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Efficacy of diquat treatments on Brazilian waterweed, effects on native macrophytes and water quality: A case study

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ABSTRACT

Brazilian waterweed (*Egeria densa* Planch) is an invasive aquatic plant that has spread to 27 countries and 39 states in the United States. Movement has been facilitated by its popularity as an aquarium plant. Once established, the plant can adversely alter ecosystems. Brazilian waterweed spreads solely by fragmentation, which suggests that it may be easier to control than plants with propagules such as seeds, tubers, and turions. Herbicides have the potential to offer targeted control; however, their use on Brazilian waterweed has yielded mixed results, and collateral damage to desirable native species is a concern. Fence Rock Lake is a 7-ha manmade impoundment in Guilford, CT, USA. Brazilian waterweed was first documented in small patches in 2009. By 2014, the plant covered most of the littoral zone, and diquat (6,7-dihydrodipyrido[1,2-a:2′,1′-c]pyrazine-5,8-diium dibromide) was applied by bottom injection at a rate of 1.8 kg active ingredient ha^{-1}. Control of Brazilian waterweed and effects on native species were assessed by the point intercept method. One year after treatment, in 2015, Brazilian waterweed was absent from all points except one. Another diquat treatment was performed in an effort to eradicate the plant from the lake. In 2016 and 2017, no Brazilian waterweed was found. The native plant community was resilient with an increase in species richness from 11 pretreatment to 18 two years posttreatment. Combined native species showed little change in frequency of occurrence. Frequency of occurrence of individual native species exhibited losses, gains, or little change depending on species. Bottom injected diquat concentrations remained low near the bottom and highest near the surface. No diquat was detected 10 days after treatment. Littoral zone dissolved oxygen fell to near, but not below, levels considered harmful to warm water fish. Transparency and total phosphorus were not substantially affected by the diquat treatment. This study confirms that Brazilian waterweed is highly controllable in a Connecticut lake with two successive yearly diquat treatments.

Key words: aquatic herbicide, aquatic plant management, *Egeria densa*, invasive species.

INTRODUCTION

Native to South America, Brazilian waterweed (*Egeria densa* Planch) has invaded 27 countries and 39 states in the United States (Pistori et al. 2004, USDA-NRCS 2019). Spread is facilitated by its popularity as an aquarium plant where it is often sold as *Anacharis canadensis* var. *gigantean* or Giant Elodea (Countryman 1970, June-Wells et al. 2012). Once established, the plant can form dense monospecific stands capable of adversely altering ecosystems (Yarrow et al. 2009). Brazilian waterweed is a close taxonomic relative to hydrilla [*Hydrilla verticillata* (L.f.) Royle] but lacks hydrilla's difficult to control axillary turions and subterranean tubers (Netherland 1997). Brazilian waterweed seed set is rare in both nature and cultivation (Haynes 1988), leaving only fragmentation and lateral expansion from the plant's base as a means of spread. This absence of difficult-to-control propagules suggests Brazilian waterweed should have increased susceptibility to control practices.

Brazilian waterweed control includes harvesting, grass carp (*Ctenopharyngodon idella*), benthic barriers, drawdown, and herbicides (Pennington 2014). Harvesting is a short-term solution due to regrowth from roots, crowns, and fragments produced and spread during the process (Curt et al. 2010). Grass carp prefer Brazilian waterweed over many other aquatic macrophytes (Pine and Anderson 1991) and can provide control; however, state restrictions, uncertain stocking rates, offsite movement, and collateral damage to native species make this a nonviable option for many sites (Colle 2014). Goldsby and Sanders (1977) reported that consecutive drawdowns in Black Lake, LA, eradicated Brazilian waterweed. The success of a drawdown is dependent on several factors such as degree of desiccation, the composition of substrate, air temperature, and the presence of snow (Csurhes et al. 2016). Herbicides can offer targeted control; however, their use on Brazilian waterweed has yielded mixed results. Hofstra and Clayton (2001) found that the control of Brazilian waterweed with the herbicides endothall (dipotassium), triclophyr, and dichlobenil was poor. Diquat (6,7-dihydrodipyrido[1,2-a:2′,1′-c]pyrazine-5,8-diium dibromide) has been shown to be an effective herbicide in some studies and less effective in others. In Battle Ground Lake, WA, the frequency and biomass of Brazilian waterweed were significantly reduced for 2 yr after treatment (Parsons et al. 2007). Treatments of Chickahominy Reservoir, VA, with a combination of diquat and endothall (dipotassium) resulted in a 94% Brazilian waterweed reduction in deep sites but only a 6% reduction in

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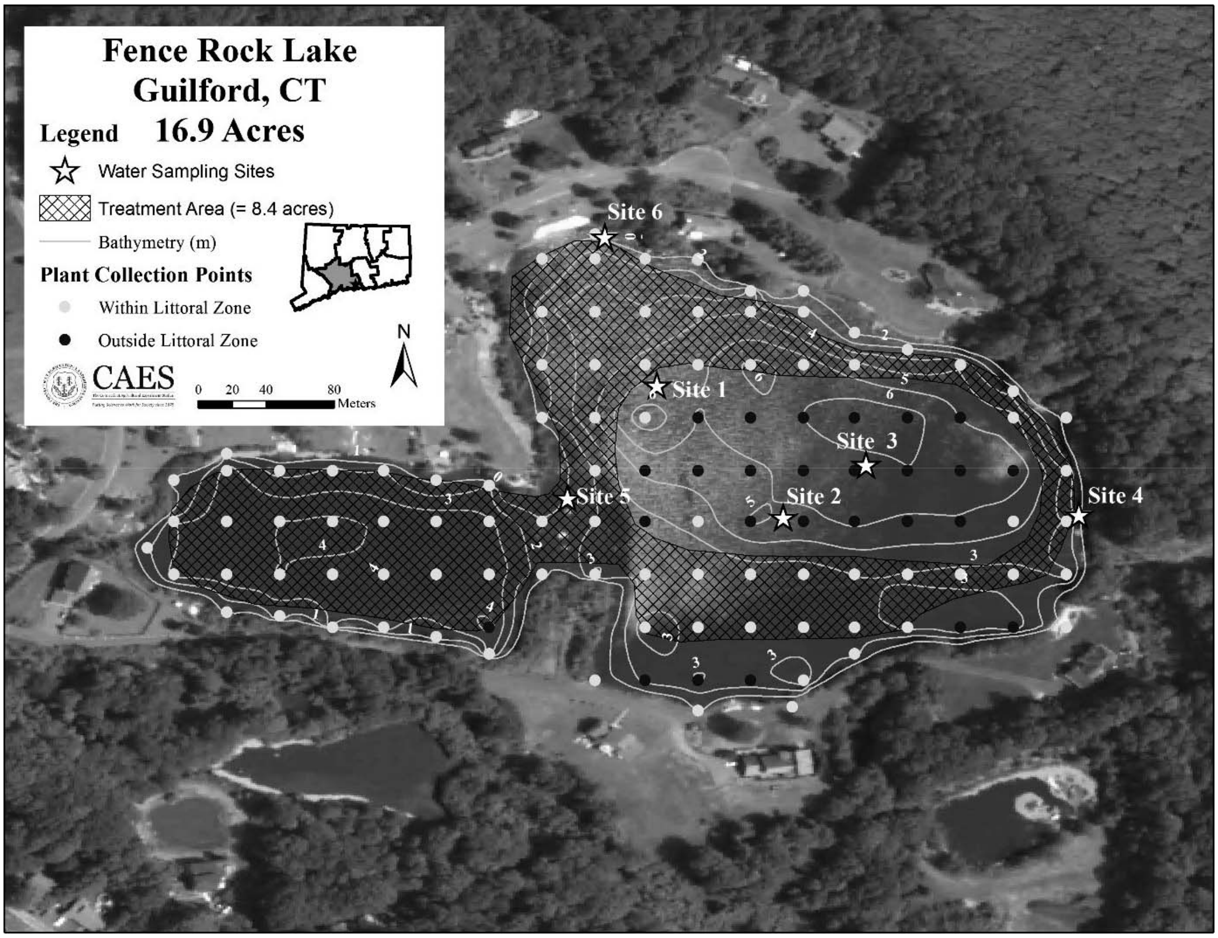

Figure 1. Bathymetry, treatment area, plant and water sampling sites in Fence Rock Lake. Solid white points are within the littoral zone and used for statistical analysis.

shallow sites after 1 yr (Berry et al. 1975). Lake Rotoroa in New Zealand was treated with diquat three times (1978, 1982, and 1985), and Brazilian waterweed increased in abundance and displaced native species (Tanner et al. 1990).

Whether invasive aquatic species are managed with herbicides or other means, collateral effects on desirable native species are a concern. Returning the aquatic ecosystem to a preinvasion assemblage of native species can be attained through proper herbicide selection, dosage, timing, and site selection (Netherland 2014). Bugbee et al. (2015) found that consecutive early season diquat treatments could reduce curlyleaf pondweed (*Potamogeton crispus* L.) to non-nuisance levels while promoting native species. Parsons et al. (2007) found a similar result at Battle Ground Lake, where the abundance of native plant species increased in areas where Brazilian waterweed was eliminated.

Fence Rock Lake is a 7-ha manmade impoundment located in Guilford, CT. It consists of a 5-ha eastern basin and a 2-ha western basin separated by a narrow shoal (Figure 1). The eastern basin reaches a maximum depth of approximately 6 m near its center, and the deepest part of the western basin is about 4 m. Brazilian waterweed was first documented in Fence Rock Lake in 2009 during a Connecticut Agricultural Experiment Station (CAES) Invasive Aquatic Plant Program (IAPP) survey (CAES IAPP 2019). The 2009 survey found several small patches of the plant (Figure 2) interspersed with a sparse community of 14 non-nuisance native species. These included low watermilfoil (*Myriophyllum humile* Raf. Morong), slender waternymph (*Najas gracillima* A. Br. Magnus), watershield (*Brasenia schreberi* J. F. Gemel), white water lily (*Nymphaea odorata* Aiton), and yellow water lily (*Nuphar variegata* Engelm. ex Durand). Because Brazilian waterweed is rarely found in CT and a changing climate could facilitate its becoming a new problem, CAES conducted additional surveys from 2010 to 2013 to determine its ability to spread within the impoundment. Unexpectedly, the small population quickly spread to the majority of the lake's littoral zone (Figure 2). Concerns that Fence Rock Lake was suffering rapid degradation and that the Brazilian waterweed could spread to other lakes prompted an effort on the local, town, and state levels to address the problem with an aquatic herbicide. The goal was to reduce or eradicate the Brazilian waterweed to levels not needing control for many years while preserving the native plant community. In 2014, a state permit was procured to apply diquat with a condition

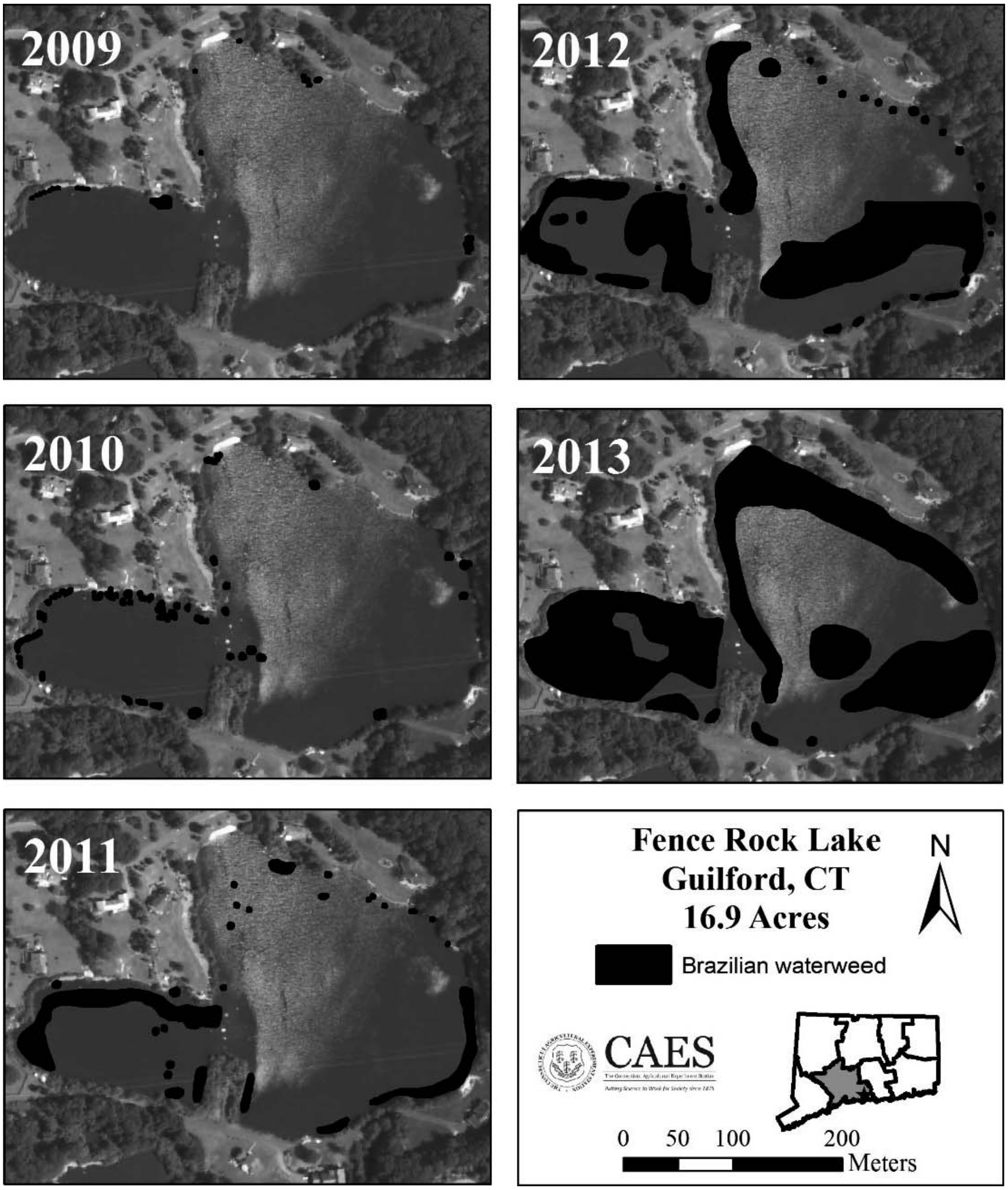

Figure 2. Spread of Brazilian waterweed from 2009 to 2013 in Fence Rock Lake.

that only half the surface area of the lake be treated. Reasons for the half lake restriction included concern that plant dieback might harm fish by lowering dissolved oxygen levels or result in the release of nutrients that could cause an algal bloom. The following study explores the efficacy of diquat on Brazilian waterweed and native macrophytes, tracks diquat concentrations, and documents dissolved oxygen and other water quality parameters in Fence Rock Lake.

MATERIALS AND METHODS

Aquatic plant surveys and treatments

In the pretreatment years 2009 to 2013, aquatic plant surveys were performed to track the spread of Brazilian waterweed utilizing methods established by CAES IAPP (2019) for its statewide monitoring efforts. Surveys were conducted from a small boat traveling over areas within the littoral zone. Plant species were documented by visual observation or collection with a long-handled rake or grapple. General locations were recorded by hand on a lake map and then transferred to a geographic information system (GIS)[1,2] where the final maps were produced (Figure 2). Transparency was measured with a Secchi disk in the deepest portion of the lake. These surveys were performed once during midsummer.

Diquat[3] was applied to the littoral zone of the lake at the maximum suggested label rate of 1.8 kg active ingredient (ai) ha^{-1} (USEPA Reg. No. 100-1091) on July 21, 2014, and again on July 23, 2015. This equates to an approximate ai concentration (diquat cation) of 224 $\mu g\ L^{-1}$ in the treatment area each year (Figure 1). Weather was sunny with calm winds on both days. A 1:1 ratio of diquat formulation to water was injected approximately 1 m above the bottom. This was facilitated by a power boat equipped with a 95-L electric sprayer with an injection hose attached to a weighted underwater camera.[4,5] The goal was to inject the herbicide into the weed beds and keep the product from concentrating near the surface as found in a previous study (Robb et al. 2014). The proximity to the weed beds was viewed on the onboard laptop computer linked to the global positioning system (GPS)[6] that displayed the boat's path. This ensured that the boat paths were approximately 15 m apart. No herbicides were applied before 2014 or after 2015.

To determine the efficacy of diquat on Brazilian waterweed, surveys were conducted before treatments on July 15, 2014, and July 22, 2015. Surveys were also conducted in posttreatment years on July 22, 2016, and July 12, 2017, to assess long-term control. Additionally, these surveys were also used to assess differences in the composition of native aquatic plant assemblages. Surveys were performed using the point intercept method (Madsen 1999) on a grid pattern established with a GPS[6,7,8,9] at 1 s latitudinal and longitudinal intervals (approx. 25 m apart). Although the grid contained 114 points, only 88 points were within the littoral zone (4 m depth) and analyzed in this study (Figure 1). Plants were collected by tossing a weighted 20 cm by 14 cm double-sided grapple with 11 tines per side. The grapple came into contact with the bottom for approximately 1 m per toss. When shoreline or other plants were not suited to removal with a grapple, they were hand harvested by collecting plants from a 1 m by 20 cm area similar to a grapple toss. Depth was measured at each point with a drop line, and the bathymetry was interpolated using a GIS.[2,7] All plant parts retrieved at each point were separated by species and identified using the taxonomy of Crow and Hellquist (2000a, 2000b). Frequency of occurrence was calculated as the percentage of littoral zone points where each species was found. ANOVA[10] and Tukey's HSD *post hoc* analysis[10] ($P \leq 0.05$) were used to determine if there were yearly differences in the frequency of occurrence of each species.

Water analysis

Water samples for diquat analysis were obtained from the littoral (treated) and deeper nonlittoral (untreated) sites at depths of 0.5 m beneath the surface and 0.5 m above the bottom (Figure 1). Samples were taken 0 (morning before treatment), 1, 4, 7, 10, 21, and 36 days after treatment (DAT). To assure consistency in the sampling locations, each site was located with a GPS.[6,7,8,9] The diquat samples were collected in 15-ml polypropylene tubes. Surface samples were obtained by hand, and bottom samples were obtained with an electric pump. The samples were immediately frozen in dry ice and stored in a cooler until delivery to the laboratory. In the laboratory, the samples were equilibrated to room temperature and passed through a 45-μm syringe filter. Samples that could not be immediately analyzed were stored at −20 C. Diquat concentrations were quantified using an HPLC-MS/MS spectrometer[11] with a diquat cation quantitation limit of 3.3 $\mu g\ L^{-1}$ (Robb and Eitzer 2011). Diquat concentrations were calculated from the mean of the three surface treated sites, the three surface untreated sites, the three bottom treated sites, and the three bottom untreated sites. Statistical differences are expressed as ± 1 SEM.

Water transparency, temperature, and dissolved oxygen (DO) concentrations were measured *in situ* on the same schedule as the diquat concentrations with the exception of the 1 DAT measurements that were taken 2 DAT. Transparency was measured with a Secchi disk at the untreated deep water sites. Transparency is highly correlated with turbidity (Steel and Neauhauser 1999) and was used as the turbidity indicator in this study. The transparency of the treated sites was not measured because of insufficient depth. Temperature and DO were measured with a calibrated digital meter[12] at 0.5 m below the surface and 0.5 m above the bottom. The total phosphorus concentration was determined on the same schedule as the diquat concentrations. Samples were placed in 250-ml Nalgene® bottles, stored on ice in a cooler, and refrigerated until analysis. Lake water pH was measured within 7 days of collection with a calibrated digital pH meter.[13] Water samples were then stabilized and frozen until tested for total phosphorus. Total phosphorus was determined using the ascorbic acid method preceded by digestion with potassium persulfate (APHA 1995). Phosphorus was then quantified using a spectrometer[14] with a light path of 2 cm and a wavelength of 880 nm. Statistical differences in water chemistry are expressed as ± 1 SEM.

RESULTS AND DISCUSSION

Response of the plant community to diquat treatments

Prior to the diquat treatment in 2014, the species richness of Fence Rock Lake on grid points consisted of Brazilian waterweed and 11 native species (Table 1). Brazilian waterweed dominated the assemblage with a frequency of occurrence (FO) of 61%, while the pretreatment compendium of native species had a FO of 57%. One year after the initial treatment (YAT) in 2015, only one Brazilian waterweed plant was found on one point, and the plant's FO dropped significantly to 1.1% (df = 3, $F = 130.3$, $P < 0.001$). Combined native species FO, however, remained statistically unchanged at 40% (df = 3, $F = 6.17$, $P = 0.135$). Native species richness increased to 16 one YAT (Table 1). Surveillance 2 YAT in 2016 found no Brazilian waterweed,

Table 1. Aquatic macrophytes found in Fence Rock Lake on georeferenced points (o = untreated years, • = treated years). Invasive species in bold.

Common name	Scientific name	2014[1]	2015[1]	2016	2017
Brazilian waterweed	***Egeria densa* Planch.**	•	•		
Brittle waternymph	***Najas minor* All.**				o
Arrowhead	*Sagittaria* species L.				o
Bur-reed	*Sparganium* species L.				o
Coontail	*Ceratophyllum demersum* L.	•	•	o	o
Golden hedge-hyssop	*Gratiola aurea* Pursh	•	•		
Humped bladderwort	*Utricularia gibba* L.	•	•	o	o
Leafy pondweed	*Potamogeton foliosus* Raf.		•		o
Low watermilfoil	*Myriophyllum humile* Raf. Morong	•	•	o	o
Nodding waternymph	*Najas flexilis* Willd. Rosk. & Schmidt	•	•	o	o
Pickerelweed	*Pontederia cordata* L.		•	o	o
Primrose-willow	*Ludwigia* species L.		•	o	o
Quillwort	*Isoetes* species L.	•	•	o	o
Slender waternymph	*Najas gracillima* A. Br. Magnus		•		
Small pondweed	*Potamogeton pusillus* L.	•	•	o	o
Snailseed pondweed	*Potamogeton bicupulatus* Fern.	•	•	o	o
Spikerush	*Eleocharis* species R. Br.	•	•	o	o
Water starwort	*Callitriche* species L.				o
Watershield	*Brasenia schreberi* J. F. Gemel	•	•	o	o
Waterwort	*Elatine* species L.		•	o	o
White water lily	*Nymphaea odorata* Aiton	•	•	o	o
Yellow water lily	*Nuphar variegata* Engelm. ex Durand	•		o	o
	Native Species Richness	11	16	14	18
	Invasive Species Richness	**1**	**1**	**0**	**1**
	Total Species Richness	12	17	14	19

[1] Pretreatment.

14 native species, and no change in native species FO (49%) compared to pretreatment levels in 2014 (df = 3, $F = 6.17$, $P = 0.791$). Surveillance 3 YAT in 2017 found no Brazilian waterweed, an increase in native species richness to 18, and an unchanged native species FO (70%) from pretreatment (df = 3, $F = 6.17$, $P = 0.186$). In 2017 brittle waternymph (*Najas minor* All.) was found on one point. This is an invasive plant frequently found in Connecticut lakes, which through prolific seeding takes advantage of ecosystem disturbance (Bugbee et al. 2019).

Changes in the native aquatic macrophytes assemblage caused by the diquat treatments were species specific but generally not statistically significant (Figure 3). Coontail (*Ceratophyllum demersum* L.) was the only native species that had a significant FO decrease from 2014 to 2017 (df = 3, $F = 29.9$, $P < 0.001$). Snailseed pondweed (*Potamogeton bicupulatus* Fern.), low watermilfoil, and watershield exhibited no statistical change in any year ($P > 0.05$). Nodding waternymph (*Najas flexilis* Willd. Rostk. & Schmidtt) increased in FO in 2016 from pretreatment conditions (df = 3, $F = 29.9$, $P = 0.029$) but returned to pretreatment conditions in 2017 (df = 3, $F = 29.9$, $P = 0.163$). Small pondweed (*Potamogeton pusillus* L.) significantly increased in FO in 2017 from 2014 (df = 3, $F = 6.00$, $P = 0.938$). Populations of other native species were small, and changes were difficult to assess.

Lake water analysis

Effective herbicide applications require plant exposure to a sufficient concentration of active ingredient for a critical length of time. Diquat is a quick acting contact herbicide requiring relatively short periods of plant exposure (Funderburk and Lawrence 1964). Skogerboe et al. (2006) found a greater than 90% reduction in the biomass of Brazilian waterweed when exposed to a 370 μg L^{-1} concentration of diquat over a half-life of 2.5 h. No diquat was found at any sites prior to treatment (0 DAT, Figure 4). One DAT the mean diquat concentration in the treated and untreated surface sites were 57 and 52 μg L^{-1} respectively, indicating rapid upward and lateral mixing. Both the treated littoral and untreated nonlittoral bottom water, however, contained no detectable diquat 1 DAT. This trend is similar to 1 DAT findings by Robb et al. (2014) in Crystal Lake, CT, where diquat concentrations were <1 μg L^{-1} near bottom compared to 270 μg L^{-1} near the surface and by Berry et al. (1975) in Chickahominy Reservoir where diquat concentrations were 30 μg L^{-1} near the bottom and 730 μg L^{-1} near the surface. Turbidity is known to decrease the efficacy of diquat (Netherland 2014), and diquat concentrations can be reduced by adsorption to suspended clay (Poovey and Getsinger 2002). Although differences in surface and bottom water turbidity were not determined in this study, the high efficacy of the diquat application on Brazilian waterweed suggests this was not the case. Plant removal of diquat may explain the discrepancy between surface and bottom concentrations. Davies and Seaman (1968) found diquat uptake in *Elodea canadensis* Michx was rapid but later released back into the water. If the metabolism of Brazilian waterweed is similar, this could account for the observed low treated bottom water diquat concentration 1 DAT (uptake) and the rise 4 DAT (release).

Temperature and DO were similar at the surface and bottom treated littoral sites (Figure 5), indicating stratification was not likely to keep the herbicide from dispersing upward. Mean diquat concentrations in the treated and untreated surface sites 4 DAT declined to 21 and 24 μg L^{-1}, respectively, while they rose to 32 μg L^{-1} in the treated bottom water. In the deeper untreated nonlittoral bottom water,

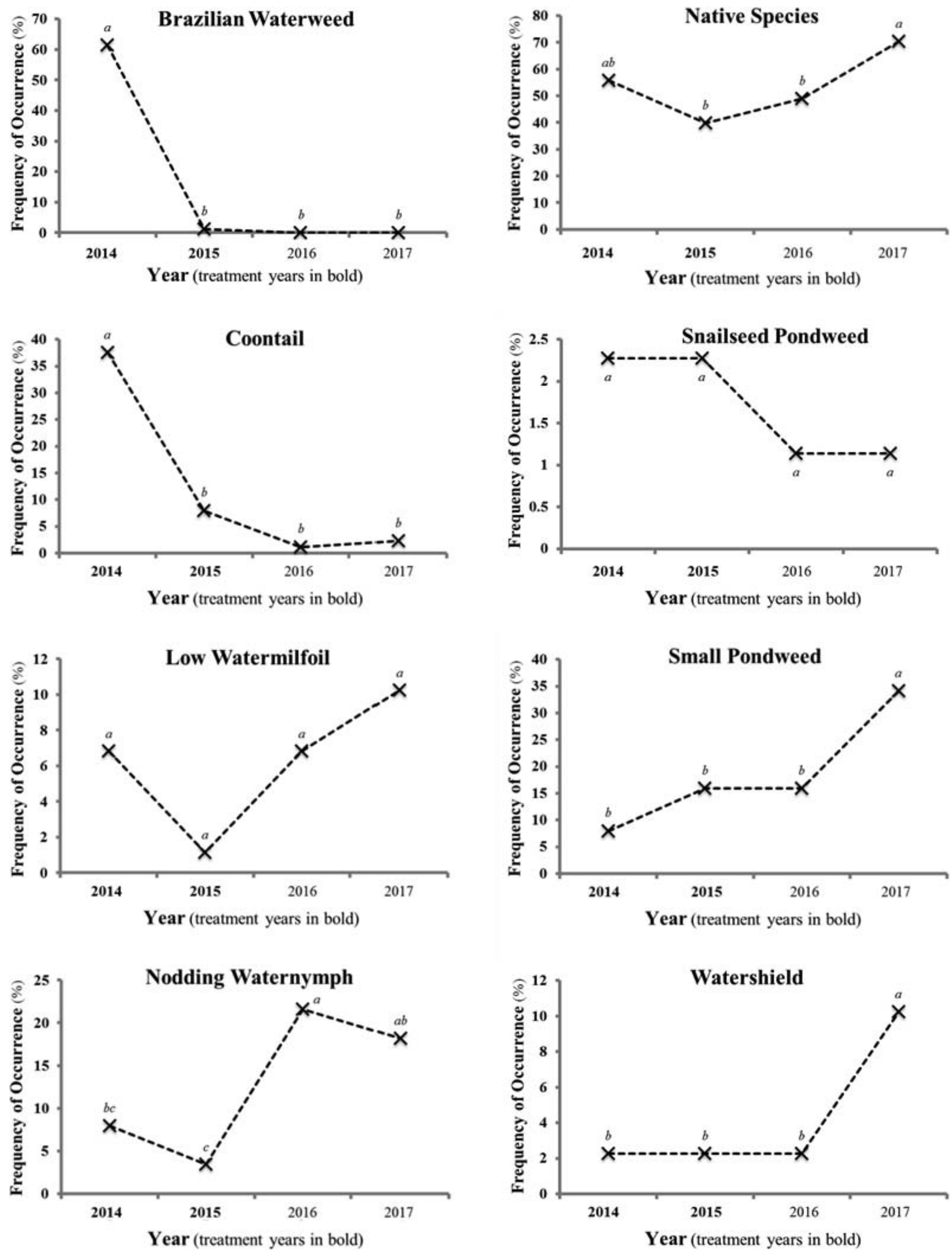

Figure 3. Frequency of occurrence (%) of Brazilian waterweed, combined native species, and select native species in Fence Rock Lake before and after diquat treatments. Years labeled in bold are treatment years. Upper and lowercase letters represent statistical differences ($P \leq 0.05$) among years detected by *post hoc* analyses for frequency.

diquat concentrations remained below detection limits. Between 4 and 10 DAT diquat concentrations in the surface treated and untreated sites followed a linear decline to below detection while the untreated deep water sites remained below detection. From 10 to 36 DAT diquat concentrations remained below detection at all sites and depths.

Plant dieback can effect water chemistry by lowering DO and releasing nutrients that favor algal blooms (Strange and Schreck 1976, Murphy and Barrett 1990). Prior to treatment, the surface treated, surface untreated, and bottom treated sites were highly aerobic with DO concentrations of 7 to 8 mg L^{-1}. By 36 DAT, the mean DO had declined to 3 to 4 mg L^{-1} in the surface treated and surface untreated sites and to 2.5 mg L^{-1} in the bottom treated sites. The deeper bottom untreated sites dropped from a mean of 2.5 mg L^{-1} pretreatment to a range of 0.6 to 1.2 mg L^{-1} 1 to 36 DAT. In the pretreatment years 2011 to 2013, the midsummer surface aerobic (7.9 to 8.6 mg L^{-1}) and deep water anaerobic

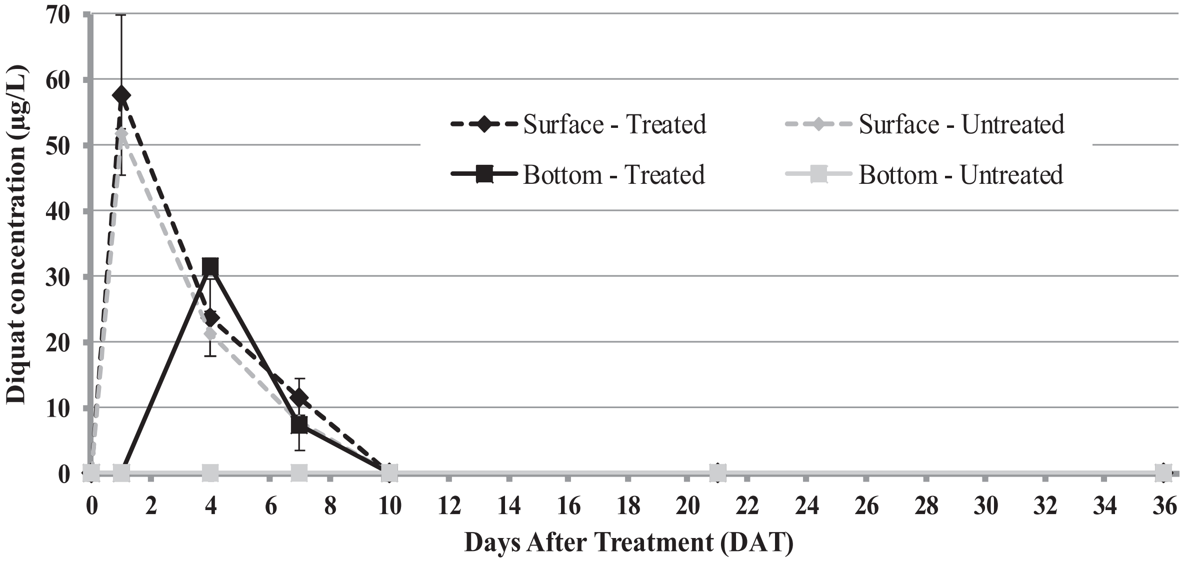

Figure 4. Diquat concentrations in surface and bottom water from treated and untreated areas of Fence Rock Lake over time (DAT 0 = July 21, 2014). Error bars equal ±1 SEM.

(0.0 to 0.8 mg L$^{-1)}$ conditions were documented at Site 1 and suggests this is normal for the lake (CAES IAPP 2019). Low DO concentrations are a particular concern for fish and other aquatic organisms. USEPA (1986) water quality criteria for freshwater suggest that as DO concentrations fall from 3 to 1 mg L^{-1} harmful effects to fish become more acute. Cool water fish such as salmon and trout are injured at the upper range, while warm water species such as largemouth bass and crappie are harmed at the lower range. Fence Rock Lake is a warm water lake with no known populations of cool water fish, and thus no harmful effects would be expected. No dead fish were observed or reported during this study.

Plant decomposition can add nutrients, particularly phosphorus that can cause algal blooms (Nichols and Keeney 1973, Schindler 1974). Prior to treatment, total phosphorus in the surface littoral treated, surface nonlittoral untreated, and bottom littoral treated sites ranged from 10 to 13 µg L^{-1} (Figure 5). From 1 to 36 DAT the total phosphorus in the surface and bottom littoral treated sites and the surface untreated nonlittoral sites increased slightly to within a range of 11 to 23 µg L^{-1} (Figure 5). Total phosphorus in the deep untreated bottom water was similar during this study with a mean concentration of 19 µg L^{-1} pretreatment, a peak of 28 µg L^{-1} 1 DAT, and a return to 19 µg L^{-1} 36 DAT. Summer

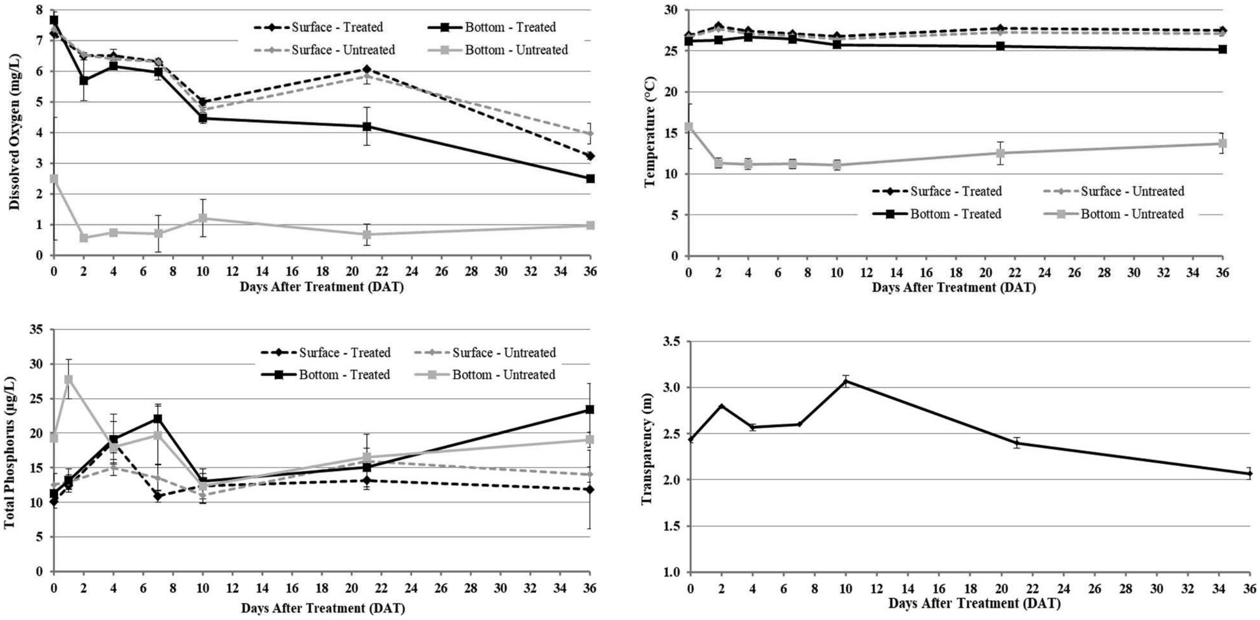

Figure 5. Transparency and water chemistry in surface and bottom water from treated and untreated areas of Fence Rock Lake over time (DAT 0 = July 21, 2014). Error bars equal ±1 SEM.

transparency in the pretreatment years 2009 to 2013 (Figure 5) ranged from 2.0 to 2.6 m (CAES IAPP 2019). In the first treatment year (2014), the transparency ranged from 2.1 to 3.1 m. Although the lowest transparency occurred 36 DAT, conditions were similar to previous years.

This study confirms that Brazilian waterweed is highly controllable in a Connecticut lake with two successive yearly diquat treatments. The native plant community was resilient with an increase in species richness from 11 pretreatment to 18 2 YAT with no change in FO. Individual native species FO exhibited losses, gains, or little change depending on species. Bottom injected diquat concentrations peaked near the surface and were low near the bottom 1 DAT in treated sites. Treated surface diquat concentrations declined, and bottom treated concentrations rose to similar levels 4 DAT. No diquat was detected at any sites 10 DAT. Littoral zone DO concentrations fell to near, but not below, levels considered harmful to warm water fish. Transparency and total phosphorus also were not substantially affected by the diquat treatment.

SOURCES OF MATERIALS

[1] Pathfinder® 5.85, Trimble Inc., 935 Stewart Dr., Sunnyvale, CA 94085.

[2] ArcGIS Desktop 10.6.1®, ESRI Corp., 380 New York St., Redlands, CA 92373.

[3] Reward®, Syngenta Corp., 341 Silverside Rd., Wilmington, DE 19810.

[4] TOV-1 Towed Video®, JW Fisher Inc., 1953 County St., East Taunton, MA 02718.

[5] SplashCam Deep Blue®, Ocean Systems, Inc., 3901 Smith Ave., Everett, WA 98201.

[6] ProXT®, Trimble Inc., 935 Stewart Dr., Sunnyvale, CA 94085.

[7] GeoXaT®, Trimble Inc., 935 Stewart Dr., Sunnyvale, CA 94085.

[8] GPS76®, Garmin International Inc., 1200 E. 151st St., Olathe, KS 66062.

[9] R1 GNSS®, Trimble Inc., 935 Stewart Dr., Sunnyvale, CA 94085.

[10] SYSTAT 11®, Systat Software, Inc., 225 W. Washington St., Suite 425, Chicago, IL 60606.

[11] 1200 HPLC®, Agilent Technologies, Inc., 5301 Stevens Creek Blvd., Santa Clara, CA 95051.

[12] YSI 58®, YSI, Inc., 1700/1725 Brannum Ln., Yellow Springs, OH 45387.

[13] Accumet XL20®, Thermo Fisher Scientific Inc., 168 Third Ave., Waltham, MA 02454.

[14] Thermo LTQ linear ion-trap mass spectrometer, Thermo Fisher Scientific Inc., 168 Third Ave., Waltham, MA 02454.

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