weed in aquatic environments for effective catchment scale management. The study evaluated the efficacy of herbicides and surfactants on all of the key alligator weed response metrics and concluded that glyphosate (isopropylamine salt) provides more effective control of overwater alligator weed than imazapyr and metsulfuron-methyl and minimises viable stem fragment production and therefore potential for dispersal throughout catchments and waterways. This study determined that metsulfuron-methyl and imazapyr should not be used for control of alligator weed growing overwater, as the number of viable stem fragments produced from these herbicides treatments were 75-90% greater than from glyphosate (isopropylamine salt) (Chapter 7; Clements et al. accepted). Further, fragments derived from imazapyr treatments took longer to sprout new roots or shoots than the viable fragments from glyphosate or metsulfuron-methyl treatments. This delay in time to sprouting of imazapyr treated stem fragments could provide a false sense of effective alligator weed control in the short term, viable fragments disperse throughout catchments before lodging and rooting into embankments, creating new infestations. In response to the research conducted on viable alligator weed stem fragment production post-herbicide application presented in this thesis (Chapters 5-7), the eradication program in Victoria, Australia, now utilises glyphosate only for control of overwater alligator weed.

While viable stem fragment production is far lower using glyphosate than using metsulfuron-methyl or imazapyr, viable stem fragmentation can still be a problem with glyphosate and the extent of the problem is likely to vary greatly with situation. These results suggest that as patches get larger and denser more viable stem fragments will be produced from glyphosate treatments (Chapters 5 and 7). Therefore, applying glyphosate to small infestations in an early stage of invasion will limit viable fragment production and potential for dispersal throughout catchments, ultimately optimising the management of this invasive aquatic plant. It is likely that reducing the time to extirpation will more than offset the extra cost of applying control techniques more frequently. Until further methods to limit viable stem fragment production post-herbicide application are established (Clements et al. 2012), viable propagules entering waterways need to be restricted. This can be achieved by utilising barriers (e.g. floating booms) to prevent stem fragment escape post-herbicide application (Dugdale et al. 2010) or utilising physical removal (Clements et al. 2014a) where there is a risk of dispersal to surrounding areas where alligator weed is absent or limited in distribution.
Further, undertaking management works strategically in an upstream to downstream direction, clearing a catchment from top to bottom, will enhance management efforts.

Regrowth is rapid from plant material rooted in the embankment following glyphosate treatments, particularly after initial treatment (Clements et al. 2014a). In contrast, imazapyr and metsulfuron-methyl both provided more effective control than glyphosate for alligator weed rooted on exposed embankments (Chapter 7; Clements et al. accepted). This data suggests that an effective management strategy for aquatic alligator weed would be to conduct initial applications of glyphosate to control overwater biomass and effectively limit dispersal of viable stem fragments. Once infestations have been forced back to the embankment utilising glyphosate and there is negligible overwater alligator weed present, imazapyr or metsulfuron-methyl treatments will provide more effective and longer term control than glyphosate. It is likely that imazapyr will be more effective than metsulfuron-methyl for this purpose (Chapter 7; Dugdale and Champion 2012). Utilising this approach will likely reduce the number of applications required each year to control aquatic alligator weed. However, this approach should be field tested to confirm effectiveness.

Herbicide efficacy differed between two formulations of the active ingredient glyphosate for control of aquatic alligator weed. Glyphosate as isopropylamine salt formulation provided more effective control of aquatic alligator weed than glyphosate as isopropylamine + mono-ammonium salt formulation (Chapter 7; Clements et al. accepted). This trial indicated that there were large differences in the efficacy of control between the two different glyphosate formulations. However, this study was conducted in small aquaria trials (modelling alligator weed in an early stage of invasion) and no other studies have reported the efficacy of different formulations of glyphosate for control of aquatic alligator weed. Further experiments to test a range of glyphosate formulations on both small and large infestations should be conducted to guide the use of this active ingredient used in eradication programs against aquatic alligator weed. Until further trials are conducted, glyphosate as the isopropylamine salt should be utilised in eradication programs for control of overwater alligator weed, as the efficacy of this formulation has been extensively studied in these published works (i.e. the formulation utilised in Chapters 4, 5, 6 and 7).

Future research to develop effective control measures for alligator weed in aquatic environments needs to integrate each of the key response metrics and evaluate above and belowground biomass, as well as viable stem fragment production (Clements et al. accepted).

Further research should be conducted to optimise control techniques by evaluating novel combinations of herbicide treatments (i.e. spiking glyphosate with imazapyr or metsulfuron-
methyl) or new chemistries, to try and obtain effective control of both overwater and marginal alligator weed infestations with fewer applications. However, environmental toxicity needs to be carefully considered.

The use of surfactants (in combination with herbicides) on larger field infestations also needs to be elucidated, as adding surfactants to any herbicide treatment provided negligible benefit for control of aquatic alligator weed in aquaria trials (Chapter 7; Clements et al. accepted).

Effective management strategies for the control of alligator weed in aquatic environments has been developed and evaluated in this thesis (Chapters 4-7) and although further research to enhance our ability to control one of the world’s most invasive aquatic plant species will continue, this data provides significant direction for waterway managers targeting extirpation of alligator weed from catchments and waterways.

The research presented in this thesis has implications for other aquatic weed management programs. The management techniques and principles developed for alligator weed in this thesis provide a model for programs that aim to optimise the management of invasive aquatic plants targeted for extirpation from catchments and waterways. Aerial detection techniques can be developed and evaluated particularly for invasive floating aquatic weeds species (including water hyacinth and salvinia) at both a landscape scale (large spatial scales) and at a site scale (small spatial scales), similar to the research conducted for alligator weed in this thesis (Chapters 2 and 3). However, advancement of aerial surveillance detection techniques must be quantified (quantitatively compared to current detection methods as demonstrated in Chapter 3) for these methods to be adopted by agencies responsible for the eradication of high risk invasive aquatic plants.

The interaction of control strategies used to extirpate invasive aquatic plants and the subsequent plant response needs to be evaluated in aquatic systems to prevent dispersal of propagules within waterbodies. Many the world’s invasive aquatic plant species do not produce viable seed in their introduced range and reproduce solely by vegetative means (whereby unspecialised stem fragment production is a common and efficient reproduction and dispersal mechanism (Barrat-Segretain 1996)), requiring these plant dispersal units to be managed effectively to achieve extirpation. The research presented in this thesis (Chapters 5-7) is the first of its kind designed to determine the viability of stem fragments produced post-herbicide application of any invasive aquatic plant. This aspect of control is likely to be important for effective control of many other invasive aquatic plants (including submersed aquatic weeds) that reproduce vegetatively and are likely to be spread by fragmentation post-
herbicide application. This aspect of control and subsequent dispersal is critical to understand when targeting extirpation (Panetta and Timmins 2004), particularly for invasive aquatic plant species.

8.3 Concluding remarks

In conclusion, alligator weed is a problematic weed species in many regions of the world that is difficult to control once naturalised. It poses a significant threat to agricultural productivity, biodiversity and social amenity of aquatic environments, requiring effective management strategies to be available to limit its impact. This research project has optimised the management techniques for this invasive aquatic plant and enhanced the likelihood of extirpation from catchments and waterways. The management techniques and principles developed for alligator weed can be utilised to model the development of efficient management strategies for other invasive aquatic plants that pose a biosecurity risk and those targeted for extirpation from catchments and waterways.

The research presented in this thesis has quantified, for the first time, all of the key criteria (Panetta and Lawes 2005) required to achieve extirpation of alligator weed from catchments and waterways. Waterway managers can utilise this data to inform the strategic direction of an eradication program and optimise the likelihood of a successful management program. The major findings of the body of published work include: (1) high altitude aerial imagery (orthophotos) can be utilised to detect large infestations of floating aquatic alligator weed; (2) low altitude aerial imagery captured by an unmanned aerial vehicle (UAV) can be utilised effectively to detect smaller patches of alligator weed; (3) alligator weed produces viable stem fragments in response to herbicide treatment, limiting extirpation attempts; (4) plant growth regulators (PGRs) were not effective at reducing the number of viable fragments produced post-herbicide application; (5) effective alligator weed management strategies are available but require a consistent approach with multiple applications over multiple years to achieve extirpation of individual patches of alligator weed within two to three years; (6) development of effective management strategies for alligator weed in aquatic environments requires control of above and below ground biomass and viable stem fragment production.
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