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How to establish aquatic field trials

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This chapter outlines the next step in the establishment of herbicide use to control aquatic weeds, following on from laboratory- and mesocosm-scale herbicide screening trials outlined in Chapter 7. The most promising results of those trials are then applied to natural aquatic situations (the field) with a number of complex and complicating factors. These factors may relate to management of other waterbody user values, environmental values, and regulatory constraints designed to protect those values. Unlike most terrestrial situations, herbicides are applied into the aquatic environment surrounding target plant species, or those target weeds grow in situations where herbicide contact with the water may occur.

Having a clear understanding of the purpose of the field demonstration provides the framework for how to establish the aquatic field trial. It is important at the outset to define the goal, research question, or hypotheses. What is the desired outcome, and what is the question the research aims to address that cannot be answered at the mesocosm or lab scale? In considering this chapter we describe six points or general considerations that are pertinent to all field demonstrations, followed by case studies of field trials with different goals (i.e., eradication, effective control, and product comparison) that provide examples of approaches for implementing an aquatic field trial.

1. GENERAL CONSIDERATIONS

1.1. Purpose—Why do a field study?

The reasons for establishing field trials are wide ranging. Common examples include comparison of different herbicides for performance on target macrophytes, assessment of efficacy on target species and the potential for off-target impacts in the natural environment, or eradication trials, all of which could lead to new herbicide use patterns. There is a fundamental need for clarity of purpose, as this drives the design (e.g., scale, replication, application rates), site selection, and monitoring that will be necessary to demonstrate results, most likely within a specified budget and time frame. For example, if the purpose were to assess whether (or not) a particular herbicide and application protocol could be used for the eradication of a submersed weed, then the time frame for monitoring would need to be sufficiently long to determine if eradication was achieved. Obviously, this means not only achieving zero biomass, but also monitoring for a posttreatment period that is related to the likelihood of residual propagules (e.g., buried fragments, root crowns, turions, seeds) losing their viability. Perhaps less obvious are the specific challenges posed by the aquatic environment compared with a terrestrial trial, such as product placement and retention in a fluid environment, impacts of rotting plant biomass killed by the herbicide on dissolved oxygen (DO), and the detection of small numbers of propagules under water (e.g., Section 2.1). These aquatic challenges are also some of the key reasons to undertake a field study, and address conditions that cannot readily be replicated at the smaller mesocosm scale.

1.2. What is concentration exposure time and why is it important?

In the case of submersed macrophytes, herbicides are not applied directly onto foliage; rather they are discharged into the aquatic environment surrounding the foliage to achieve the effective herbicidal concentration. Unlike mesocosm studies, field studies need to consider factors such as water movement and dilution that can reduce herbicide concentration and exposure time (CET) with the target vegetation. To test the efficacy of a product to reduce the biomass of submersed macrophytes, the trial design must address how a sufficient concentration can be achieved in water for a sufficient contact time around the target plants to achieve desired efficacy. For example, CET is dependent on the size of the waterbody and the treatment plots, weather patterns, wave action, flow, thermocline, and how these effect herbicide dilution and dispersion (e.g., Section 2.2). For comparison of multiple herbicides, the trial design must also incorporate a method to separate plots effectively in an otherwise contiguous aquatic space to prevent cross-contamination among plots (e.g., Sections 2.2.3 and 2.3).

1.3. Regulatory requirements

Regulatory requirements may be present at the local, state, or national level. A legal requirement and important component for all field demonstrations is to ensure that the appropriate legislation is understood and adhered to, and that relevant permits (consents) are in place before operations commence. This represents that legal obligations to ensure environmental, public, and personnel safety are being upheld. The herbicide label and material safety data sheet (MSDS) provide details for product use, concentrations required to achieve desired efficacy, and species controlled. Additionally, if new use patterns are being developed, consent conditions may require further monitoring and data gathering, such as documenting the potential for and ways to mitigate off-target impacts, that are pertinent to future registrations for herbicide usage.

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These additional data may inform future legislation and/or regulations for the use of the herbicide being tested. On the other hand, the data may be used to result in fewer regulations for future product use.

Monitoring and reporting also may be required as part of the field demonstration compliance process. Examples include the use of tracer dye prior to herbicide application to understand likely water movement, sampling for herbicide dissipation posttreatment, monitoring of DO, assessment of abundance of local native flora and fauna pre- and posttreatment, and demonstration of survival of caged fish/invertebrates within the treated area (e.g., Section 2 [also see Chapter 8, Herbicide dissipation]). Typically, caged animals are deployed when determining worst-case exposure for accumulation of residues in tissue—rather than acute impacts. Other complications can include the nature and proximity of threatened and endangered species, or species of special concern, to treatment plots.

1.4. Site selection

After the purpose of the trial has been determined, selection of the trial site requires careful consideration. As it is often not possible to find comparable water bodies with the same abiotic and biotic conditions (including the abundance and condition of the target weed species), a single large water body is frequently selected for a field trial. A general rule of thumb is to have treatment plots within the water body that are as large as possible, to minimize edge effects and maximize the likelihood of achieving target CET in the middle of the field plot. Where possible the trial site should be large enough to incorporate a minimum of three replicate plots per treatment with additional reference or untreated control plots. More plots are desirable (see Chapter 15).

Site ownership and access must be determined. The distance to and from the field site, requirements for boat or shore access, and likelihood of trial disruption by other waterbody users need to be contemplated. Discussions with landowners and regulatory authorities are a must prior to undertaking a reconnaissance of the selected site. Use of the area by boaters, waterfowl hunters, and fishers and the proximity to water takes, aquaculture ventures, and their use patterns should be determined, and peak use events (e.g., duck shooting season, fishing tournaments, boating regattas, recreational holidays, etc.) should be avoided if possible. Other specific considerations relating to biotic and abiotic features of the site are discussed in the sections below.

1.4.1. Biotic factors. The target plant must be in sufficient quantity, condition, and life stage (e.g., actively growing) to undertake the field trial at the selected site. Not only is the plant condition important relative to its growth stage, but also the presence of biofilms coating the vegetative portions of the plant can have a significant effect on efficacy (Clayton and Matheson 2010). Particularly for contact herbicides, biofilms, detritus, and suspended sediments may reduce the ability of the product to contact the plant, and in the case of diquat deactivation occurs (Clayton and Matheson 2010). The ionic bonds between diquat and charged particles result in adsorption to surfaces, negating herbicidal activity (Netherland 2014).

Where a comparison of different herbicides or herbicide rates is desired, discrete beds of the target weed can permit evaluation without significant cross-contamination of treatments. How far apart these weed beds need to be is dependent on water-movement patterns that could lead to herbicide drift out of the treated plots (see Sections 1.5 and 2.2). Ideally, the distance between treatment plots should be maximized and distance validated by tracer dye studies. To evaluate a concentration series of one herbicide in flowing water, the lowest concentration should be applied furthest upstream and the highest concentration furthest downstream (see Chapter 14). If a number of different herbicides are being assessed it is best to select separate waterways, with similar characteristics, for each product.

In addition to the target species, characterization of other biota in the affected area needs to be considered. A literature/information search should be undertaken to uncover previous ecological studies for the site and any resultant designation (e.g., ecological significance or reserve status). Surveys of flora and fauna are usually required to determine the diversity and abundance of other species and their conservation status. This provides the baseline to compare any nontarget effects resulting from the herbicide trial (see Section 1.6).

1.4.2. Abiotic factors. As described earlier, an essential difference between terrestrial and aquatic trials is the intimate connectivity of the submersed aquatic weed with its habitat and the goal of optimizing herbicide application to achieve control of the target species with minimal off-target impacts. Primary factors to consider are water movement (e.g., water velocity, tidal influence), clarity (e.g., turbidity), temperature, and DO in the environment. Effective weed control is reliant on achieving a desired CET of herbicide to impact target plants (Getsinger et al. 2008). Diffusion results in herbicide dilution, and this is accelerated by water movement through wave action and in particular by water flow. Failure to take account of water flow on CET can result in poor or no control of target weeds where a long CET is required. For example, in a trial to evaluate the use of endothall (dipotassium) to control *Ceratophyllum demersum* in a drainage system, excellent control was achieved in the still and slow-flowing channel sections, but little to no control was seen in faster-flowing areas (Champion and Taumoepeau 2007).

In still water bodies, development of thermal stratification (a thermocline) can effectively reduce the volume of water in which the herbicide can dilute, with a thermal barrier preventing mixing with colder bottom waters during summer (Haller 2014). This therefore requires either a recalculation of application rates or, if target weed beds occur beneath the thermocline, then injection into the bottom waters or a negatively buoyant gel carrier or clay granule formulation (Section 1.5.1) is required. A similar process that leads to thermocline development can occur during warm and still summer conditions. A layer of warm surface waters can prevent surface application of aqueous herbicides from reaching weed beds, with diffusion occurring within this thin warm water layer away from the target area. Use of tracer dyes can reveal this application issue, along with water-movement patterns (see Section 1.5).

Beds of the target weed species have a major influence on abiotic factors. In flowing water, dense macrophyte beds are effective in retarding water velocity and increasing waterbody volume (Champion and Tanner 2000), providing large areas with low velocity that promotes deposition of sediment. Large submersed weed beds can improve water clarity in this way. These beds can also severely impact DO and pH by accentuating diurnal fluctuations or spatial partitioning of these parameters. Water temperature is another important consideration; the warmer the temperature, the lower the concentration of DO. Emergent (especially mat-forming sprawling species) and submersed plants affect these parameters differently. The foliage of emergent species sits above the water surface, and the roots, the decomposition of naturally senescent parts, and the deposition of sediments potentially lead to degraded anoxic habitat beneath (Wilcock et al. 1995; also see Section 2.3). Submersed plant beds photosynthesize during the day and often create highly oxygenated waters surrounding them. But water pH is also affected, and pH values exceeding 9 are not uncommon. At night respiration of plants can depress DO and lead to acidic pH. Thus, a diurnal variation in these parameters occurs. Extreme biomass of either emergent or submersed plants create a stressful habitat for aquatic fauna, and monitoring has shown that organisms inhabiting such areas are typical of polluted systems (see Section 1.6). Habitats with DO levels < 4 mg L^{-1} are not suitable for the survival of many fish species (see Section 2.3). Therefore, it is important to quantify the aquatic environment prior to commencing the herbicide trial, with the use of data loggers to record DO, temperature, and pH at regular intervals through a period spanning a week prior and one or more weeks after herbicide application. The time frames suggested are indicative only; actual time frames will be dependent on the baseline conditions, rate of vegetation decay, and assessment of likely outcomes.

Regulations to restrict the further impact of deoxygenation resulting from the decay of target weed after herbicide treatment may apply. However, such regulations may differ from place to place, and between products, depending on their mode or speed of herbicidal activity. An example in New Zealand is a restriction (25 to 33%) on the portion of a waterbody that can be treated at any one time. However, this restriction is waived for the treatment of emergent weed beds should DO measurements determine that levels are below 4 mg L^{-1} prior to treatment (New Zealand Environmental Protection Authority [NZEPA] 2013). This guidance recognizes the degraded nature resulting from weed invasion. Application of herbicide into warm waters is more likely to accentuate DO effects, because at higher temperatures decay rates (and therefore respiration rates) are higher, leading to more rapid oxygen depletion, with the additional effect of lower maximum oxygen concentrations present in warmer water compared to cooler water. Deciding the best time to undertake an aquatic trial is a balance between suitable target weed condition and cooler conditions where deleterious DO impacts are mitigated. Day et al. (2014) documented the occurrence of fish deaths in a New South Wales lake resulting from *Cabomba caroliniana* control using carfentrazone. Thermal stratification and neutral pH led to all submersed weed beds being eradicated, in this case because of the smaller effective water volume treated and longer herbicide persistence. The Australian Environmental Protection Authority (AEPA) determined that appropriate assessment of risks and implementation of mitigation actions had occurred in this trial. Posttreatment fauna surveys showed no long-term impacts in Glenbrook Lagoon, with healthy, breeding populations of four native fish, whereas *C. caroliniana* has not been detected in the waterbody posttreatment (Day et al. 2014).

1.5. The herbicide trial

The herbicide trial can now proceed informed by the consideration of trial goals and site biotic and abiotic characteristics, with all legal and landowner permissions gained.

Legal permissions (including the herbicide label) are likely to outline the signage, notification, maximum application rate (per hectare), maximum allowable environmental concentration and applicator requirements for the trial (e.g., NZEPA 2013). Any trial must strictly comply with those permissions. The application technique used should be selected to match the weed species, extent, and abiotic conditions experienced at the site. There are a wide range of application methods from knapsack to aerial application, the choice of method is largely dependent on the size and configuration of the area to be treated and accessibility issues.

Where desirable flora and/or sedentary fauna are known to be present, impacts can be mitigated by either:

- ensuring those species are tolerant of the concentration of herbicides used in earlier laboratory and mesocosm-scale trials (e.g., tolerance of charophytes to maximum application rates of diquat and endothall; Hofstra and Clayton 2001, Kelly et al. 2012)
- relocating individuals to unaffected habitat within the same water body (e.g., freshwater mussels)
- collecting and culturing individuals for release after the trial (e.g., the rare *Myriophyllum robustum* was collected from Lake Otamatearoa and cultivated prior to the herbicide trial (Wells et al. 2014). Herbicide application had no impact on this species in this lake (Figure 1).
- covering the affected area to ensure no direct herbicide application (e.g., covering desirable plants with a tarpaulin)

1.5.1. Submersed weeds. Where a submersed weed bed is the target for control, application options include the use of granular formulations for broadcast, gelling agents (e.g., guar gum–based products; Chandrasena et al. 2012), or application techniques such as trailing hoses or subsurface injection, all in order to obtain sufficient CET. Inert dye tracers such as rhodamine water tracer (RWT) have been effectively used to demonstrate likely herbicide diffusion

Figure 1. *Myriophyllum robustum,* a rare endemic watermilfoil in Lake Otamatearoa, New Zealand (photo by R. Wells).

within weed beds (see Section 2.2.2) beforehand to enable effective CET (Getsinger et al. 2008).

In flowing water systems, it can be difficult to achieve sufficient CET from a single herbicide application. Automatic dosing systems and other practical considerations to mitigate water movement are discussed in Chapter 14.

Where the target area is a small discrete submersed weed bed, or where there is only a limited area of weed available for comparison of herbicide rates (or different herbicides), creating a series of treatment plots may be achieved by creating a barrier to water movement in or out of the designated area. In one trial aimed to demonstrate the selective nature of the herbicide diquat, a 20 by 10–m experimental plot was established with the use of warratahs (metal fence posts) enclosed by shade cloth and retained for 1 h posttreatment (see Section 2.2.3). This trial successfully demonstrated selective control of *Lagarosiphon major* with diquat with no damage to the nationally endangered submersed macrophyte *Isolepis lenticularis* (Champion 2016).

Some herbicides are rapidly degraded at high pH (e.g., carfentrazone and flumioxazin), and in those cases an early-morning application is likely to provide optimal conditions for herbicide persistence and CET (see Section 1.4.2 discussion on DO and pH).

Polar herbicides like diquat (strong cation) can be rapidly adsorbed onto clay particles and organic material such as epiphyton (strong anions). Clayton and Matheson (2010) developed a "dirtiness" scale to assess the likely success of diquat treatment, where dirtiness was correlated to silt coatings on leaf and shoot surfaces. Moderately dirty plants may still be effectively treated by using maximum allowable concentrations, but herbicide use was not recommended on the dirtiest plants.

1.5.2. Emergent/wetland weeds and floating leaved plants. Approaches to herbicide application to field trials involving plants with foliage on or above the water surface are much more aligned to typical terrestrial field trials, especially those for emergent weeds. Most herbicides used for emergent weeds are unlikely to be present at concentrations that would be damaging to submersed vegetation where contamination of water from inadvertent over-spray might occur. Herbicide trials can be laid out in a typical randomized block design (see Section 2.3). Where polar herbicides such as glyphosate are being evaluated, it is important that emergent plants that are coated in silt (e.g., in tidal areas) are cleaned before herbicide application. This can involve gun and hose application of water prior to herbicide application, allowing time for drying before herbicide application. Generally, the monitoring of herbicidal impacts should be carried out in the central part of a treated area to ensure there is no edge-effect bias in sampling.

1.6. Monitoring

Wherever possible the BACI (before, after, control, impact) design should be followed. Control sites should preferably be selected within the same water body and therefore experiencing similar environmental conditions. In flowing, nontidal water bodies this can be simply achieved by selecting control sites immediately upstream of treated areas. In lentic systems, a bay with similar exposure and aspect could be chosen far enough from the treatment site to prevent exposure of the target weed to herbicidal concentrations of the product. The distance between treatment and untreated control or reference site that is large enough to ensure separation of water and therefore herbicide treatment, can be determined by understanding water-movement patterns and validated by tracer dyes (Section 1.4). Occasionally, situations occur that permit the treatment of discrete small water bodies to allow assessment of a dilution series of herbicides (Section 2.1.1) or comparison of different herbicides.

When a field trial is in a single large waterbody, the size of the system and the scale of the weed beds need careful consideration to optimize the design. Although BACI or replicated trial plots are ideal for statistical analysis, if for example, the target weed within the different plots is dissimilar at the outset, then the result may not be readily interpretable. Further, if whilst ensuring replication, the plots (areas being treated) are too small, such that edge effects are apparent (e.g., neighboring weed encroaches during the monitoring period, artificially increasing the plant cover), or the target CET is not achieved, then the trial design will still lack robustness. In this regard the importance of pretreatment surveys to understand and characterize the trial site cannot be overstated. Collecting pretreatment or "before" data provides the information against which changes posttreatment are compared where replicated plots are not possible. The trial focus, then, is on change at the site over time (before and after) in both treated and untreated reference plots. In this example multiple sample points within single-treatment plots, while not statistical replicates, will provide better data for comparison and for determining the treatment outcome.

Monitoring for efficacy of the herbicide treatment(s) on target weeds can be undertaken using a variety of survey methods (see Chapter 9). Specific measures for assessing herbicide efficacy may include the change in weed cover, abundance (density), biomass, and viability.

Figure 2. Macroinvertebrate sampling to assess the potential for off-target impacts from herbicide application. Waikato River Delta control site.

Change in weed cover and abundance (density) can be assessed visually, but this relies on the skill and repeatability of the individual assessors. More objective and statistically sound approaches may include photo points, remote imagery, or point-intercept methods.

Biomass is an absolute measure of efficacy routinely used in mesocosm studies. It is suitable for field studies where discrete (known) areas of weeds within plots can be subsampled. However, accurate biomass sampling is often not practical (and can be hazardous) in dense weed beds and/or in deep water (> 1 m).

In addition to quantifying the effect of the herbicide on plant abundance, the viability of remaining plant parts (e.g., root crowns, defoliated stems, underground propagules) may require evaluation. Representative plant material could be assessed, by sampling and cultivating (growing out) under controlled conditions, or monitored over relevant timescales in the field. Relevant timescales are those sufficient to have observed recovery, or mortality of the treated weeds. Further, if fragmentation results from herbicide treatment, then measures of fragment numbers and their viability, compared with untreated plants, need to be quantified by sampling and cultivating under controlled conditions, as described above.

Depending on the purpose of the field-trial assessment of off-target effects on other flora and fauna, impact on water quality (especially DO) and measurement of herbicide dissipation in the environment may be monitored routinely. The following paragraphs and case studies provide examples of monitoring programs to illustrate approaches.

Monitoring example 1: Impact and environmental fate of herbicides

A trial to monitor the impacts and environmental fates of the herbicides metsulfuron-methyl and imazapyr isopropylamine used for the control of the emergent *Alternanthera philoxeroides* (alligator weed) was undertaken on the lower

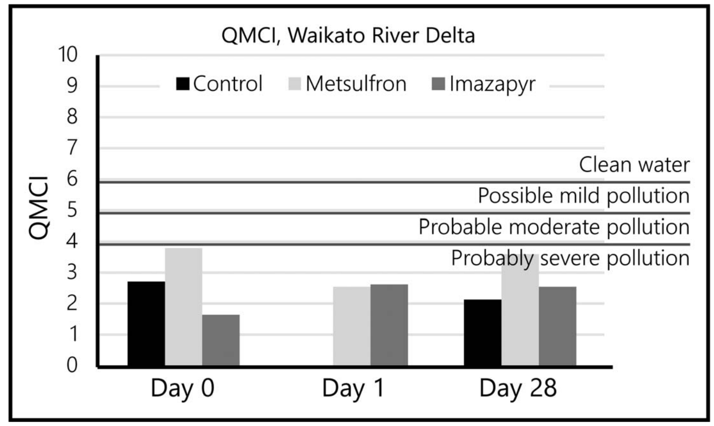

Figure 3. Quantitative macroinvertebrate community index from the Waikato River Delta before, 1 and 28 d after treatment with metsulfuron methyl and imazapyr isopropylamine herbicide (source: Champion et al. 2014).

Waikato River, near Tuakau (Waikato Region, North Island, New Zealand). Use of the two herbicides was permitted for this purpose by the NZEPA and by a resource consent from Waikato Regional Council (Champion et al. 2014). Benthic macroinvertebrates were sampled from surface sediment and macrophytes of three 25 by 25–cm square quadrats was scraped into a Wisconsin net (Figure 2) and bulked for each of the four herbicide sample sites and their controls (adjacent untreated reference sites). Samples were taken at time zero and ca 28 d after treatment (DAT) for the control at 0, 1, and ca 28 DAT for the treated sites.

In the laboratory, the animals were sorted and identified to the lowest taxonomic level practicable (usually genus and species level). Additionally, the metrics %EPT (percentage of the most sensitive insect larvae families; Ephemeroptera, Plecoptera, and Trichoptera) and quantitative macroinvertebrate community index (QMCI) were calculated for freshwater samples. The QMCI is an index of invertebrate sensitivity to organic enrichment, where individual taxa have been assigned scores based on their ability to tolerate organic enrichment (Stark and Maxted 2007). Scores for all taxa collected from a site were averaged on weighted abundance to give an overall site score of between 1 and 10.

There were no differences between the treated and control sites or pre- and posttreated samples. All samples of freshwater invertebrates were regarded as depauperate, with samples comprised of taxa tolerant of organic enrichment, and very low numbers or absence of the most sensitive EPT taxa. The QMCI indicates the sample sites on the Waikato River and Te Kowhai pond are probably severely polluted (highly degraded) (Figure 3).

Monitoring example 2: Dissolved oxygen consequences

A second trial to eradicate the giant emergent grass *Zizania latifolia* was undertaken in Lake Kereta (Auckland Region, North Island, New Zealand) with the grass-specific herbicide haloxyfop-methyl, a herbicide permitted for this purpose by the NZEPA and by a resource consent from the Auckland Council (Champion et al. 2014). Lake Kereta is a

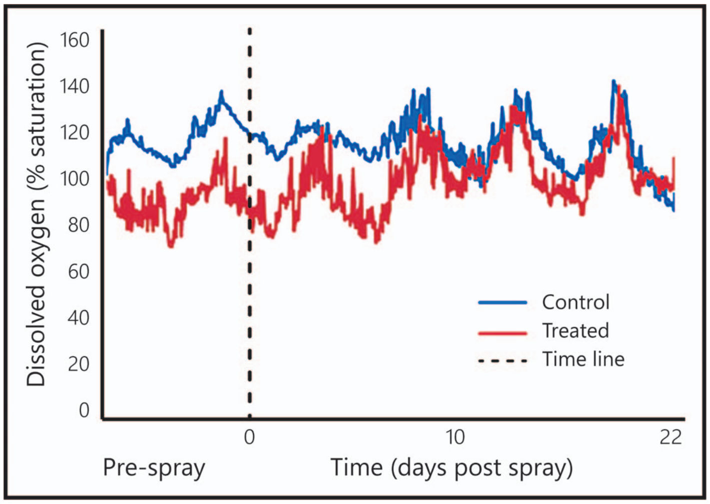

Figure 4. Dissolved oxygen in Lake Kereta at treated and control sites before and after application of haloxyfop-R-methyl to control *Zizania latifolia* (source: Champion et al. 2014).

natural dune lake with no extensive submersed macrophyte beds as a result of grass carp stocking in 2008 (Hofstra et al. 2014) to eradicate *C. demersum*. The water quality was regarded as poor, being highly nutrient enriched and regularly supporting large planktonic algal blooms (Gibbs et al. 1999). The DO loggers were deployed at 50% water depth, one in the treatment area, the other in an untreated area with similar water depth and other environmental variables. The DO and temperature were logged every 15 min for a minimum of 7 d prior to herbicide application and 22 DAT (Figure 4). The DO within the treated zone was similar to pretreatment levels until ca 10 DAT, after which DO was more similar to DO in the control zone (Figure 4). There was no significant difference between treated and untreated (control) measurements.

2. CASE STUDIES

2.1 Eradication

2.1.1. Achieving the unexpected—Endothall trials to optimize submersed weed control. Endothall was evaluated in mesocosm-scale trials in New Zealand (Wells and Clayton 1993, Hofstra and Clayton 2001, Hofstra et al. 2001) providing a high level of control of the aquatic weeds *Hydrilla verticillata*, *C. demersum*, and *L. major*, and was registered for use in 2005. Wells and Clayton (1993) reported it required 48 and 22 h contact time, respectively, at 2.5 and 5 mg L^{-1} to kill *L. major*. *Lagarosiphon major* was more susceptible to endothall than *H. verticillata* and less than *C. demersum* in comparative tests. Hofstra and Clayton (2001) reported that 0.5 mg L^{-1} endothall killed planted shoots of *L. major* in tanks with 3 to 7 d contact time, or by 3 d at 2 mg L^{-1} or more.

A field trial was conducted in a series of eight small gravel extraction ponds in Oreti, Southland, South Island to demonstrate the use of endothall to control *L. major* (Wells and Champion 2010). The concentration of endothall (as Aquathol® K or Aquathol® Super K pellets) was selected to include estimated application rates from 5 mg L^{-1} (maximum label rate) down to 0.1 mg L^{-1} (Table 1). As the ponds were not of natural origin, the regional authority (Environment Southland) ruled that this trial did not require a resource consent under relevant regulations (Resource Management Act 1993). However, herbicide residue analyses were undertaken on samples from each pond, along with temperature data, to assist in interpreting results. The data demonstrated that desired herbicidal concentrations were maintained for up to 38 d (Figure 5). No detectible endothall residues were sampled 8 mo after treatment (MAT). Temperatures were cool (ca 16°C) in the Oreti ponds during the trial, which is expected to have contributed to the slow product breakdown, resulting in long contact times and efficacy.

The ponds were assessed before treatment, for species present, height (maximum and average), and plant cover with a quick survey method (Clayton 1983). Location of selected areas of dense *L. major* were noted specifically to enable monitoring of endothall efficacy on this target species. A repeat assessment was made at 53 d, 10 mo, and 2 yr after treatment and compared with the pretrial assessment. Of 23 macrophyte species recorded, only 5 were adversely affected (Table 2), and, with the exception of Ponds 4 and 8 (Table 1), *L. major* was eradicated (Wells and Champion 2010).

This trial demonstrated the potential for endothall to be used as a highly selective control tool in cool temperatures, but under conditions with a long CET also demonstrated its potential as an eradication tool for sensitive species such as *L. major*.

2.1.2. Eradication trials. Following the pilot demonstration in the Oreti ponds (above), larger-scale eradication trials have been successfully undertaken in several small New

Table 1. Endothall treatment rates for Oreti ponds (source: Wells and Champion 2010).

Pond	Area (m^2)	Average depth (m)	Average cover of *Lagarosiphon major*	Endothall treatment
1	1,660	1.2	40%	3 mg L^{-1} Aquathol® K with gel
2	1,012	0.8	10%	3 mg L^{-1} Aquathol Super K (pellets)
3	290	1.2	50%	2.5 mg L^{-1} Aquathol K
4	1,450	1.2	100%	0.5 mg L^{-1} Aquathol K
5	224	1.2	75%	5 mg L^{-1} Aquathol K
6	14,000	1.3	Patches 100%	0.11 mg L^{-1} endothall diluted from 6a and 6b
6a, cove	200	1.4	80	3 mg L^{-1} Aquathol K
6b, cove	400	1.4	80	3 mg L^{-1} Aquathol K + gel
7	522	1.2	80%	1.0 mg L^{-1} Aquathol K
8	1,416	1.4	< 10%	Control, untreated

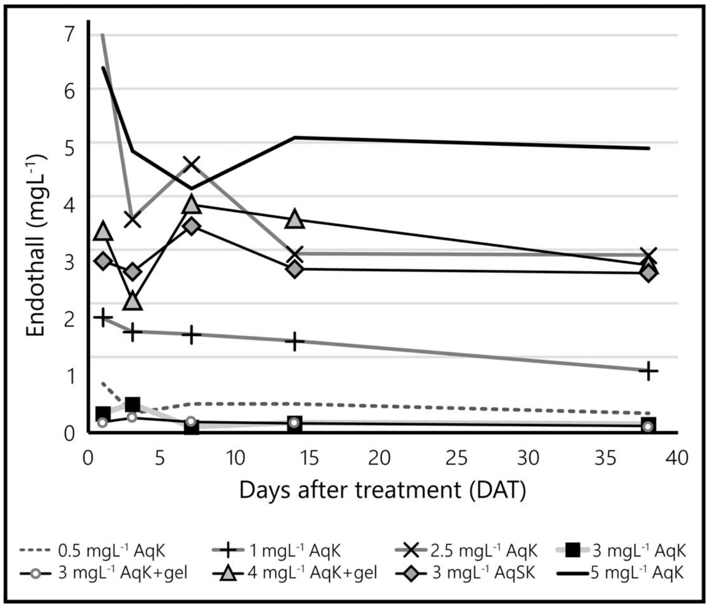

Figure 5. Endothall concentrations in treated Oreti ponds (source: Wells and Champion 2010). Aquathol K and Aquathol Super K are represented by AqK and AqSK, respectively.

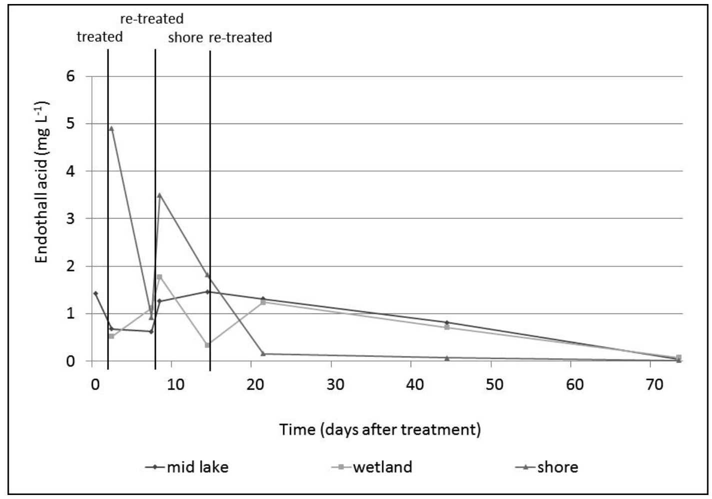

Figure 6. Endothall concentrations postapplications for midlake, wetland, and shore locations. The first vertical lines (black) represent endothall applications with aqueous product to open water, pellets amongst marginal emergent vegetation, and pellets in the retreated shoreline (source: Wells et al. 2014).

Zealand waterbodies, with eradication of *L. major* achieved in two lakes (Wells and Champion 2010). In addition, the one known South Island field population of *C. demersum* was eradicated (Wells et al. 2014) following one application of endothall.

However, another whole-lake trial of endothall to eradicate *C. demersum* was not successful (Wells et al. 2014). Otamateroa is a small (10 ha) shallow (4 m maximum depth) dune lake with submersed vegetation dominated by the invasive weed *C. demersum*, whilst the shallow margins have a range of native emergent species. In winter (June 2011), three applications of endothall were applied over 2 wk to maintain levels above 1.5 mg L^{-1} for 3 wk (Figure 6).

The weed beds were reduced to decaying fragments within 7 d, and 9 wk later only the occasional viable fragment was found buried within the bottom detritus. Six months later only a few scattered plants, mostly less than 0.5 m tall, were found. But within 3 yr *C. demersum* had returned to pretrial levels, highlighting the need to monitor for a sufficient period of time to determine trial outcomes that relate to the initial goal.

The desired goal of eradication was not achieved in this case. It is likely that groundwater inflows to the dune lake margin rapidly diluted endothall to below herbicidal rates in the shallow lake margins, as indicated in Figure 6. This was predicted, and to mitigate this endothall pellets were distributed in these areas. Despite the use of pellets, *C. demersum* was likely to have survived amongst shallow emergent vegetation and subsequently recolonized the main body of the lake.

2.2. Effective control

2.2.1. The efficacy of endothall (Aquathol K) to control H. verticillata. The purpose was to verify at field scale the efficacy of endothall on *H. verticillata*, following successful tank-scale studies (Hofstra and Clayton 2001), and provide a method with which to control *H. verticillata*. The New Zealand *H. verticillata* was not susceptible to diquat or fluridone.

Lake Waikōpiro is ca 10 ha in area, with a maximum depth of 18 m. *H. verticillata* formed an almost continuous monospecific band around the shallow margins of the lake from less than 1 m to 6.5 m depth. In contrast, the other lakes with *H. verticillata* were either much larger (up to ca 174 ha), or not entirely dominated by *H. verticillata* in the littoral zone, which meant they were less suitable as a trial lake compared with Lake Waikōpiro.

Table 2. Macrophyte response to endothall treatment in the Oreti ponds (source: Wells and Champion 2010).

Susceptible Species (Notes)	Species that Were Not Susceptible
Lagarosiphon major[1] (highly susceptible down to < 0.11 mg L^{-1})	*Nitella* sp. aff. *cristata*, *Nitella hyalina*, *Nitella leonhardii*, *Chara globularis*, *Tolypella nidifica* (charophytes)
Ranunculus amphitrichus (no recovery observed)	*Myriophyllum votschii*, *Lilaeopsis novae-zelandiae*, *Hydrocotyle hydrophila*, *Triglochin striata*, *Ruppia polycarpa* (low-growing turf species)
Ranunculus trichophyllus[1] (recovered, most likely from seedbank)	
Myriophyllum triphyllum (recovered from rhizome and stems, dominant macrophyte 10 mo after treatment)	*Callictriche stagnalis*,[1] *Eleocharis acuta*, *Glyceria declinata*,[1] *Juncus articulatus*,[1] *Nasturtium officinale*,[1] *Persicaria decipiens*, *Schoenoplectus tabernaemontani* (emergent species)
Azolla rubra (floating species, not found after treatment)	*Lemna disperma* (floating species)

[1]Denotes species that are not native to New Zealand.

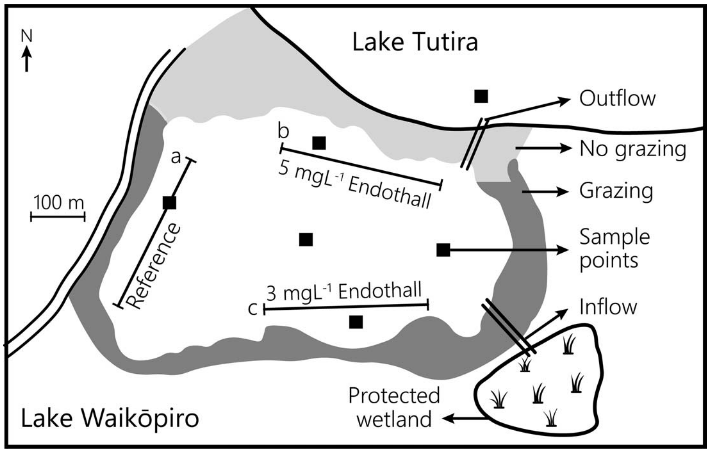

Figure 7. Diagram illustrating the trial design used to evaulate the efficacy of endothall to control hydrilla in Lake Waikōpiro. Treatment and reference plots are as marked, and the black squares show sampling sites for endothall dissipation.

There were two treated areas, one using endothall at the highest rate permissible (5 mg L^{-1}) and a lower rate (3 mg L^{-1}). Each trial area comprised one vegetated hectare (approximately 300 m of shoreline). The treated areas were on opposite sides of this small, trapezoid-shaped lake, with a designated reference (control) plot along a third section of the shoreline. Treatment plots were a minimum of 150 m from each other, 100 m from the reference plot (Figure 7). The herbicide was applied with the use of a trailing hose at the surface of the weed bed during late summer (March 2001) by a registered applicator, in accordance with the experimental-use permit (under the Pesticides Act 1979) and U.S. product label recommendations.

Water temperature, DO, and pH were monitored in the 5–mg L^{-1} treatment plot and the reference plot prior to and for 1 MAT. The purpose of this data collection was to address the concerns of local authorities that weed-bed decay would depress the DO. Despite warm water temperatures (ca 20°C) in late summer there was no evidence of DO depletion attributable to endothall treatment or subsequent plant decay. The DO levels were close to saturation in treatment and reference sites. No significant changes in pH were noted.

Water samples were taken from the treatment and reference plots, the center of the lake, and adjacent areas of neighboring Lake Tutira (there is a culvert between the two lakes) and analyzed for endothall (Figure 7). Sampling was carried out immediately before and following herbicide application, as well as 1, 7, and 28 DAT. By 1 DAT the maximum concentration of endothall outside of treatment plots was 0.282 mg L^{-1}; by 7 DAT endothall was below 0.2 mg L^{-1} at all sampling points and was no longer present by 28 DAT.

Impacts of herbicide application on *H. verticillata* and other submersed vegetation were assessed at 1, 6, and 12 MAT. Impact on flora was assessed by visual survey by scuba divers of species presence in the treatment and reference sites and compared with pretreatment data. A survey of fauna associated with the vegetation was carried out 1 mo after treatment and compared with the pretreatment survey. There was no notable change in the occurrence of any biota that were recorded pre- and posttreatment other than the *H. verticillata*. The main difference between pretreatment and posttreatment results was in the height of the hydrilla beds present. Pretreatment *H. verticillata* had 90 to 100% cover from ca 0.6 to 6.5–m water depth, with a maximum height of ca 5 m. Posttreatment the *H. verticillata* height was reduced to an average of 0.5 m in the treatment plots. There was approximately 70% less *H. verticillata* in treated areas than in the reference plot 1 MAT. However, by 12 MAT the *H. verticillata* abundance was again the same in all plots.

The data collected in this field demonstration were targeted to address specific concerns as well as provide proof of concept for *H. verticillata* weed-bed control by endothall. By addressing the specific questions, this research, along with another field demonstration and existing data packages for the product, enabled the registration of endothall for aquatic use in New Zealand. Subsequently, endothall was used as one of the primary control tools in the national eradication program for *H. verticillata*. Several years later, when endothall was used in one of the same areas of Lake Waikōpiro, a better result was achieved (80% *H. verticillata* reduction by 1 MAT) with an early-summer application. This better result was attributed to the position of the thermocline that minimized depth dilution of the herbicide (Hofstra et al. 2003). The location of the thermocline is known to be a factor that can determine the outcome of herbicide use in aquatic situations (e.g., Haller 2014).

2.2.2. The use of diquat to reduce C. demersum weed beds—dealing with dilution and dispersion. Lake Karāpiro is a 777-ha lake that was formed in 1947 by the damming of a river for hydropower generation. The invasive species *C. demersum* forms extensive weed beds along most of the shallow-water zone to ca 5-m water depth. *In situ* weed beds and weed drift pose a significant threat to amenity and utility functions of this highly valued lake. Cost effective and environmentally safe weed control options are a priority for lake managers to ensure the uses and values of the lake are maintained. Diquat is currently the primary control tool, and a number of weed control trials have been undertaken to optimize its use, particularly with respect to dilution, dispersion (optimizing CET), and plant conditions.

A field demonstration using RWT dye was initiated in 2007. This study aimed at improving the understanding of the effects of water-exchange patterns (flow) on herbicide movement. The primary purpose was to determine which weed beds could be treated with diquat and a predictable level of reduction achieved, compared with those weed beds that could not (i.e., areas with faster flow, or relatively isolated patches of weed). Additional research priorities were the potential for herbicide residues to disperse from treatment zones to a municipal water take and the impacts on DO and nontarget biota (Matheson et al. 2010).

Compared with an aquatic environment the treatment of small target areas (such as isolated plants) on land is relatively simple because once the herbicide is on plant surfaces it remains there (assuming no rain immediately

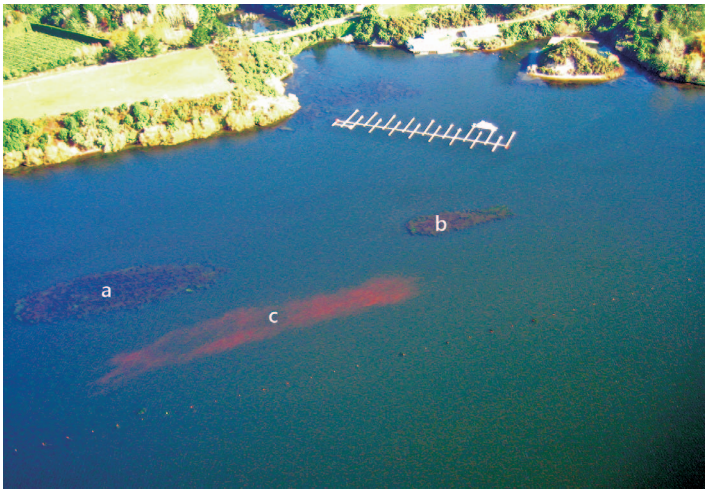

Figure 8. Rhodamine water tracer dye is rapidly moved away from weedbeds on two submerged islands. The image was taken 8 min after application shows that neither of the submerged islands (a, b) are covered by dye (c) (source: Clayton et al. 2006).

following application). However, effective treatment of small target areas or narrow bands of submersed weeds can be challenging because herbicide in water is subject to dilution and dispersion factors and the smaller the area treated—relative to area of surrounding untreated water—the shorter the potential contact time achievable within the area of application. If the target area treated is quite small then the herbicide can quickly disperse from the target area without adequate contact time to achieve an effective result. As the size of the area treated increases, then so too does the length of contact time achieved within the target area (Clayton et al. 2006).

RWT dye was applied by helicopter to some of the largest submersed weed beds, comparatively isolated weed beds (i.e., on submerged islands), as well as potentially sensitive areas (e.g., weed bed near a water intake) prior to treatment with diquat (Clayton et al. 2008). Aerial photographs taken at intervals (ca 1, 15, 30, and 60 min) after dye application were used to assess the rate and direction of water drift, to interpret contact time, and to assess the potential for successful weed control (Matheson et al. 2010). The dispersion of the dye illustrated that in the large areas of weed bed successful control would likely be achieved (given appropriate plant condition) because the dye dispersed little within dense weed beds. In contrast, the isolated weed beds on the submerged islands could not be adequately targeted by aerial dye application, with dye rapidly moving into the main channel and downstream (Figure 8). These underwater islands are surrounded by deep water and lie in the direction of water flow towards the hydropower station. Targeting of small pockets of weed in open water has a high risk of not achieving good control (Matheson et al. 2010). A more quantitative method of determining real-time bulk water-exchange processes following an RWT application can be achieved with the use of calibrated field fluorometers, capable of measuring aqueous dye concentrations as low as 0.1 $\mu g\ L^{-1}$.

Table 3. Water analyses for diquat cation (mg L^{-1}) pre- and post-herbicide application (source: Clayton et al. 2008).

	Sampling Period for Diquat			
Sample Site Description	1 HAT	2 HAT	3–5 HAT	24 HAT
Water intake (10 m deep)	< 0.001	0.002	< 0.001	0.012
Water treatment station		< 0.001		
Adjacent diquat-treated weed bed	< 0.001	0.110[1]	0.030	0.006[2]
Open water	< 0.001	0.003	0.002	0.008

Diquat detection limit was 0.001 mg L^{-1}. HAT = hours after treatment.
[1]Eleven percent of target treatment rate.
[2]Less than 1% of target treatment rate.

Application of RWT dye at a distance of 500 m (the herbicide exclusion zone) away from the municipal water intake was used to test specifically for the potential for subsequent herbicide treatments to disperse towards the water intake. After 10 min there was lateral dispersion of dye, but after 1 h it was apparent that the direction of water movement was away from the water intake and rather moved southwards towards the center of the lake (Clayton et al. 2008). Alongside identifying water (RWT dye) dispersion patterns relative to the water intake, water samples were collected from the intake at 10-m depth and from within the water-treatment station, during the subsequent diquat treatment. The standard precautionary use of activated carbon at the treatment plant effectively removed any trace of diquat from the water intake with levels below detection within 2 h after treatment (Table 3). The 500-m exclusion limit combined with the use of activated carbon at the treatment station guaranteed removal of any residual low levels of diquat if it were to reach the intake (Clayton et al. 2008).

Compliance monitoring confirmed DO exceeded the ecological habitat standard of 80% (the level considered acceptable for healthy aquatic life) at all diquat treated sites in Lake Karāpiro as required by regulations. Eels in New Zealand support a valuable commercial and traditional fishery, and as such, eel populations were sampled by netting to monitor any changes in response to the use of herbicide. No significant changes in eel condition or numbers were attributable to diquat application, although eel numbers did change in response to seasonal changes in water temperature in treatment and reference plots (Table 4). Control of nuisance weed beds was not associated with

Table 4. Eel catch per unit effort (CPUE; number of eels/number of nets, ± standard error) expressed per treatment and species (source: Clayton et al. 2008).

	Eel species	
Treatment	Shortfin (*Anguilla australis*)	Longfin (*Anguilla dieffenbachia*)
Treatment plot		
Before diquat application	2.91 ± 1.44	0.25 ± 0.17
After diquat application	1.16 ± 0.54	0.16 ± 0.16
Untreated reference plot		
Before	3.25 ± 1.04	0.25 ± 0.13
After	0.08 ± 0.08	0.08 ± 0.08

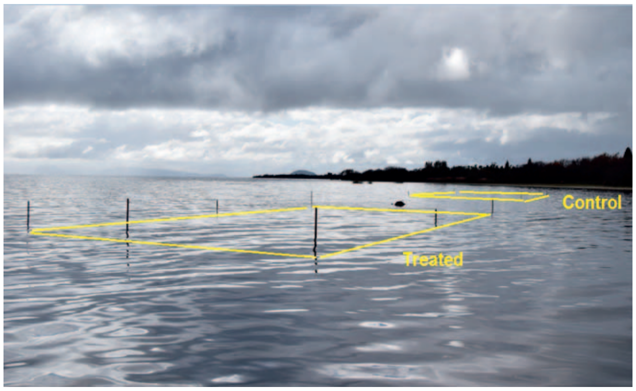

Figure 9. Herbicide demonstration and control plots in Lake Taupō (photo by R. Wells).

impacts on eels, other fish, or water-quality parameters (Matheson et al. 2010).

2.2.3. Determining level of risk to a nontarget plant (I. lenticularis). This field demonstration in Lake Taupō was undertaken as part of a consent condition, with the information required to validate the approach taken to use diquat against the target weed (*L. major*) in the presence of a threatened native plant species (*I. lenticularis*) (Champion 2016).

Two plots (20 by 10 m in ca 0.9 m of water) were established marked by warratah stakes (Figure 9). Submersed vegetation was assessed in both areas (plots) for species present and average cover values (Table 5) by using the quick survey method of Clayton (1983). *Lagarosiphon major* was the dominant plant in both areas, growing to 0.5 m tall, with most of the other plants growing beneath a canopy of this plant. Exceptions were *I. lenticularis*, *Juncus bulbosus*, and the *Myriophyllum* species which were of similar height, and the taller *Eleocharis* species. Immediately prior to the application of diquat (1 mg L^{-1}), the treatment plot was enclosed in shade cloth netting (95%), which was removed 1 h after treatment (HAT).

A repeat assessment of the vegetation in the plots (i.e., damage to target and nontarget species) was made 33 DAT. All of the species were still present in both areas with similar covers, although additional native species *Nitella hyalina*, *Chara fibrosa*, and *Glossostigma diandrum* were noted in the treated areas, all at very low covers (< 1%).

Lagarosiphon major was affected by herbicide application in the treated area with discoloration (brown rather than green in color), loss of growing tips, shorter stature, and greater epiphytic algae cover compared to the untreated (control) area (Figure 10). These symptoms were consistent with the expected progressive decline of *L. major* by 33 DAT. However, no other plant species showed symptoms of damage from the diquat application, with healthy *I. lenticularis* plants present posttreatment (Figure 11). The additional species seen in the treated plot are likely to have been present during the inspection prior to treatment but were obscured by the taller, denser growth of *L. major* (Champion 2016).

The approach utilized in this field demonstration was very specific to the site, a localized area in a much larger lake, and was to meet the conditions for the use of diquat (consent conditions), enabling assessment of off-target impacts on a threatened species. The utility of the shade cloth barrier to restrict water movement temporarily and maintain the desired 1-h contact time was successfully demonstrated as evidenced by the decline of the *L. major* in the treated area. Based on this demonstration, an area no less than 2 ha was planned for annual treatment to enhance *I. lenticularis* habitat.

Table 5. Cover values (%) for plant species in the reference and treated plots before herbicide application (source: Champion 2016).

Species	Species Average Cover (%)	
	Reference (Control) Plot	Treated Plot
Callitriche petriei subsp. *petriei*	> 1	> 1
Chara fibrosa	> 1	–
Crassula sinclairii	> 1	–
Eleocharis acuta	> 1	> 1
Eleocharis sphacelata	> 1	> 1
Glossostigma elatinoides	> 1	> 1
Isolepis lenticularis	15	15
Juncus bulbosus	> 1	> 1
Lagarosiphon major (target weed)	90	90
Lilaeopsis ruthiana	1	1
Lobelia perpusilla	1	3
Myriophyllum propinquum	5	5
Myriophyllum triphyllum	5	5
Nitella pseudoflabellata	> 1	> 1
Potamogeton crispus (nonnative)	–	> 1
Ranunculus amphitrichus	–	> 1
Ranunculus limosella	> 1	> 1

2.3. Product comparison

A field trial was undertaken to evaluate three herbicides for the control of *Myriophyllum aquaticum* (Hofstra et al. 2006). The wetland site had a near monoculture of dense *M. aquaticum* (95 to 100% cover) in shallow water. Water depth was usually 0.3 m, but fluctuated with stream flow and local rainfall events. Eighteen treatment plots of 5 by 5 m (triplicates for each treatment, in a randomized block design) and three untreated reference plots were marked out in a region of the wetland. Each herbicide was applied at two rates, referred to as high or low as follows: 8.8 and 14.8 kg a.i. ha^{-1} endothall, 2.0 and 4.0 kg a.i. ha^{-1} triclopyr, and 6.8 and 20.3 kg a.i. ha^{-1} dichlobenil (Hofstra et al. 2006). The plots were separated by untreated regions to provide a buffer (20 m) between herbicide treatments. Water sampling was carried out in treatment plots, reference plots, and downstream of the treatment site to monitor for herbicide dispersion. There was some off-site herbicide movement, evidenced by the detection of herbicide outside of the target areas; however, none of these residues were excessive (i.e., they were only a small fraction of that applied), nor were residues present in the downstream reference area. For example, 7 DAT low levels of endothall (equating to less than 0.2% of the initial application rate), were found in the midregion (lagoon) of the wetland.

During the trial, a change in weather patterns meant the plots became drier (dewatered), enabling a second treatment (51 d after initial treatment) under these new

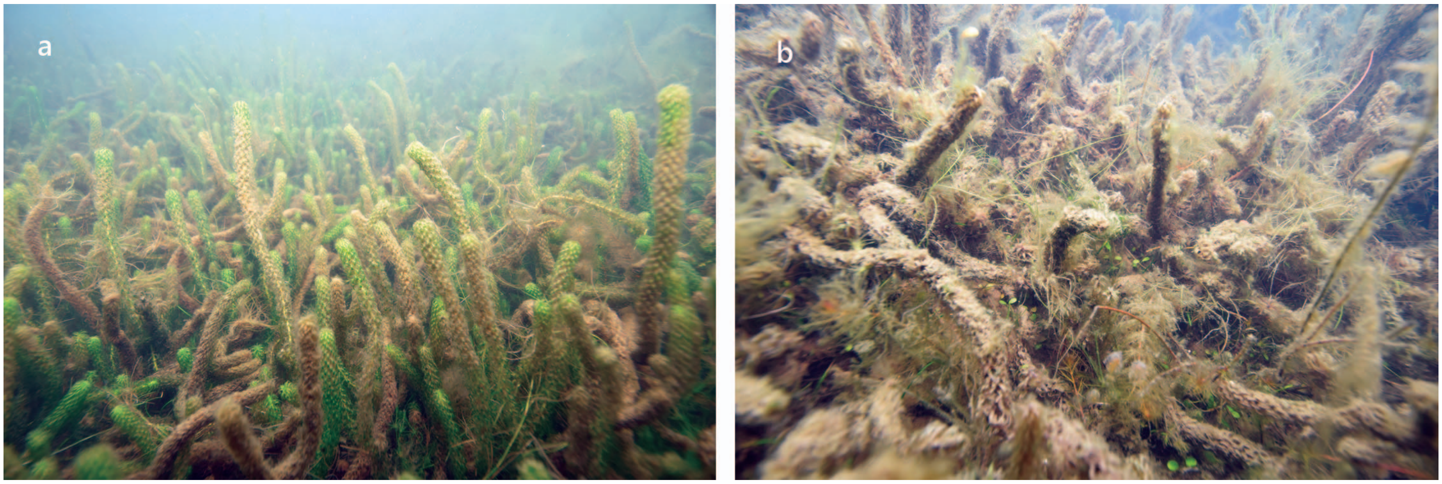

Figure 10. *Lagarosiphon major* dominated the vegetation in the control (a) and in the treated plot (b) (photo by R. Wells).

conditions where a longer exposure period was guaranteed. The trial plots were assessed visually (species present and percent vegetation cover) prior to the application of herbicides, and at 1, 4, 7, 11, 30, and 54 wk after treatment (WAT) from the initial application (Hofstra et al. 2006).

There was an initial reduction of *M. aquaticum* in all treatment plots; however, by 4 WAT recovery (new shoot development) was substantial and coincided with the drop in water level in the wetland. Following the respray, successful reduction of *M. aquaticum* was maintained for a longer period of time than the initial spray, with percent cover in treatment plots increasing to between 60 and 90% cover by 30 WAT (150 d after respray) (Figure 12), largely as a result of encroaching plants from outside of the spray zone rather than recovery from within the treatment plots. The better spray result was achieved when the plants were no longer submersed but rather were in a terrestrial setting (dewatered), likely facilitating direct herbicide contact with basal plant parts during the respray.

This field trial also demonstrates how depressed the DO can be in some aquatic situations, regardless of herbicide application. Data loggers were utilized during the trial to record water-quality parameters every 15 min. The data presented in Figure 13 from the lagoon (untreated reference) show that little DO was present compared with the adjacent stream outflow. These results directly relate to the more recent regulations in New Zealand surrounding the use of herbicides for control of emergent weeds in aquatic situations that are already recognized as having low DO prior to the treatment (see Section 1.3.2).

The native fish inanga (*Galaxias maculatus*) and eels (*Anguilla australis*) were regarded as the most important components of the fish fauna in this wetland; as such there was a requirement to address the potential for herbicide residues to have a negative impact on these species. Inanga and eels were caged (60 of each species in separate 1.5 by 0.5–m–wide, 1.5 by 0.5–m–deep cages) and placed at two downstream sites from the trial and in the main outflow

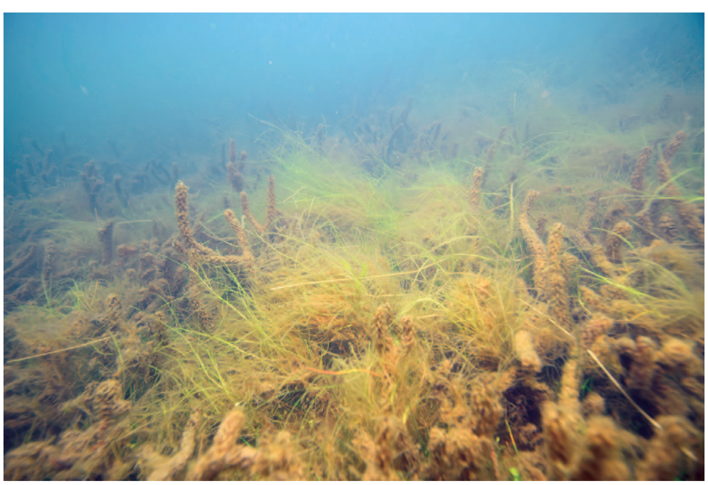

Figure 11. Healthy *Isolepis lenticularis* plants growing amongst damaged *Lagarosiphon major* dominated vegetation in the treated plot 33 days after treatment (photo by R. Wells).

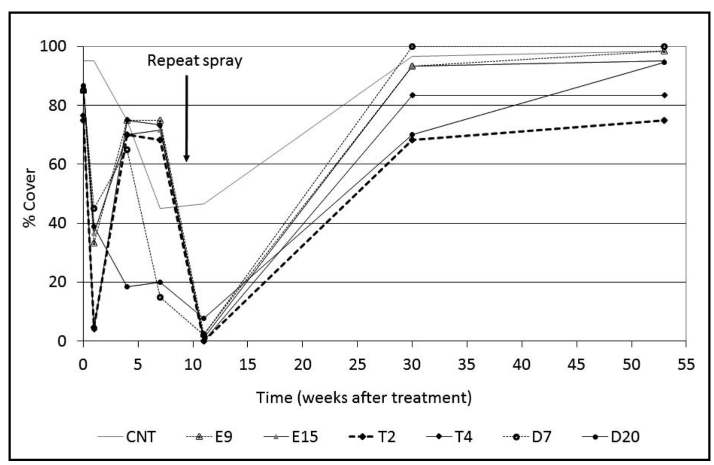

Figure 12. Percent cover of *Myriophyllum aquaticum* in wetland plots treated with herbicide. Legend abbreviations are as follows: CNT, E, T and D represent control, endothall, triclopyr, and dichlobenil, respectively. Numbers after each treatment represent the rate of application in kg ai/ha. Timing of the respray is indicated.

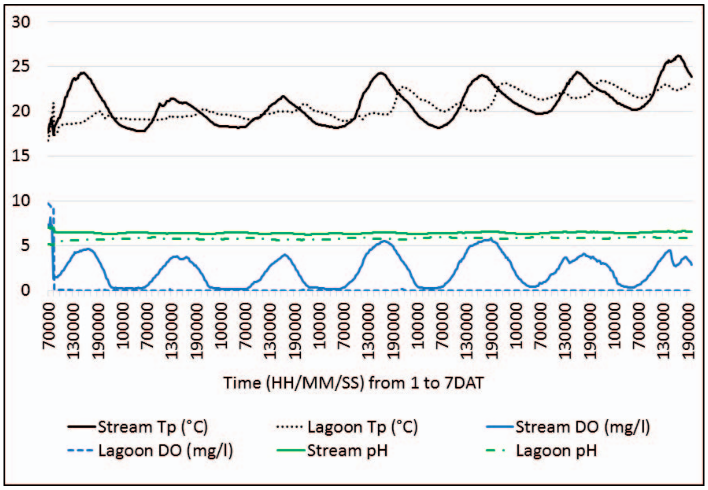

Figure 13. Posttreatment temperature (Tp), dissolved oxygen (DO), and pH in the lagoon (untreated area) and the outflow stream.

from the treatment plots to assess any potential acute toxic impacts of the herbicides (Figure 14). No impacts were observed, and subsequent data indicate no residues were found in the outflow. Rather, the fish were at greater risk from naturally low DO and reducing water levels.

CONCLUSIONS

Improved product placement and use patterns that achieve desired levels of control of target weed species, whilst minimizing the potential for off-target effects, will continue to be sought to reduce the impacts of invasive aquatic plants on the environmental, amenity, and utility values of freshwaters. The specific challenges associated with an aquatic environment need careful consideration, and may require bespoke solutions when designing and implementing an appropriate field demonstration to ensure that it addresses the research needs or goal.

Figure 14. Floating cages in the outflow stream used to assess any potential acute toxic impacts of the herbicides.

Field demonstrations are an essential step in providing real-world proof of concept for new products and extending the use patterns of existing products.

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