Charophyte germination responses to herbicide application

CHRYSTAL L. KELLY, DEBORAH E. HOFSTRA, MARY D. DE WINTON AND DAVID P. HAMILTON[*]

INTRODUCTION

Charophyte species form one of New Zealand's prominent native submerged freshwater plant communities and are widely recognized as a beneficial component of lake ecosystems (Coffey and Clayton 1988). They are known as rapid colonizers and are usually the first aquatic plant species to re-establish after a disturbance event (Van den Berg et al. 1998, de Winton et al. 2000, van Donk and van de Bund 2002). New Zealand has four charophyte genera; *Chara, Lamprothamnium, Tolypella,* and *Nitella* (Wood and Mason 1977, de Winton et al. 2007), with *Chara* and *Nitella* species being the most common charophyte genera found in freshwater (Coffey and Clayton 1998).

The spread of tall-growing monospecific stands of aquatic invasive weed species has detrimental effects on native aquatic plants and biota. These effects include replacing native plants (de Winton and Clayton 1996, Champion et al. 2002) or displacing them to either deeper water where light is limited or to shallow areas where they are more exposed to wave action and desiccation (Closs et al. 2004). Displacement of native plants has detrimental impacts on native seed banks, thus limiting the emergence, growth, and reproduction of charophytes (de Winton and Clayton 1996, Bonis and Grillas 2002).

In New Zealand, the impact of invasive plants and the management objectives to remove or decrease weed beds of alien species have led to the development of aquatic control technologies, in particular the use of aquatic herbicides (Leonard and Creenland 1965, Clayton 1986, Wells et al. 1986, Clayton and Tanner 1988, Tanner et al. 1990, Wells and Clayton 1993, Hofstra and Clayton 2001b, Hofstra et al. 2001, Champion et al. 2002, Closs et al. 2004, Hofstra and Champion 2008, Clayton and Matheson 2010). For large-scale weed control in New Zealand, aquatic herbicides are the most widely used method (Champion et al. 2002), and diquat and endothall are the only aquatic herbicides registered for use on submerged invasive plants in standing and flowing water in New Zealand (Hofstra and Champion 2006). Diquat[1] is formulated as dibromide salts (6,7-dihydrodipyrido[1,2-a:2',1'-c] pyrazinediium ion) and is a fast-acting contact herbicide that disrupts the electron transport system in plant photosynthesis and damages cell membranes and cytoplasm, leading to desiccation or defoliation (Simsiman et al. 1976, Clayton 1986,

Hofstra et al. 2001, Netherland 2009). Endothall[2] is formulated as a dipotassium salt (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid) and is known to inhibit protein synthesis and cause disruption of membranes and respiration, resulting in wilting, desiccation, and collapse of the treated plants (Simsiman et al. 1976, Hofstra and Clayton 2001a, Hofstra et al. 2001, MacDonald et al. 2002, Hofstra and Champion 2008, Netherland 2009).

Fluridone[3] is widely used throughout the United States for invasive weed management (Siemering et al. 2008) and, although not registered in New Zealand, it has been evaluated in herbicide efficacy trials (Wells et al. 1986, Hofstra and Clayton 2001b). Fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone), also known as a "bleaching herbicide," is a systemic herbicide that inhibits the formation of carotenoid pigments essential for normal plant growth. The impact is observed in new shoot growth, which is often white, as the chlorophyll is destroyed by sunlight (Wells et al. 1986, Doong et al. 1993, Netherland 2009).

Chelated copper[4] is a compound derived from a copper triethanolamine complex and copper hydroxide. Copper is a plant cell toxicant used as an algaecide for control of aquatic vascular plants as well as algae. Copper is a natural element and does not biodegrade in the water column but becomes biologically inactive by sorption to sediments and subsequent sedimentation. Copper in a chelated compound does not readily precipitate in the water column, allowing it to remain active for longer (Leslie 1990, Guha 1995, Durborow et al. 2007, Morris 2009, Netherland 2009).

Numerous field observations have demonstrated that the aquatic herbicides used in the present study are successful on target weeds such as *Lagarosiphon major* and that there is little to no impact (i.e., injury symptoms or biomass reduction) on established charophyte meadows (Leonard and Creenland 1965, Starling et al. 1974, Clayton 1986, Wells et al. 1986, Clayton and Tanner 1988, Hofstra and Clayton 2001a). However, their impact on critical early life stages of charophyte species has received relatively little attention. When oospores germinate, the initial primary protonema that emerges from the oospore is colorless and only becomes pigmented after cell division to form an intermodal cell with a nodal cell at both ends (Smith 1950, Bold and Wynne 1978). Early germling survival is dependent on the starch reserves accumulated in the oospores (de Winton et al. 2004) until the germling becomes pigmented; the germling then moves to a reliance on photosynthetic energy sources. These features of germlings and young charophyte plants may make them more susceptible to herbicide effects, especially diquat, endothall, and fluridone, which have modes of action that disrupt and inhibit photosynthesis (Netherland 2009).

*First and fourth authors: Graduate Student and Professor, Department of Biological Sciences, University of Waikato, Private Bag 3105, Hamilton 3240, New Zealand; Second and third authors: Research Scientists, National Institute of Water and Atmospheric Research, PO Box 11115, Hillcrest, Hamilton 3216, New Zealand. Corresponding author's E-mail: chrystallkelly@gmail.com. Received for publication March 11, 2012 and in revised form July 6, 2012.

In this study, we hypothesised that charophyte germlings are a potentially vulnerable life cycle stage in herbicide treatments used for invasive weed control and could therefore represent a "bottleneck" in the charophyte life cycle under such treatments. Our main objective was to examine effects of selected herbicides (diquat, endothall, fluridone, and chelated copper) on charophyte germling growth.

MATERIALS AND METHODS

Seed bank material. Approximately 20 L of lake sediment containing oospores, hereafter referred to as seed bank material, was collected from Lake Tarawera (38°10'S, 176°23'E, depth 5 to 7 m), Lake Tikitapu (Blue Lake; 38°11'S, 176°19'E, depth 5 to 7 m), and Lake Rotoroa (Hamilton Lake; 37°47'S, 175°16'E, depth 1.5 m) to give a mixed age and composition of different *Nitella* and *Chara* species.

Each of the lake sediments was separately passed through a large garden sieve (12 mm mesh) into containers to remove any plant matter and large debris from the sediment. The sieved sediment from each lake was thoroughly homogenized (stirred for 5 minutes) before subsamples were taken to estimate the density and composition of oospores. Four subsamples of 10 mL (Lake Tarawera and Lake Tikitapu) or 100 mL (Lake Rotoroa) of sediment were passed sequentially through a sieve sequence of 500 μm and 250 μm mesh size. The contents of the sieves were placed into separate glass petri dishes and examined under a stereo microscope (Leica MZ 9.5, Leica Microsystems, Bio-Strategy Ltd). The oospores present were identified according to de Winton et al. (2007), counted, and recorded.

Once the oospore composition and concentration were determined for each lake, the three lake sediments were mixed together according to a predetermined mixing ratio to optimize the potential germination response. The mixing ratio was calculated based on 5% oospore germination as the lower range of published rates from natural sediment (de Winton et al. 2004). The goal was to have a mixing ratio that potentially yielded 100 germinating oospores per 120 mL of the mixed lake sediment. Once the lake sediments were mixed, they were stored at 2 C for approximately 5 months.

Experimental design. Mixed seed bank material (120 mL) was measured and placed into 130 mL plastic (HDPE) pots (Labserv®); a sufficient number of pots was prepared to ensure germling replicates for testing. The pots were placed into two water-filled storage containers (115 L, 79 by 55.5 by 39 cm). After 10 d, additional pots were filled with 120 mL of mixed seed bank material each, and three *L. major* apical shoots (15 cm) were planted into the sediment of each pot. The 10 d time delay was to minimize *L. major* shoot growth to ensure plants did not exceed the capacity of the jars used in the subsequent experiment, while allowing the pots of germlings time to germinate. The pots with *L. major* were placed into a third water-filled storage container (60 L, 63 by 44 by 34 cm). The three water containers were placed in a constant temperature room (20 C, 14 h:10 h light:dark cycle), with an average water temperature of 19.56 C (Onset HOBO® pendant temperature logger), 5.02 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR; Onset HOBO® pendant light logger), and under constant aeration. The pots remained in the water-filled containers until widespread germination activity was noted (approximately 42 d). Sixty-five pots containing germlings and 25 pots of *L. major* were individually placed into 2 L glass jars (22.8 cm, 11.6 cm diameter, Arthur Holmes Ltd).

The jars with charophyte germlings received one of three treatment concentrations per herbicide; diquat (0.1, 1.05, or 2 ppm), endothall (0.1, 2.55, or 5 ppm), fluridone (0.005, 0.0775, or 0.15 ppm), and chelated copper (0.5, 0.75, or 1 ppm), as well as an untreated control. The jars with the *L. major* shoots were treated with one concentration (maximum label rate) per herbicide; diquat (2 ppm), endothall (5 ppm), fluridone (0.15 ppm), and chelated copper (1 ppm). Five replicates were utilized for each treatment and control. All treatments and the untreated controls were randomly assigned to the 2 L jars. The jars were set up under controlled photoperiod (14 h:10 h light:dark cycle), receiving an average of 36.6 μmol m^{-2} s^{-1} PAR (Onset HOBO® pendant light logger) at 20.34 C temperature (Onset HOBO® pendant temperature logger). After 24 h, aeration was started and maintained for the duration of the experiment.

Harvesting. Plants were harvested 14 d after treatment. Pots were removed from each jar and above-ground germling or *L. major* shoot height was measured. The germlings were removed and placed onto preweighed (±0.0001 g) foil sheets while *L. major* shoots were placed into separate brown paper bags. The foil sheets and paper bags were placed into an 80 C drying oven (Contherm Thermotec 2000 series oven, Bio-Strategy Ltd) and dried to constant weight. The foil sheets were weighed (±0.0001 g), initial foil weight deducted, and the charophyte biomass determined and recorded. The *L. major* shoots were weighed (±0.0001 g; without bag) and recorded.

An ANOVA (GenStat 12th Edition) and post-hoc test (Tukey HSD) was used to distinguish any significant differences ($p \leq 0.05$) between the control and each of the treatment doses for each herbicide and algaecide.

RESULTS AND DISCUSSION

Lagarosiphon major. Over the 14 d experimental period, *L. major* shoots in the untreated control jars remained healthy and intact. Four days after treatment, the *L. major* shoots treated with diquat and endothall turned necrotic, and by day 12 there was total collapse of the shoots tissue (no possible recovery), although there were visible green charophytes growing in the *L. major* pots. *L. major* shoots treated with fluridone remained green with pink tips developing after 12 d of exposure. Four days after treatment, the *L. major* shoots treated with chelated copper showed darkening leaf color while the stem remained light green color. Over time, the darkened leaves became flaccid (day 12) and lost buoyancy (day 14) while the stems remained healthy and new lateral shoots began to grow.

There was no difference in *L. major* shoot height between the control, fluridone, and chelated copper treatments (Figure 1). In addition, there were no recoverable shoots in the diquat and endothall jars 14 d after treatment (Figure 1). Diquat and endothall achieved complete *L. major* control, and no biomass was recovered from these treatments. In compari-
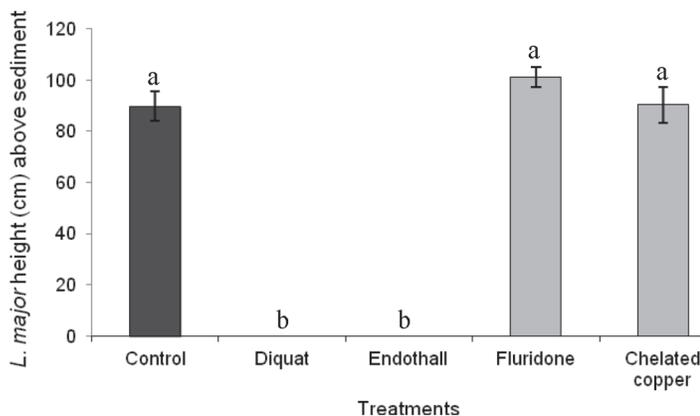
Figure 1. *L. major* treatment height (cm) above sediment. Treatment means ±1 SE, n = 5. Bars sharing the same letters do not differ according to Tukey HSD test at p ≤ 0.05 level of significance.

son with the control (Tukey HSD test), *L. major* had a significantly lower biomass in the diquat, endothall, and chelated copper treatments while biomass of fluridone-treated *L. major* was similar (Figure 2).

All four treatments (diquat, endothall, fluridone, and chelated copper) severely affected *L. major* as expected. Diquat and endothall in particular had high efficacy on the *L. major* shoots causing total collapse of the shoots as observed in previous studies (Clayton 1986, Wells and Clayton 1993, Hofstra and Clayton 2001a). Fluridone impacted only *L. major* shoots as visual injury symptoms of chlorosis and purple coloration of new shoots were noted, while older plant tissue remained generally healthy as noted previously in research by Wells et al. (1986) and Hofstra and Clayton (2001b). The efficacy of fluridone on *L. major* biomass may have been limited by the duration of the experiment (approximately 2 weeks) because fluridone is a slow-acting herbicide that requires long exposures (45+ d) to be efficacious (Netherland 2009).

Charophyte germlings. Calculations of germination potential (Table 1) suggested oospore concentrations present in the experimental sediment were sufficient to enable germination and establishment of charophytes.
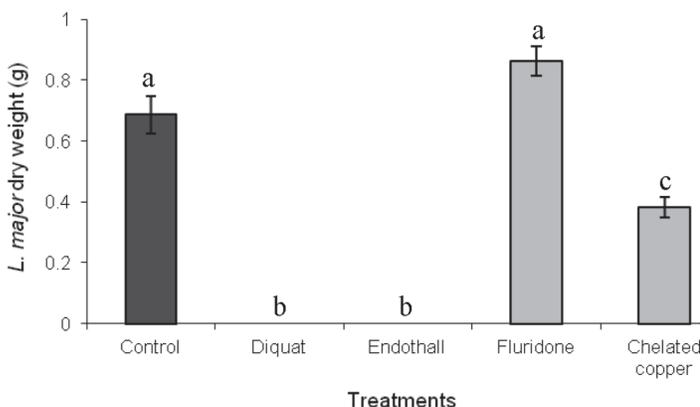

Figure 2. Average *L. major* biomass (g) 14 days after treatment. Treatment means ±1 SE, n = 5. Bars sharing the same letters do not differ according to Tukey HSD test at p ≤ 0.05 level of significance.

With the exception of the germlings treated with chelated copper, germlings were present in the treatment pots throughout the 14 d experimental period, with noticeable oospore germination and new germlings emerging. The germlings treated with chelated copper were mostly brown and opaque with only a few new germlings beginning to emerge through the sediment by the conclusion of the experiment. These germlings in the chelated copper treatment (the positive control) were affected as expected, confirming charophyte susceptibility to algaecide effects under the experimental conditions used in this study.

Despite visual evidence of algaecidal effects in the chelated copper treatment, there was no overall difference in germling height between untreated germlings of the control and germlings in any of the herbicide or algaecide treatments by the end of the experiment (Figure 3). The charophyte biomass was lowest in the chelated copper treatments (Figure 4); however, there were no significant differences (p > 0.05, Tukey HSD test) in charophyte biomass between the untreated germlings and the treated germlings (Figure 4).

Although significant differences were apparent between some herbicide treatments (e.g., compared with the chelated copper treatment), visual injury symptoms associated with diquat, endothall, and fluridone were not detectable relative to the control treatment. Differences in charophyte biomass occur naturally as part of the germling response and growth. For example, chance timing of germination may drive the differences in the composition of germlings, such as the plants with earliest germination gaining a competitive advantage to resources such as light (Casanova and Brock 1990, de Winton et al. 2000). Moreover, the observed regeneration of new plants through germination of oospores still in the seed bank may have compensated for any herbicide impacts on the treated germlings.

Our results indicate that charophyte germlings were not susceptible to herbicide treatment, and that oospores were able to germinate continuously from sediments where they are present. This result has two important implications for field application of the herbicidal products for invasive weed management in lakes and waterways. First, if successive herbicide applications are carried out, germling establishment is still likely to be supported by continual germination from the sediment providing a large enough oospore population is present. Therefore the timing of herbicide applications for invasive aquatic plant management in lakes and waterways is not crucial for charophyte regeneration. Second, given the same level of target weed control (i.e., the level of target weed control is not compromised by product choice), the use of one product over another (with exception of copper algaecide) does not confer any advantage or benefit to native charophyte regeneration.

Further research in charophyte germling responses to herbicide application would involve repeating this experiment over a longer experimental timeframe.

ACKNOWLEDGMENTS

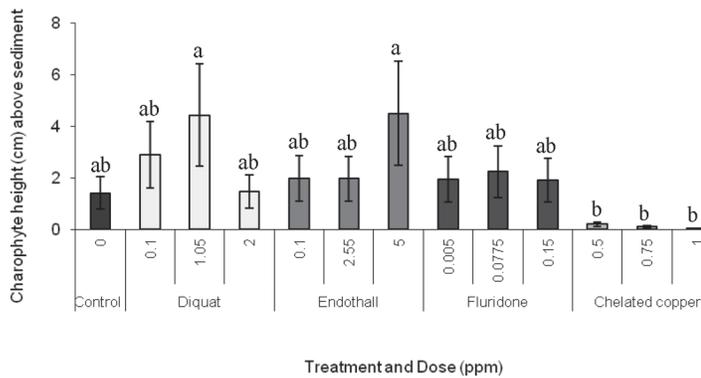| Lake	Oospores (120 mL sediment)	Potential germlings (120 mL sediment*)	Mixing Ratio	Potential germlings (120 mL sediment*)
Lake Tarawera	2922	146	13/34	56
Lake Tikitapu	2559	128	11/34	41
Lake Rotoroa	46	2	10/34	1
Total				98

*based on 5% germination

Figure 3. Charophyte height (cm) above sediment. Treatment dose means ±1 SE, n = 5. Bars sharing the same letters do not differ according to Tukey HSD test at p ≤ 0.05 level of significance.

SOURCES OF MATERIALS

[1]Reglone®, Syngenta Crop Protection Ltd., Auckland, New Zealand
[2]Aquathol® K, United Phosphorous Inc, King of Prussia, PA
[3]Sonar® AS, SePRO, Carmel, IN
[4]K-Tea®, SePRO, Carmel, IN

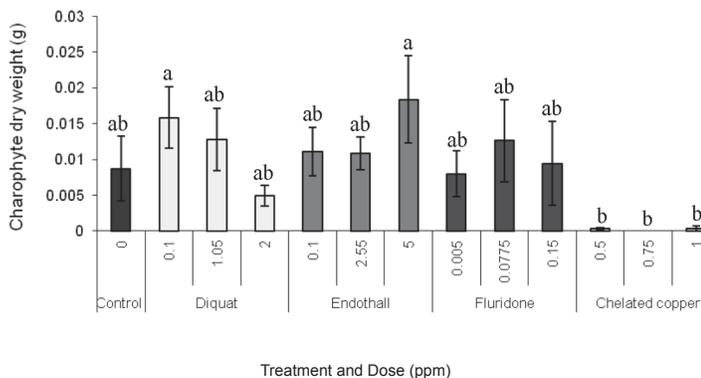Figure 4. Charophyte biomass 14 days after treatment. Treatment dose means ±1 SE, n = 5. Bars sharing the same letters do not differ according to Tukey HSD test at p ≤ 0.05 level of significance.

LITERATURE CITED

Bold HC, Wynne MJ. 1978. Introduction to the algae: structure and reproduction. Prentice-Hall Inc, Englewood Cliffs, New Jersey. 706 pp.

Bonis A, Grillas P. 2002. Deposition, germination and spatio-temporal patterns of charophyte propagule banks: a review. Aquat. Bot. 72:235-248.

Casanova MT, Brock MA. 1990. Charophyte germination and establishment from the seed bank of an Australian temporary lake. Aquat. Bot. 36:247-254.

Champion PD, Clayton JS, Rowe J. 2002. Lake managers handbook: alien invaders. Ministry for the Environment Publication, Wellington, NZ. ME number 4444. 51 pp.

Clayton JS. 1986. Review of diquat use in New Zealand for submerged weed control. Proceedings of the EWRS/AAB 7th symposium on Aquatic Weeds: 73-79.

Clayton JS, Matheson F. 2010. Optimising diquat use for submerged aquatic weed management. Hydrobiologia. 656:159-165

Clayton JS, Tanner CC. 1988. Selective control of submerged aquatic plants to enhance recreational uses of water bodies. Verh. Internat. Verein. Limnol. 23:1518-1521.

Closs G, Dean T, Champion PD, Hofstra DE. 2004. Chapter 27: Aquatic invaders and pest species in lakes, pp. 1-14. In: J. Harding, P. Mosley, C. Pearson, B. Sorrell (eds.). Freshwaters of New Zealand. The Caxton Press, New Zealand.

Coffey BT, Clayton JS. 1988. New Zealand waterplants. A guide to plants found in New Zealand freshwaters. Ruakura Agricultural Centre, New Zealand. 65 pp.

de Winton MD, Casanova MT, Clayton JS. 2004. Charophyte germination and establishment under low irradiance. Aquat. Bot. 79:175-187.

de Winton MD, Clayton JS. 1996. The impact of invasive submerged weed species on seed banks in lake sediments. Aquat. Bot. 53:31-45.

de Winton MD, Clayton JS, Champion PD. 2000. Seedling emergence from seed banks of 15 New Zealand lakes with contrasting vegetation histories. Aquat. Bot. 66:181-194.

de Winton MD, Dugdale TM, Clayton JS. 2007. An identification key for oospores of the extant charophytes of New Zealand. New Zeal. J. Bot. 45:463-476.

Doong RL, MacDonald GE, Shilling DG. 1993. Effect of fluridone on chlorophyll, carotenoid and anthocyanin content of *Hydrilla*. J. Aquat. Plant Manage. 31:55-59.

Durborow RM, Tucker CS, Gomelsky BI, Onders RJ, Mims SD. 2007. Aquatic weed control in ponds. Kentucky State University Aquaculture Program, a KSU Land Grant Program. 24 pp.

Guha P. 1995. Exploring ecological control of Chara. Crop Prot. 14(6):527-528.

Hofstra DE, Champion PD. 2006. Management options assessment report for *Hydrilla verticillata*. NIWA client report HAM2006-159 Prepared for Biosecurity New Zealand (MAF07204). 20 pp.

Hofstra DE, Champion PD. 2008. Assessment of environmental effects for the use of Endothall in four hydrilla affected lakes in Hawke's Bay. NIWA client report HAM2008-086 Prepared for Biosecurity New Zealand (MAF08208). 50 pp.

Hofstra DE, Clayton JS. 2001a. Evaluation of selected herbicides for the control of exotic submerged weeds in New Zealand: I. The use of endothall, triclopyr and dichlobenil. J. Aquat. Plant Manage. 39:20-24.

Hofstra DE, Clayton JS. 2001b. Control of dioecious New Zealand *Hydrilla* using fluridone in mesocosms. J. Aquat. Plant Manage. 39:125-128.

Hofstra DE, Clayton JS, Getsinger KD. 2001. Evaluation of selected herbicides for the control of exotic submerged weeds in New Zealand: II. The effects of turbidity on diquat and endothall efficacy. J. Aquat. Plant Manage. 39:25-27.

Leonard WF, Creenland AR. 1965. Control of aquatic weeds. Proceedings of

the NZ Weed and Pest Control Conference. 18:95-104.

Leslie AJ. 1990. Aquatic use of copper-based herbicides in Florida. Bureau of Aquatic Plant Management, Florida Department of Natural Resources, Tallahassee, Florida, USA. 14 pp.

MacDonald GE, Querns R, Shilling DG, McDonald SK, Bewick TA. 2002. Activity of endothall on *Hydrilla*. J. Aquat. Plant Manage. 40: 68-71.

Morris JE. 2009. Managing Iowa fisheries - Aquatic plant management. Iowa State University Extension Publication PM-1352J, Iowa State University, Ames, IA. 10 pp.

Netherland MD. 2009. Chapter 11: Chemical control of aquatic weeds, pp. 65-78. In: L. A. Gettys, W. T. Haller, M. Bellaud (*eds.*). Biological control of aquatic plants: a best management practices handbook. Aquatic Ecosystem Restoration Foundation, Marietta GA.

Siemering GS, Hayworth JD, Greenfield BK. 2008. Assessment of potential aquatic herbicide impacts to California aquatic ecosystems. Arch. Environ. Contam. Toxicol. 55:415-431.

Simsiman GV, Daniel TC, Chesters G. 1976. Diquat and endothall: their fates in the environment. Residue Rev. 62:131-74.

Smith GM. 1950. The fresh-water algae of the United States (2nd ed.): McGraw-Hill Book Company, New York. 719 pp.

Starling MB, Chapman VJ, Brown JMA. 1974. A contribution to the biology of *Nitella hookeri* A. Br. in the Rotorua Lakes, New Zealand. II. Organic nutrients and physical factors. Hydrobiologia. 45:157-168.

Tanner CC, Clayton JS, Coffey BT. 1990. Submerged-vegetation changes in Lake Rotoroa (Hamilton, New Zealand) related to herbicide treatment and invasion by *Egeria densa*. New Zeal. J. Mar. Fresh. 24:45-57.

Van den Berg MS, Coops H, Meijer M-L, Scheffer M, Simons J. 1998. Clear water associated with a dense *Chara* vegetation in the shallow and turbid Lake Veluwemeer, The Netherlands, pp 339-352. In: E. Jeppesen, M. Sondergaard, M. Sondergaard, K. Khristoffersen (*eds.*). The structuring role of submerged macrophytes in Lakes. Springer-Verlag, New York.

van Donk E, van de Bund WJ. 2002. Impact of submerged macrophytes including charophytes on phyto- and zooplankton communities: allelopathy versus other mechanisms. Aquat. Bot. 72:261-274.

Wells RDS, Clayton JS. 1993. Evaluation of endothall for aquatic weed control in New Zealand. Proceedings of the NZ Plant Protection Conference. 46:102-106.

Wells RDS, Coffey BT, Lauren DR. 1986. Evaluation of fluridone for weed control in New Zealand. J. Aquat. Plant Manage. 24:39-42.

Wood, RD, Mason, R. 1977. Characeae of New Zealand. New Zeal. J. Bot. 15:87-180.