Strategies to minimise oxygen depletion associated with glyphosate control of Mexican water lily: Ammended May 2012

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Strategies to minimise oxygen depletion associated with glyphosate control of Mexican water lily: amended May 2012

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1. Introduction

This report constitutes the final report for the project:

Minimising deoxygenation resulting from control of Mexican water lily with glyphosate.

AGM-250303; TATDOC#3295047 v1; CMI103852

The deliverable for this project was:

> A manuscript, approved for submission to a scientific journal or conference proceedings by DPI internal review, will be provided. Note, if data are found to be inappropriate for publication a technical report will be produced instead.

Background

Goulburn-Murray Water (G-MW) currently controls Mexican waterlily growing in assets with glyphosate herbicide. However, rapid dieback and then decay can result in very low oxygen levels in the waterbody, with associated potential for nutrient pulses and fauna deaths.

The goal of this project is to determine if a modified herbicide application strategy (lower rates and application during cooler times of the year) can be used to minimise deoxygenation, without compromising efficacy of control.

The field component of this project has been completed by G-MW over the last two-three years. It consists of a replicated and randomised trial applying herbicide to plots at different rates and at different times of year at three separate experimental sites and assessing the effect on the Mexican waterlily.

This aim of this project is to apply robust statistical and scientific analyses to these field results and publish finding in a scientific journal so that robust and defensible decisions about the management of Mexican waterlily can be made in the future by G-MW or other Victorian and Australian waterbody managers.

Deliverable

Upon inspection and analysis of the data and the trial design it was decided that the results warranted publication in a peer reviewed scientific journal.

The paper written will be submitted to the Journal of Aquatic Plant Management (JAPM). This is a United States based journal that specialises in aquatic weed management and is freely available online (http://www.apms.org/japm/japmindex.html) from one year after publication.

The manuscript that we have written for JAPM has been approved for submission by DPI and NIWA internal review.

The paper includes the findings of the study along with recommendations for future management of Mexican water lily. Additional information that is not appropriate for inclusion in a scientific paper has been provided in this report.

Authors

The authors of the paper and their association with the project are listed below:

Deborah Hofstra and Paul Champion

> Aquatic plant management scientists from NIWA (National Institute of Water and Atmospheric Research) in New Zealand. They were contracted by DPI to write the manuscript to enable the project to be finished within the timelines required by NCCMA and G-MW.

Tony Dugdale

> Aquatic weed scientist at BRD (Biosciences Research Division), DPI. The project manager at DPI.

Masha Fridman

> Biometrician at BRD (Biosciences Research Division), DPI, who undertook the statistical analyses.

Roger Baker and Mark Finlay

> Aquatic plant managers at G-MW. They designed, established and undertook the field trials.

2. Manuscript for journal submission

The following MS is that which has been approved by NIWA and DPI and will be submitted to JAPM:

Strategies to control Mexican water lily to minimize potential for dissolved oxygen depletion

D. E. HOFSTRA, P. D. CHAMPION, T. M. DUGDALE, M. FRIDMAN, R. BAKER, M. FINLAY*

ABSTRACT

Mexican water lily (*Nymphaea mexicana* Zuccarini) is a perennial aquatic plant that originates from Mexico and southeastern USA. It has been widely distributed and is known to rapidly colonise shallow nutrient-rich waters, resulting in dense infestations that restrict water movement, contribute to siltation and decrease the economic utility, recreational and environmental values of waterbodies. In the state of Victoria (Australia), Mexican water lily occurs in several lakes and waterways that are managed for environmental values and supply of irrigation, stock and domestic water. Following the expansion of Mexican water lily, and associated concerns about low dissolved oxygen, a control program using glyphosate was implemented. The current research project was undertaken to determine (a) the efficacy of glyphosate application during spring and autumn compared with standard summer treatment, (b) the efficacy of a low application rate compared to the label rate and, (c) the rate of dieback, and oxygen depletion potential, following each of the above scenarios, to develop a herbicide application strategy to minimize the potential for low oxygen levels in the water and maintain control of Mexican water lily. The two experimental sites in the Goulburn Weir system had replicate treatment plots with two herbicide rates (3 and 6 L ha-1 and untreated control plots) and applications at different times (months) over two successive years. Time of

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application was the most significant factor in determining efficacy, with treatment during late summer/autumn resulting in the longest duration of low Mexican water lily. There were no differences in efficacy attributed to herbicide rate. This study illustrates that low cover of Mexican water lily in the warmest months, and the potential for increased gas exchange between water and atmosphere and increased dissolved oxygen, was only achieved with late spring to early summer herbicide application. To achieve both low cover of Mexican water lily in summer, and for a long duration, may require two applications annually (eg., spring to early summer application to provide low lily cover in summer, followed by another when temperatures cool in autumn). Alternatively longer term reduction and subsequent site eradication could be achieved with higher rates of glyphosate.

Keywords: herbicide efficacy, aquatic plant management, glyphosate, seasonal treatment, *Nymphaea mexicana*, oxygen

INTRODUCTION

Mexican water lily (*Nymphaea mexicana* Zuccarini) is a yellow flowered perennial aquatic plant that originates from Mexico and southeastern USA. It is a parent of many hybrid cultivars of ornamental water lilies that have been distributed to many countries as pond plants. Naturalised plants in Australia and elsewhere outside its native range, including New Zealand, southern Africa and Spain, are likely to be of hybrid origin but have characters diagnostic of the species (Queensland Government 2011). These include upright rhizomes (erroneously known as tubers), creeping fleshy stolons with attached ‘brood bodies’ consisting of a number of buds and a bunch of short yellow banana-shaped roots, giving the plant its alternative common name of banana water lily (Rickettson 1995). Plants can rapidly colonize shallow (up to 2 m deep) nutrient rich waters, and can result in heavy infestations in still-water lakes, reservoirs, ponds and slow moving channels (DiTomaso and Healy 2003).

Heavy infestations can restrict water movement, contribute to siltation, reduce gas exchange between air and water and decrease the recreational value of a waterbody (Capperino and Schneider 1985).

Mexican water lily (along with other aquatic weed species) was likely introduced into lakes and waterways of northern Victoria as a result of careless disposal of aquarium plants (G-MW 2009). It is presently in Lake Nagambie, Goulburn Weir Pool, Gunbower Creek and Broken River as well as in Lake Benalla, and continues to spread (G-MW 2009).

G-MW manages the major dams, irrigation supply and drainage systems, surface water diversions and groundwater in the Goulburn River catchment, including rivers and streams that are natural carriers, as well as Goulburn Weir pool (and associated backwaters) that were formed upstream of the Goulburn weir (constructed in 1891). These lakes and waterways have multiple uses and many stakeholders, with stored water used for crop irrigation, stock and domestic water supply, recreation as well as the recognized biodiversity values of the system.

Within the G-MW managed waterbodies, Mexican water lily has a negative impact on both water quality and amenity values of these systems (G-MW 2009) such as reduced habitat value for fauna and flora, restricted water exchange between backwater areas and river channels, impeded access, reduced aesthetic values, including foul odour associated with declining water quality (Finlay et al. 2008). In response, G-MW implemented a spray program with glyphosate over a seven year period (1996 to 2003) during which the area of Mexican water lily within the weir pool was reduced from 200 to ca 30 ha (Finlay et al. 2008, G-MW 2009). Water Eco Science study found no direct impact of the spray program on water quality (Francis and Crapper 2004). However the program was suspended from 2004 to 2008 following fish deaths in the Goulburn River downstream of the weir. It was suspected that water with depleted oxygen levels passed over the weir and caused the fish deaths downstream, but an audit by Environmental Protection Authority Victoria was unable to establish a cause.

Although it is recognized that rapid plant dieback and decay can result in very low oxygen levels in the water, with associated potential for nutrient pulses and fauna death, G-MW has responded to complaints from stakeholders about poor water quality following expansion of Mexican water lily in the system and currently controls Mexican water lily in water-bodies they manage with glyphosate, under a strict risk management framework (Finlay et al. 2008).

The current research project was undertaken to determine if herbicide could be applied at lower rates or cooler times of the year to minimize deoxygenation of the water, without compromising efficacy of control. Application at lower rates might result in a slower decomposition process and therefore maintenance of higher oxygen levels in the water column. The oxygen holding capacity of the water is greater in cool water so application at cooler times of year may also result in greater oxygen level in the water.

The objectives were to determine (a) the efficacy of application during spring and autumn compared with standard summer application, (b) the efficacy of a low application rate compared to the label rate and, (c) the rate of dieback, and therefore oxygen depletion potential, following each of the above scenarios. The purpose then was to use the research data to develop a herbicide application strategy to minimize low oxygen levels in the water and maintain satisfactory control of Mexican water lily.

MATERIALS AND METHODS

A replicated and randomised trial was set up in the summer of 2008 and 2009 that included two different experimental sites (Basleys' backwater and Picnic point) within the Goulburn Weir Pool backwater (Figure 1), with two herbicide rates and applications at different times (months) over two successive years. Both locations had shallow water with little or no water flow and were characterized by 100% surface cover of Mexican water lily, with few other aquatic plant species present (eg., the submerged *Cabomba caroliniana).*

At each location 60 adjacent treatment plots (5 by 4 m) were marked out with stakes that emerged above the water. There were three replicate plots for each herbicide treatment (3 L ha^{-1} or 6 L ha^{-1} glyphosate (1.1 and 2.2 kg ai ha^{-1} of Weedmaster Duo, 360 g ai L^{-1})) for each treatment month, and three untreated control plots at each of the two locations. Herbicide application was monthly from spring (November) through to early winter (June to July) in the first year of treatment. In the second year herbicide was re-applied to plots at the same rates, and month of treatment from November through to July at Basleys' backwater and through to June at Picnic point. Treatment plots were sprayed with glyphosate from a spray boat using calibrated equipment with a foam jet nozzle (0010) to minimize drift. A total of nine applications per treatment were undertaken and monitored at least monthly. Monitoring involved a visual assessment of percent cover of Mexican water lily above the water by two assessors. A 0.5 m band around the perimeter of each plot was ignored when estimating cover to negate the impact of leaves originating from adjacent plots. During application and monitoring events, water temperature and dissolved oxygen (DO) were recorded ca 30 cm below the water surface with an InsiteIG 310® optical oxygen meter, and water depth was recorded, along with comments on general plant health (leaf size and condition), ambient temperature and weather conditions. To provide an indication of the background DO dynamics, DO was measured 10 cm above the substrate in an area adjacent to the plots at Picnic point, under Mexican water lily with 100% cover during an 8 week period in summer 2010. The probe was a Greenspan®ODO3000 fitted with an optical DO sensor, set to record and log at 30 min intervals.

Statistical analysis:

Visual assessments of percent cover of Mexican water lily were used to estimate the duration of low water lily cover following herbicide application and the time to low cover. The duration of low cover was defined as the total number of months with ≤20% water lily cover during the period of one year following herbicide application. Time to low cover, was then determined as the time interval (weeks) between herbicide application and the reduction in water lily cover to ≤20%. The duration of low cover and time to low cover were estimated for each month of herbicide application (November-August), herbicide rate (control, 3 L ha^{-1} or 6 L ha^{-1}) and location (Basleys or Picnic point). Time to low cover was stratified by the frequency of assessment (2 per month or 3 per month) as the frequency of assessment affected the precision of estimation. Months of herbicide application with fewer than two assessments per month (May, June and July in year 1 and all of year 2) were excluded.

T-tests were used for binary comparisons of outcome measures between herbicide rates and locations. Ordinary least squares (OLS) multiple regression analysis was used to model non-linear and multivariate relationships. Stata/SE 12.1 was used for all statistical analyses.

RESULTS AND DISCUSSION

Mexican water lily showed clear seasonal trends with winter dieback of mature plants, new growth in spring, and increased cover over summer months within the control plots (Figure 2). This resulted in rapid increase in plant cover to ca 100% by mid spring (early November). At monthly site visits water temperatures ranged from 9 to 25°C, DO fluctuated from 4.8 mg L^{-1} (January) to 10 mg L^{-1} (June and July) and water depth varied between 0.3 to 1.2 m. During January and February 2010 average daily DO ranged between 0 and 6 mg L^{-1} and daily DO fluctuations were large (Figure 3).

Herbicide treatments were efficacious and did not differ between the two treatment rates (Tables 1 and 2), with both 3 and 6 L ha^{-1} (1.08 or 2.16 kg ai ha^{-1}) treatments resulting in a dramatic reduction in the cover of Mexican water lily a month from application, for all months in which the herbicide was applied (Figure 2).

Dieback of aquatic plants is associated with a drop in dissolved oxygen (DO) as the plant decays, and where dieback is rapid a more intense local DO demand could be expected than where it is slow. Hence the rate of Mexican water lily dieback was used as a theoretical indicator of local DO demand, and therefore potential for low water column DO. Although cover appeared to decline more quickly when treated at 6 L ha^{-1} compared to 3 L ha^{-1}, there was no difference detected in the speed of dieback with application rate, location or application month for the February to April period (Tables 3 and 4), as such associated differences in DO are not expected. However, there was a difference in the speed of dieback for application month for the November to January period (Table 4).

What was different between the treatment months, was the duration that the Mexican water lily cover remained low (at 20% cover or less). The duration of low cover was strongly associated with location (Basleys compared with Picnic point, $p=0.002$) and the month of herbicide application ($p<0.001$) (Table 2). The month of application was the strongest predictor; it accounted for 47% of the variability in the duration of low cover (Table 2). From the model, estimates of low cover duration were predicted (Table 2) and illustrate that late summer to early autumn herbicide application provides the longest period of low water lily cover (Figure 4).

For the treatments in the present study, Mexican water lily always grew back to problematic levels within 12 months. There are at least two possible explanations for the differences observed in duration of low cover, which are longest for applications made from midsummer to autumn. The first, is that the longest duration of low cover was achieved at that time of year, because the herbicide was translocated down into the rhizomes more effectively, thus having greater phytotoxicity. This is a common observation and is usually attributed to the net downward flow of solutes through the phloem at that time of year, taking the herbicide with it. A second explanation is that Mexican water lily exhibits a strong seasonal growth trigger, whereby growth is triggered by conditions that occur only in spring (October to December). For example, for plants at both Basleys' backwater and Picnic point, increase in cover began in November in the control plots and the plots treated from February through to August. For the February treatment plots, this represents a period of nine months that the rhizomes were dormant.

Glyphosate was shown to be a promising herbicide for water lily (both *Nymphaea* and the related *Nuphar* species) control by several early English and US studies (Barrett 1974, Riemer and Welker 1974, Welker and Riemer 1983) with total control of *Nuphar lutea, N.luteum* subsp. *variegatum* and *Nymphaea odorata,* obtained when glyphosate was applied at rates of around 2 kg ai ha^{-1}. Herbicide efficacy on *N. odorata* was evident one MAT (Riemer and Welker 1974) which is consistent with the findings of the present study. The New Zealand program to eradicate *N. lutea* used aerial application of glyphosate at 6 kg ai ha^{-1} (Champion 1991), with two applications over two years reducing floating leaf canopy from 100% pretreatment to <5%. Lower application rates were used in a previous trial but led to short-term defoliation of the water lily with dense regrowth the following summer. Continued follow-up spot-spray treatment (annually) of seedling and rhizome regrowth using 3% glyphosate (360 g ai L^{-1}) has now eradicated this species from the site (authors observations). High rates of glyphosate have subsequently been used to control infestations of Mexican water lily at two New Zealand water bodies (Lake Rotokaeo, Hamilton, Waikato; and Waitakere Wetland, Bethells Beach, Auckland). In both cases treatments were made twice a year (early summer and autumn) spot-spraying floating leaves. Eradication was achieved at Lake Rotokaeo, and at Waitakere wetland there was a reduction in area of water lily from 2 ha to 20 m^2, both after three years of treatment (Champion, 1999, 2003, 2007, Dugdale and Reeves 2003). In all cases rhizome death was apparent with masses of floating rhizomes resulting from initial treatment. At Lake Rotokaeo, situated in an urban setting, dead rhizomes were mechanically harvested following initial herbicide treatment to reduce potential odour and associated decline in DO during decomposition (G Angell, Aqua-Ag NZ, pers comm).

DO levels under areas of Mexican water lily in the Goulburn Weir Pool that were not treated, were severely depressed during warm months (Figure 3), probably due to a combination of factors reported to occur under heavy mats of floating vegetation, including natural turnover of standing crop, restricted water movement and mixing by dense floating canopies, and reduced oxygen holding capacity of warmer water (Sculthorpe 1967; Cronk and Fennessy 2001). Low cover of water lily in the warmer months of January through March has the potential to increase the surface area for gas exchange across the atmosphere water interface and in turn increase DO levels (Sculthorpe 1967) during this critical period. However in the current study, low cover of Mexican water lily in the warmest months (January through to March) was only achieved with late spring to early summer herbicide applications (Figure 2), which add organic matter (and thus oxygen demand) during this period of low ambient DO and provides only a short period of control (Table 1).

Because none of the treatments tested here provided both low Mexican water lily cover in summer and low cover for a long duration, two alternative strategies are suggested. The first is two herbicide applications annually, one in spring to early summer (eg., November, December) to provide low lily cover in summer (January, February), the second as temperatures start to cool in autumn (March, April) to achieve a longer duration of low cover. It is likely that over successive years the rhizomes will be killed, resulting in substantially less Mexican water lily (as demonstrated by Champion, 1999, 2003, 2007, Dugdale and Reeves 2003) and a corresponding decrease in the requirement for herbicide. The second strategy is to apply glyphosate at higher rates (6 kg ai ha^{-1} vs 2.2 kg ai ha^{-1}), with or without a second application in the same year. Although results in the present study do not demonstrate a change in control between the experimental rates, previous studies (Champion 1999, 2003, 2007) have shown that higher rates (eg., 6 kg ai ha^{-1}) may provide better control and significantly reduce the underwater biomass of rhizomes and stolons. Residue testing from the Goulburn weir pool during Mexican water lily control in 1997 to 1998 showed that where glyphosate was applied at 2.16 kg ai ha^{-1} maximum glyphosate concentration in the water in the spray zone was 0.39 mg L^{-1}, while downstream glyphosate levels were <0.038 mg L^{-1} (Francis and Crapper 2004). This is well below the maximum concentration of 1.2 mg L^{-1} where 95% protection of freshwater species would occur (ANZECC 2000). It is therefore unlikely that a three-fold increase in glyphosate application would breach these guidelines. Despite this, because a higher rate of glyphosate will result in greater mortality of rhizomes, which upon decay will create a larger DO demand than the current control program, it is suggested that higher rates are restricted to blocks of Mexican water lily, adding new blocks to the treatment program each year. Harvesting of dead rhizomes needs to be considered and a follow-up program to control regrowth from viable rhizome fragments, brood-bodies or seedlings needs to be instigated. As with the twice annual application strategy, it is anticipated that use of higher rate glyphosate would be reduced following the initial control season, with corresponding decrease in area impacted by Mexican water lily and consequent improvement in DO.

In summary, the present study showed time (month) of application was the most significant factor in determining efficacy as measured by duration of low cover. Although there were some differences between the two experimental sites, data from both show that treatment during late summer to autumn (February to May) will yield over eight and a half months of low Mexican water lily cover. The study also showed there were no differences in efficacy or speed of dieback attributed to treatment rate, therefore an opportunity exists to reduce both the environmental load and economic costs associated with the use of the herbicide. However, at either treatment rate, there would be a continued need for annual herbicide application. Therefore, alternative control strategies of either applying glyphosate twice a year, or applying glyphosate at a higher rate are proposed. Both of these options will reduce Mexican water lily abundance in the long term, leading to less use of herbicide. The former option, will also present a lower biomass of Mexican water lily at each application resulting in a lower potential oxygen demand as it decays.

ACKNOWLEDGEMENTS

This project was funded by Goulburn-Murray Water (G-MW), North Central Catchment Management Authority and Caring for our Country (Enhancing the values of the Gunbower Ramsar site). The authors would like to thank Geoff Angell (Aqua-Ag, New Zealand) and staff at G-MW who assisted with monitoring Mexican water lily abundance, and Dale Prewett for preparation of Figure one.

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Footnote

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Figure 1. Location of the two herbicide trial sites Basleys and Picnic point (-36.747791; 145.136175) in the backwater between Goulburn Weir Pool and Lake Nagambie, Victoria, Australia.

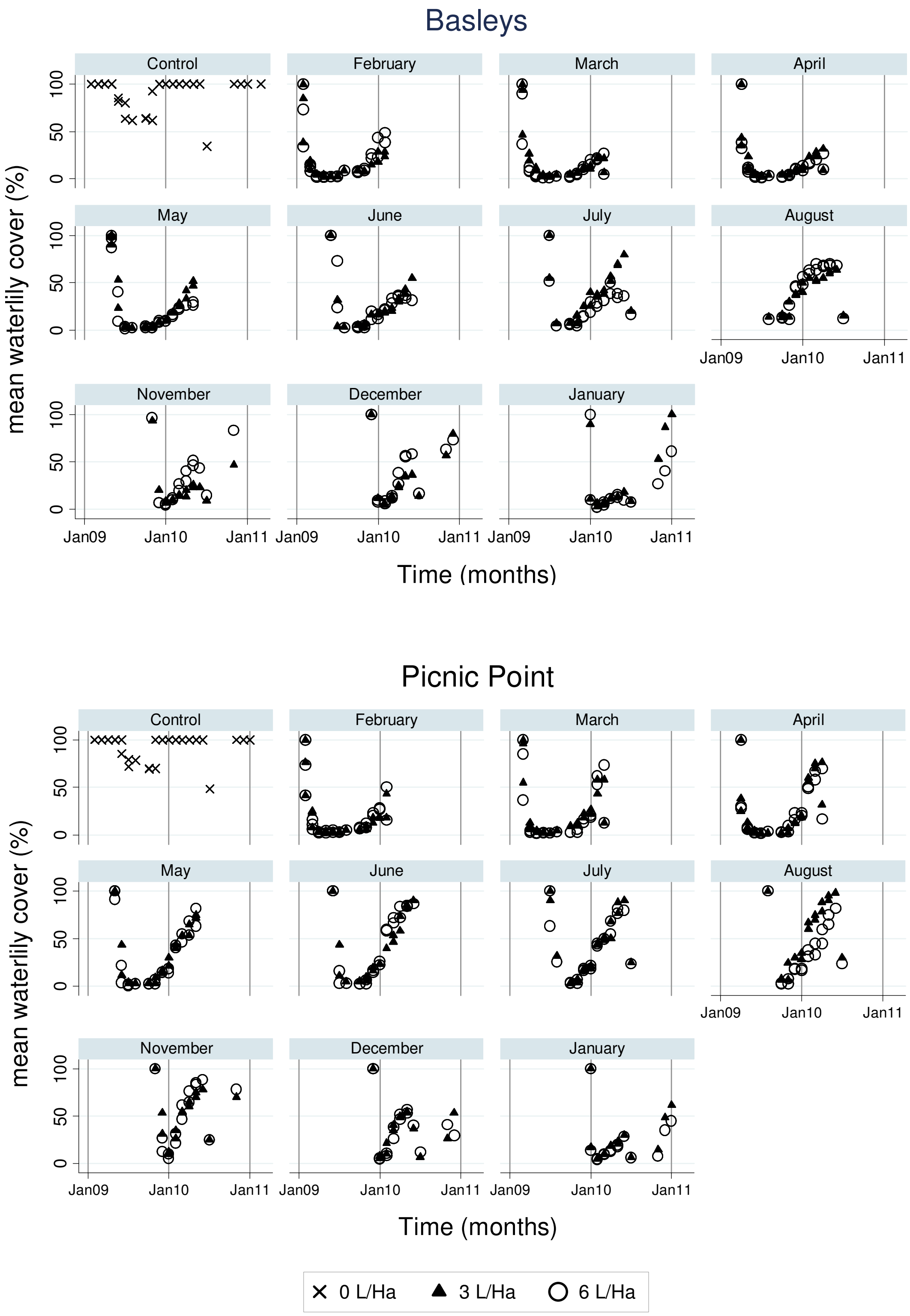


Figure 2. Mexican water lily response to herbicide application at different months, treatment rates and locations. Each estimate represents the mean cover (%) of three replicate measures, and is shown for the 12 months following the first herbicide application and the duration of the study for treatment and control plots respectively. The month of herbicide application is indicated above each graph and the vertical lines represent 1 year intervals.

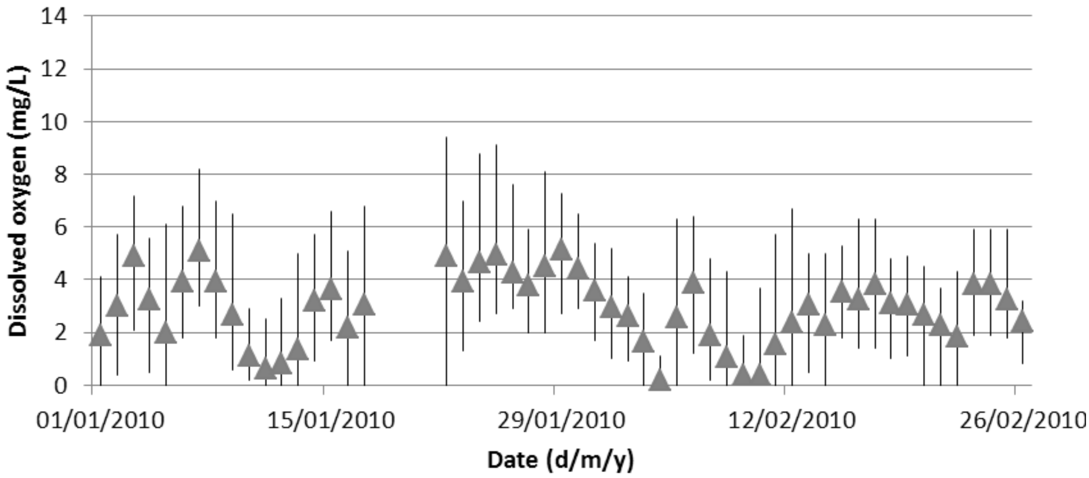


Figure 3. Daily dissolved oxygen from a reference area at Picnic point. Values shown are maximum and minimum, with average DO concentration indicated by the triangle.

Table 1. Duration of low water lily cover (≤20%) over a 12 month period following the first herbicide application. Observed mean estimates (± sd) for each location (Basleys and Picnic point) and treatment (control, 3 and 6 L ha^{-1}) are shown. The duration of low cover was measured in months.

Month of Application	Control*		3 L ha^{-1}		6 L ha^{-1}	
	Basleys	Picnic	Basleys	Picnic point	Basleys	Picnic point
January	0.3 ± 0.5**	0	5.7 ± 0.6	7.3 ± 2.9	9.0 ± 3.5	9.7 ± 1.2
February	0	0	11.0 ± 1.7	10.3 ± 1.5	10.0 ± 1.0	10.0 ± 0
March	0	0	11.0 ± 1.7	9.3 ± 0.6	11.3 ± 0.6	9.7 ± 0.6
April	0	0	9.7 ± 2.1	8.7 ± 0.6	11.3 ± 0.6	8.7 ± 0.6
May	0	0	8.7(3) ± 0.6	6.7 ± 0.6	9.3 ± 1.5	7.7 ± 0.6
June	0	0	7.7 ± 0.6	5.3 ± 0.6	7.3 ± 1.5	6.0 ± 1.7
July	0.3 ± 0.5	0	6.7 ± 1.5	5.0 ± 1.7	7.7 ± 0.6	5.3 ± 1.5
August	----	0	----	----	----	----
November	0.3 ± 0.5	0	8.3 ± 3.5	1.7 ± 1.2	5.0 ± 0	3.0 ± 1.0
December	0.3 ± 0.5	0	6.3 ± 4.0	7.0 ± 2.6	5.7 ± 3.8	6.3 ± 2.5

* N = 6 / location

** Low cover duration was observed in 2 out of 6 Basleys control plots on one occasion only (Jul, 2010)

Table 2. OLS multiple regression model of low Mexican water lily cover duration within one year since the first herbicide application with predicted estimates of low cover duration.

Independent variables	Predicted Duration (months [95 % CI])	Δ Duration [p-value]	ΔR^2**	F-test [p-value]
Rate				
3 L ha^{-1}	7.5 [6.9 – 8.1]	§	0.005	0.323
6 L ha^{-1}	7.9 [7.4 – 8.3]	0.37 [0.323]		
Place				
Picnic point	8.3 [7.7 – 8.9]	§	0.047	0.002
Basleys	7.1 [6.7 – 7.5]	1.19 [0.002]		
Month of application			0.473	<0.001
November	4.2 [3.0 – 5.4]			
December	6.6 [5.8 – 7.4]			
January	8.3 [7.7 – 8.8]			
February	9.4 [8.9 – 9.8]			
March	9.8 [9.3 – 10.3]	* [<0.001]		
April	9.6 [9.2 – 10.0]			
May	8.8 [8.4 – 9.2]			
June	7.3 [6.9 – 7.7]			
July	5.2 [4.6 – 5.9]			
Model Total	----	----	0.524	<0.001

* Variable change in Duration due to a quadratic relationship between Duration and the Time of application (see Figure 4).

**Change in R^2 after the addition of the indicated independent variable to the model

§Reference group

Table 3. Comparison between herbicide rates (3 and 6 L ha^{-1}) and the time taken to achieve low water lily cover following the first herbicide application. Mean estimates for weekly periods (± sd) of three replicates are shown for both locations, Basleys and Picnic point.

Assessments per month	Month of Herbicide Application	Basleys		Picnic point	
		3 L ha^{-1}	6 L ha^{-1}	3 L ha^{-1}	6 L ha^{-1}
3	February	4.0 ± 2.0	4.0 ± 2	4.3 ± 2.1	4.0 ± 0
	March	5.0 ± 3.6	3.3 ± 1.2	4.0 ± 0	4.0 ± 0
	April	5.3 ± 0.6	4.0 ± 1.7	4.0 ± 1.7	4.0 ± 1.7
	Average	4.8 ± 2.2	3.8 ± 1.5	4.1 ± 1.4	4.0 ± 0.9
	T-test [p-value]	0.270		0.839	
2	November	2.0 ± 0	2.0 ± 0	2.7 ± 1.2	2.7 ± 1.2
	December	4.3 ± 2.3	3.0 ± 0	6.0 ± 1.7	3.3 ± 0.6
	January	4.0 ± 0	4.0 ± 0	4.0 ± 0	4.0 ± 0
	Average	3.4 ± 1.6	3.0 ± 0.9	4.2 ± 1.8	3.3 ± 0.9
	T-test [p-value]	0.472		0.198	

Table 4. OLS multiple regression model of the time taken to achieve low water lily cover following the first herbicide application.

Models (Time restrictions)	Independent variables	Δ time-to-low cover (Weeks)			Predicted time (weeks)	ΔR^2 *	F-test [p-value]
		Δ (weeks)	p-value	95%CI			
Feb-Apr	Rate					0.034	0.299
	3 L ha^{-1}	§	§	§	4.4	.	.
	6 L ha^{-1}	-0.6	0.299	-1.6 to 0.5	3.9		
	Location					0.006	0.676
	Basleys	§	§	§	4.3	.	.
	Picnic point	-0.2	0.676	-1.3 to 0.9	4.1		
	Month					0.006	0.896
	February	§	§	§	4.1	.	.
	March	~0	<1.00	-1.4 to 1.4	4.1	.	.
	April	0.3	0.685	-1.0 to 1.5	4.3	.	.
	Constant	4.47	<0.001	3.1 to 5.8	.	.	.
	Model Total	.	.	.	.	0.046	0.575
Nov-Jan	Rate					0.062	0.064
	3 L ha^{-1}	§	§	§	3.8	.	.
	6 L ha^{-1}	-0.7	0.064	-1.4 to 0.4	3.2	.	.
	Location					0.043	0.119
	Basleys	§	§	§	3.2	.	.
	Picnic point	0.6	0.119	-0.2 to 1.3	3.8	.	.
	Month					0.380	<0.001
	November	§	§	§	4.2	.	.
	December	-0.2	0.723	-1.1 to 0.8	4.0	.	.
	January	-1.8	0.001	-2.9 to -0.8	2.3	.	.
	Constant	2.4	<0.001	1.8 to 3.0	.	.	.
	Model Total	.	.	.	.	0.484	<0.0001

§ Reference group

. Not applicable

* Change in R^2 after the addition of the indicated independent variable to the model

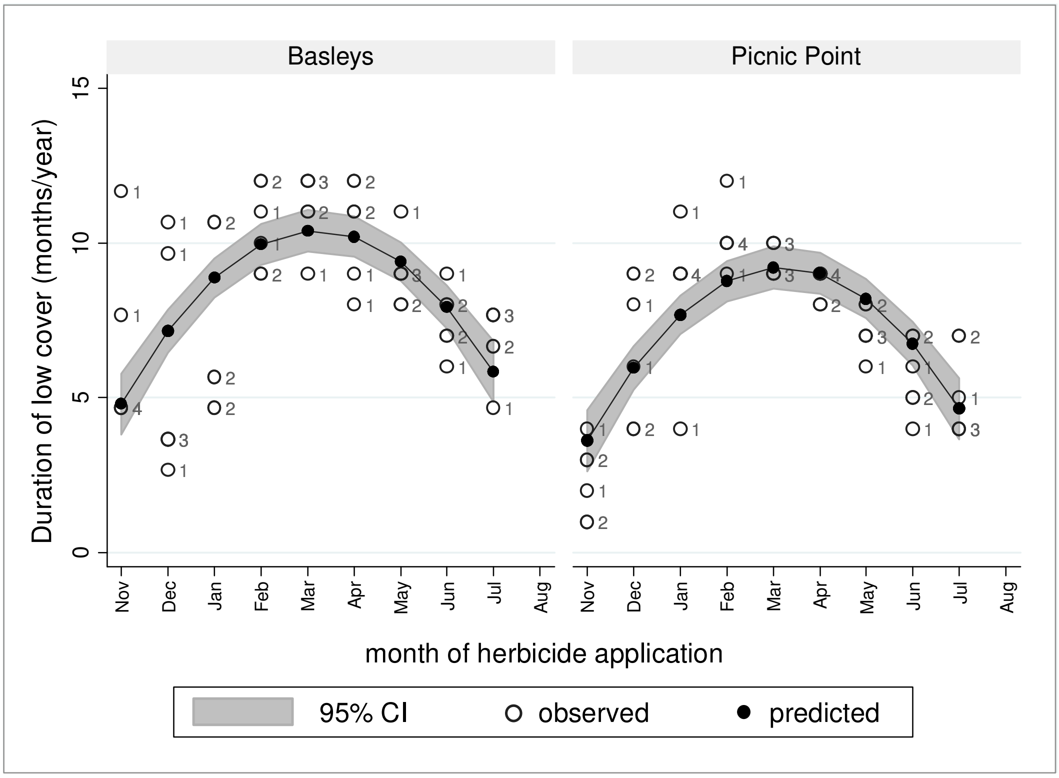


Figure 4. Illustration of the OLS regression model fit for duration of low Mexican water lily cover. The numbers beside each data point, refer to the number of observations per data point.

3. Additional information unsuitable for publication

Gunbower data

Although data exists from a trial at Gunbower that replicates the trials reported here (for Picnic Point and Basleys), the data is not used in the manuscript. This is because the trial was abandoned in January 2010 so there was not a full year of data available after each application, therefore duration of control could not be calculated. In addition, the trial site at Gunbower Creek dried over winter 2009, providing an additional impact on the Mexican water lily (Tim Nitschke, G-MW personal communication).

The available data from Gunbower were examined. The trends observed replicate what was found for the Goulburn Weir sites, i.e. duration of control and speed of die-back (where sufficient data) were within the ranges expected from the Goulburn Weir data and there were no strong differences between 3 and 6 L ha^{-1} application rates. These data are shown in Figure 3.1.

Based on this, the recommendations made in the manuscript are appropriate for the Gunbower Creek site.

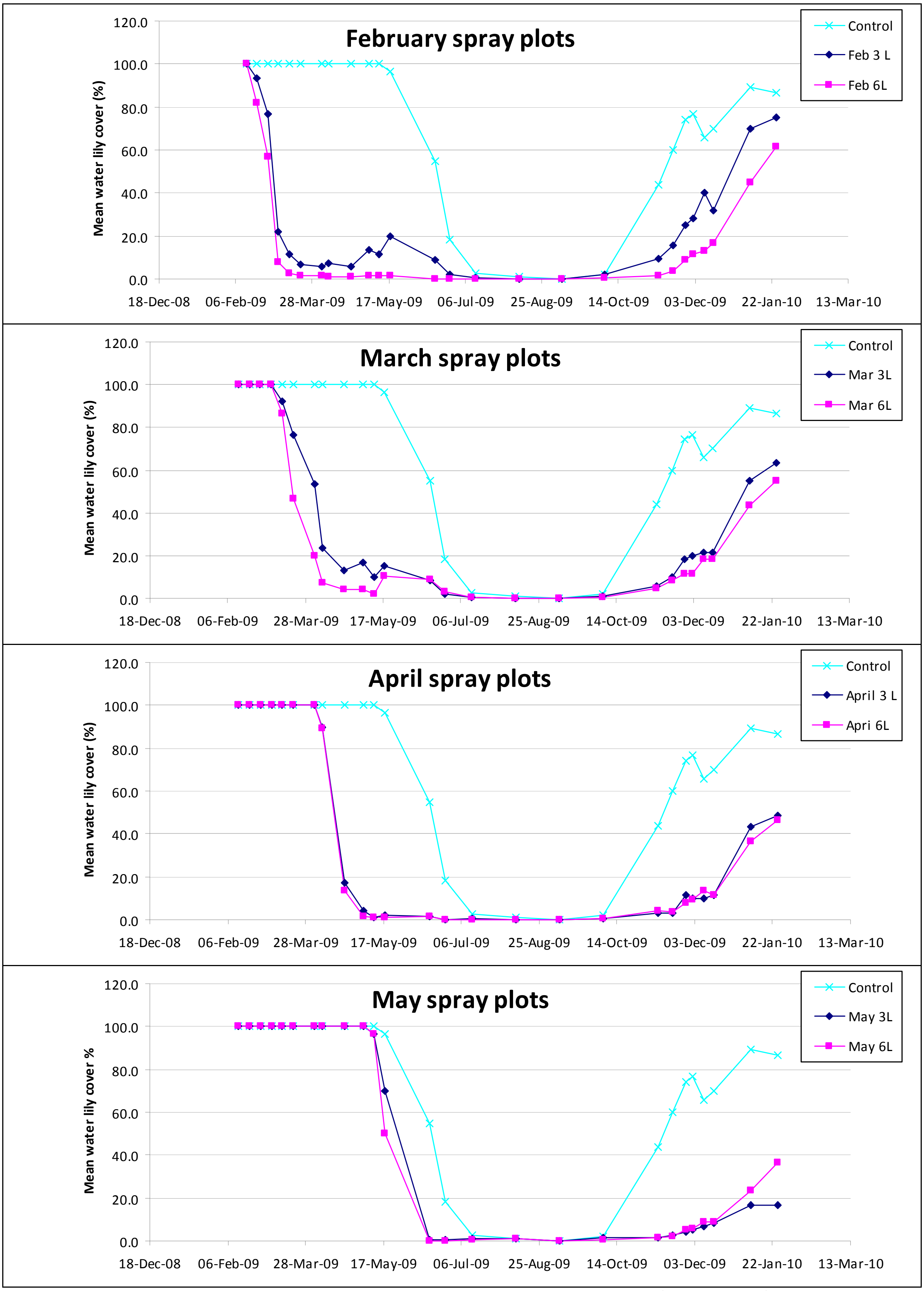


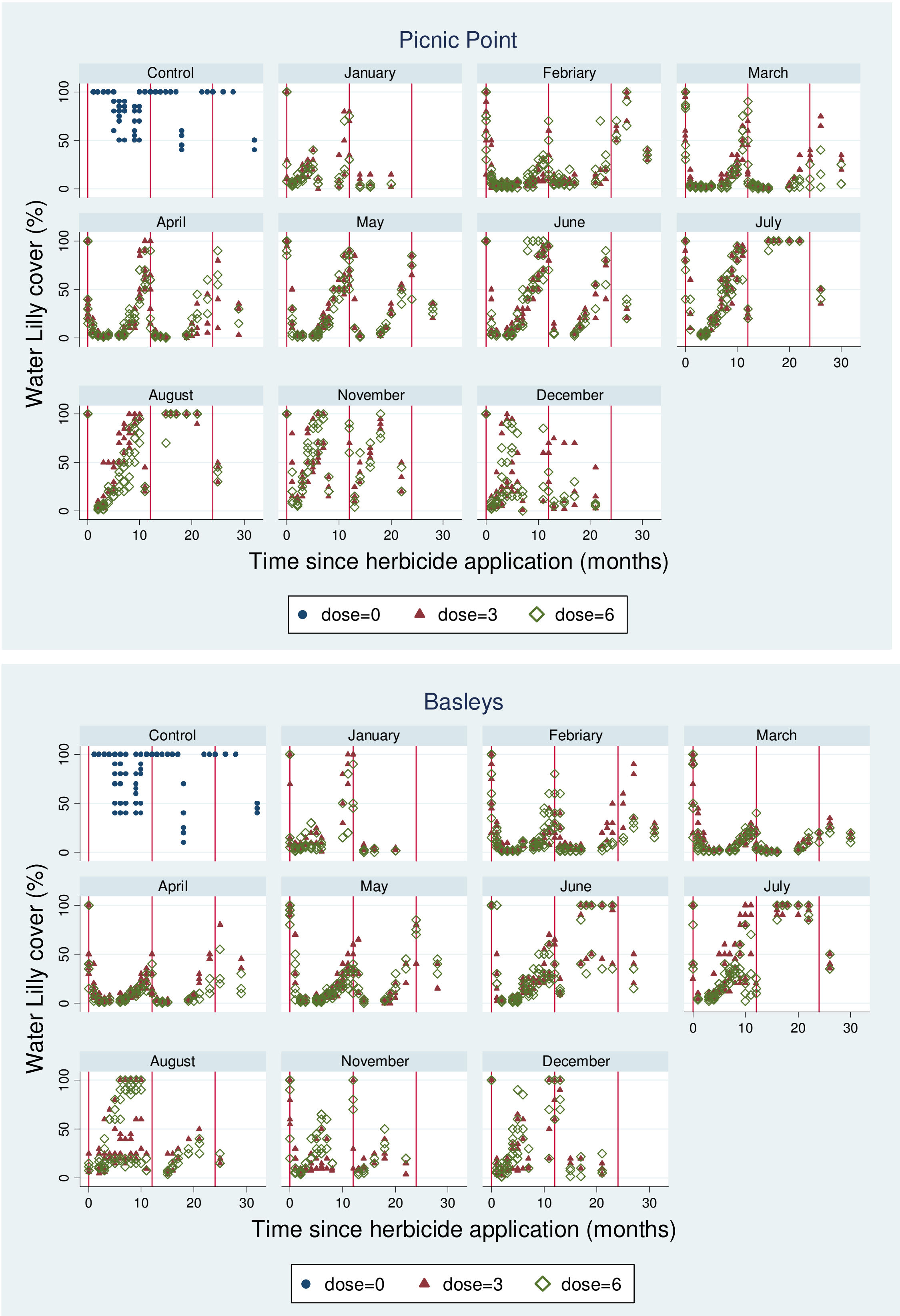
Figure 3.1. Mexican water lily response to glyphosate application at Gunbower Creek for different months and treatment rates. Each estimate represents the mean cover (%) of three replicate measures.

Dissolved oxygen data

Although dissolved oxygen data (DO) exist at 0, 3 and 6 L ha it was not used because some of the probes used to collect it were unreliable.

Additional figure

An alternative version of Figure 2 is shown below. This includes all data collected for this project. An abridged Figure 2 is included in the MS because statistical analyses were only carried out on a subset of the data (from time zero for each treatment plot to 12 months after application date). This was because the intervals between assessments in the second year were greater and irregular, making statistical analyses difficult. However, the trends observed in the figure below show that the results of the second year of application are consistent with the first.



Alternative Figure 2. Mexican water lily response to herbicide application at different months, treatment rates and locations. Each estimate represents the mean cover (%) of three replicate measures, and is shown for the 12 months following herbicide application and the duration of the study for treatment and control plots respectively. The month of herbicide application is indicated above each graph and the vertical lines represent 1 year intervals.

4. Directions

Two strategies are proposed in the paper to improve water lily control and reduce potential DO demand.

1. The first is to apply glyphosate twice per year, once in spring to early summer to provide low lily cover in summer, followed by another when temperatures start to cool in autumn.
2. The second is to apply glyphosate at higher rates to achieve longer term reduction in below ground biomass and subsequent site eradication.

Test recommended strategies

It will be useful to establish a trial to determine the effectiveness of the new strategies at reducing water lily abundance, in comparison to the current approach.

Collect DO data from large treatment areas

It will also be useful to design and conduct a rigorous program to measure DO under large areas treated with glyphosate compared to untreated areas and areas free of water lily.

Depending on initial findings this could be expanded to different treatments (i.e. rates or application frequency).