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Small Plot Applications of Florpyrauxifen – Benzyl (Procellacor SC™) for Control of Monoecious Hydrilla in Roanoke Rapids Lake, NC

Bradley T. Sartain, Erika Haug, Kurt Getsinger, Benjamin P. Sperry,
Mark Heilman, and Mike Greer

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Small Plot Applications of Florpyrauxifen – Benzyl (Procellacor SC™) for Control of Monoecious Hydrilla in Roanoke Rapids Lake, NC

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Abstract

Four demonstration plots were selected at Roanoke Rapids Lake, NC to evaluate water exchange and aqueous herbicide residues in stands of submersed aquatic vegetation (SAV) following treatment with rhodamine wt dye and florpyrauxifen-benzyl to control monoecious hydrilla. Florpyrauxifen-benzyl (Procellacor™ SC) was applied in combination with Rhodamine WT (RWT) at two of the plots. Dye measurements and herbicide residue samples were collected at specific time intervals to draw comparisons between herbicide and RWT dye dissipation. The two additional plots served as reference plots to the treatment plots. Pre- and post-treatment vegetation surveys were conducted to evaluate monoecious hydrilla control and non-target species response. RWT dye and herbicide residue data indicated rapid water exchange was occurring with each treatment plot. As a result, florpyrauxifen-benzyl concentration and exposure times (CETs) towards monoecious hydrilla were not sufficient to achieve adequate control by 4 weeks after treatment (WAT). To reduce the impact of hydraulic complexity and improve herbicide efficacy, treatments should coincide with minimal reservoir discharge events to extend herbicide CET relationships. Evaluations of florpyrauxifen-benzyl on late season, mature plants may have impacted herbicide efficacy. Evaluations should be conducted earlier in the growing season, on young, actively growing plants, to discern potential differences in efficacy due to treatment timing and phenology. More information on herbicide concentration and exposure time relationships for monoecious hydrilla should be developed in growth chamber and mesocosm settings to improve species selective management of monoecious hydrilla in hydrodynamic reservoirs.

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Preface

This study was conducted as part of the Aquatic Plant Control Research Program (APCRP). The APCRP is sponsored by Headquarters, US Army Corps of Engineers (HQUSACE), and is assigned to the US Army Engineer Research and Development Center (ERDC) under the purview of the Environmental Laboratory (EL), Vicksburg, MS. The work reported herein was performed by the Aquatic Ecology and Invasive Species (EEA) of the Ecosystem Evaluation and Engineering Division (EEED) ERDC-EL Vicksburg, MS; North Carolina State University; and SePRO Corporation.

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At the time of publication, Mr. Alan Katzenmeyer was Chief, EEA; Mr. Mark D. Farr was chief, EEED. Dr. Brandon Lafferty was the deputy director of ERDC-EL, and Dr. Jack Davis was the acting director.

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1 Introduction

1.1 Background

The effectiveness of a submersed aquatic herbicide application is ultimately driven by: (1) herbicide concentration in the water column surrounding the target plant and (2) time the target plant is exposed to a dissipating herbicide concentration (Getsinger et al. 1996; Getsinger and Netherland 1997). The association between these two variables is herbicide and target plant specific and has been defined as the concentration exposure time (CET) relationship (Netherland and Getsinger 1991; Getsinger et al. 1996; Getsinger and Netherland 1997). Since herbicides are primarily absorbed via the shoots and leaves of submersed plants, water exchange can drastically influence treatment efficacy by altering the length of herbicide exposure, plant uptake, and affecting CET relationships (Getsinger et al. 1996). Rhodamine WT (RWT) is an inert fluorescent tracer dye commonly used to characterize water exchange in stands of submersed plants. Previous research has shown dissipation of RWT dye to be highly correlated to dissipation patterns of aquatic herbicides when applied as a submersed treatment (Fox et al. 1991, 1992, 1993; Turner et al. 1994, Wersal and Madsen 2011). Therefore, RWT is a useful tool to evaluate conventional and novel application methodologies, and pattern the potential off-target movement of applied herbicides to develop site-specific treatment strategies (Fox et al. 1992, 1993; Turner et al. 1994; Getsinger et al. 1997; Getsinger et al. 2000; Poovey et al. 2004; Getsinger et al. 2013). Over the past 30 years, field demonstrations and operational-scale herbicide treatments have confirmed the importance of linking water-exchange and herbicide CETs for managing submersed aquatic plants such as hydrilla (*Hydrilla verticillata* L.f. Royle) and Eurasian watermilfoil (*Myriophyllum spicatum* L) particularly in run of river reservoirs that are more prone to constant and variable water flows (Haller et al. 1990; Fox and Haller 1992; McNabb 1993; Farone and McNabb 1993; Fox et al. 1994; Getsinger et al. 1996).

Product selection for managing plants in moderate to high water exchange areas has traditionally been limited to fast-acting contact herbicides that generally have shorter exposure time requirements than slow-acting systemic herbicides. In these settings, effective long-term control with contact herbicides can only be achieved when multiple treatments are applied over time. Therefore, a fast-acting systemic product would be

valuable to overcome limitations of managing aquatic plants in areas of rapid bulk water-exchange. Additionally, new herbicides are needed to manage populations that are less susceptible to other herbicides such as fluridone-resistant hydrilla (Michel et al. 2004) and hybrid watermilfoils with reduced sensitivity to 2,4-D and triclopyr (LaRue et al. 2013).

Florpyrauxifen-benzyl (Procellacor™), a reduced risk arylpicolinate synthetic auxin herbicide, was registered in 2018 for aquatic use (Epp et al. 2016). Preliminary screenings indicated that florpyrauxifen-benzyl is capable of providing systemic and selective control for common nuisance aquatic plants, including but not limited to milfoils, hydrilla, and crested floating heart (Netherland and Richardson 2016; Richardson et al. 2016; Haug 2018; Beets and Netherland 2018; Beets et al. 2019, Sperry et al. 2021; Mudge et al. 2021a). In addition, florpyrauxifen-benzyl is effective at low concentrations and short exposure times (< 1 hr) on highly sensitive species such as watermilfoils (Mudge et al. 2021b). Similar to the fast-acting contact herbicide endothall, florpyrauxifen-benzyl has a relatively short half-life (days); however, it exhibits systemic activity and has provided hydrilla control at 24 ug L⁻¹ following a 24-hr exposure (Beets and Netherland 2018; Taylor et al. 2014). Consequently, as a fast-acting systemic herbicide that can provide hydrilla control, many resource managers are still learning how to effectively incorporate florpyrauxifen-benzyl into their management plans. Although small-scale research trials have provided beneficial data concerning general efficacy and selectivity, few trials have documented how to effectively utilize this technology in the field. To develop and establish new and/or enhance current florpyrauxifen-benzyl use patterns, the collection of concentration and exposure time (CET), herbicide efficacy, species selectivity, and water quality data from the field is crucial.

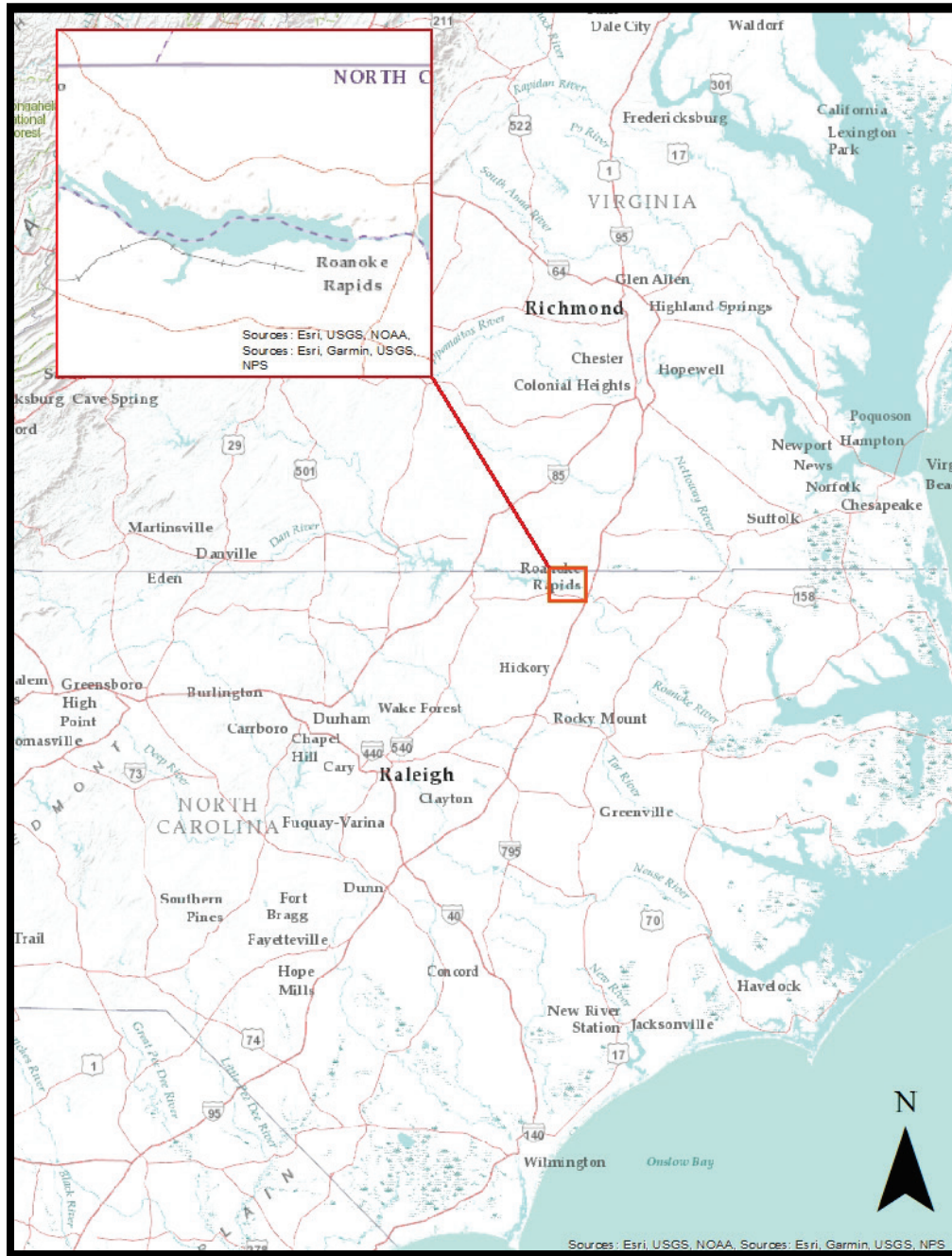
Roanoke Rapids Lake (RRL), located along the North Carolina and Virginia state line, is a ~1,860 ha (4,600 acre) impoundment on the Roanoke River (Figure 1). Constructed in 1955 primarily for hydroelectric power production, it is the smallest of three reservoirs (John H. Kerr, Lake Gaston, RRL) located along the Roanoke River and is the last impoundment before the river empties into the Albemarle Sound, NC. The lake is owned and operated by Dominion Energy and land use surrounding RRL consists of agricultural and residential areas, mixed hardwood forests, and wetlands (Dominion Power 2010). In addition to hydroelectric power and flood control, RRL and the lower Roanoke River provide

numerous recreational activities and valuable wildlife habitat (Dominion Power 2010). Water level within RRL is greatly influenced by water releases from the Lake Gaston Dam on the upstream and the Roanoke Rapids Dam downstream, with typical water level fluctuations between 1 to 1.5 m (Dominion Power 2010).

Native and non-native submersed aquatic vegetation (SAV) species occur throughout RRL. The invasive, monoecious hydrilla and Eurasian watermilfoil (EWM) have been shown to be the most dominant SAV species. Vegetation surveys conducted between 1999 and 2015 documented monoecious hydrilla and EWM presence in 60 to 72% and 18 to 36% of total samples respectively, (Howell 2017). Additional SAV species found throughout RRL include coontail (*Ceratophyllum demersum*), fanwort (*Cabomba caroliniana*), southern naiad (*Najas guadalupensis*), brittle naiad (*Najas minor*), pondweeds (*Potamogeton* spp.), and musk grass (*Chara* spp.). Emergent vegetation such as water willow (*Justicia americana*) is also found along the lake shoreline.

Pursuant to monoecious hydrilla being the dominant SAV species and the hydrodynamic characteristics associated with hydrilla infestations in RRL, the lake was selected as a suitable study site to evaluate florpyrauxifen-benzyl (Procellacor™) use in the field. This document describes a field demonstration focused on developing new and/or enhancing current florpyrauxifen-benzyl use patterns for managing monoecious hydrilla through the collection of CET, efficacy, and species selectivity data in the field.

Figure 1. Location of Roanoke Rapids Lake, NC.



1.2 Objectives

The overall goal of this research was to develop field verified florpyrauxifen-benzyl treatment strategies to selectively control monoecious hydrilla in hydrodynamic systems.

Specific objectives were to accomplish the following:

1. Develop an initial data bank linking dissipation of herbicide and RWT dye downstream from treatment plots to determine water exchange processes within SAV stands, and
2. Couple water exchange processes to treatment effectiveness on monoecious hydrilla and impacts to co-occurring non-target species.

1.3 Approach

Four demonstration plots were selected at RRL to evaluate the dissipation of aqueous herbicide residues in stands of SAV following treatment with florypyrauxifen-benzyl to control monoecious hydrilla. Florypyrauxifen-benzyl (Procellacor™ SC) was applied in combination with RWT at two of the plots. Dye measurements and herbicide residue samples were collected within each plot at specific time intervals to draw comparisons between herbicide and RWT dye dissipation. The two additional plots served as reference plots to the treatment plots. Pre- and post-treatment vegetation surveys were conducted within each plot to evaluate monoecious hydrilla control and non-target species response.

2 Materials and Methods

2.1 Water exchange and herbicide residue study

2.1.1 Plot description

Four plots (~1.2 ha in size each) were selected for evaluation (Figure 2). Plots 1 and 2 were selected for water exchange and herbicide treatment, and Plots 3 and 4 were selected as a reference and did not receive herbicide treatment. A description for each plot is provided below and plot locations within the lake are shown in Figure 2.

Plot 1 was in open water with an average depth of 1.5 m (at treatment). It was positioned on the upstream end of the lake in a shallow bay. The plot was 320 m from a peninsula that separated the bay from the main channel of the Roanoke River, ~450 m from the reservoirs northern shore, and ~2600 m from the Lake Gaston Dam.

Plot 2 was a rectangular shaped 1.2 ha plot, with an average depth of 2 m (at treatment). It was oriented parallel to the northeastern shoreline of a teardrop-shaped island at the mouth of the shallow bay. The plots downstream border was positioned 80 m upstream from the tip of the island and 1,180 and 230 m from the southern and northern reservoir shoreline, respectively.

Plot 3 was rectangular in shape and positioned along the northern shoreline of RRL and had an average depth of 1.1 m. Plot depth nearest the shoreline was roughly 0.6 m and gradually increased to a maximum recorded depth of 1.2 m along the plot edge furthest from the shore. It was located ~500 m to the east of treatment Plot 1 and ~1,400 m north northwest from treatment Plot 2.

Plot 4 was also rectangular shaped and positioned along the northern shoreline of the lake approximately 800 m downstream of reference Plot 3. The average depth was 1.3 m and gradually increased from 0.8 m along the plot edge closest to the shoreline to 1.5 m along the outer plot edge. It was located ~1,000 m east southeast from treatment Plot 1 and ~600 m north of treatment Plot 2.

Figure 2. Location of treatment (red) and reference (blue) sampling plots within Roanoke Rapids Lake, NC.



2.1.2 Rhodamine WT dye and herbicide treatment

RWT dye was utilized to characterize water exchange patterns within each Plots 1 and 2. Although RWT dye is commonly used in combination with herbicide treatments, this was the first demonstration documenting RWT dye and floraspiraxifen-benzyl herbicide being applied as a tank mix. Thus,

it was unknown if RWT dye and florpyrauxifen-benzyl dissipation would mimic one another.

Plots 1 and 2 were treated with RWT and florpyrauxifen-benzyl herbicide (Procellacor SC) on 18 August 2020 (Plot 1: start time 0953/end time 1001, Plot 2: start time 1109 end time: 1114). The target application rate for RWT and florpyrauxifen-benzyl was 10 and 48 $\mu\text{g L}^{-1}$, respectively for each plot. Weather at the time of both treatments was sunny, 26.5°C with minimal cloud cover and a northwest wind at 1.1 kph. Treatments were performed by boat using a 227 L (60-gal) spray tank and four-drop hoses. Drop hoses were adjusted to hang 60 cm below the water surface. This height was determined to stay submerged at speeds of 9.6 kph (6 mph) and under. The shortened length of the drop hoses was intended to reduce drag through dense plant beds and subsequent disturbance of bottom sediments. Average depth in each plot used to calculate RWT and herbicide application rates was adjusted based on the differential between the gauge height when bathymetry data were collected (July 10) and the gauge height at the time of treatment. This difference was +6 cm. Water quality data (temperature, dissolved oxygen (DO), and pH) was collected during the morning and evening at the midpoint of each plot and is presented in Table 1.

Table 1 Water quality measurements during the morning and evening on the day of treatment, 18 August 2020.

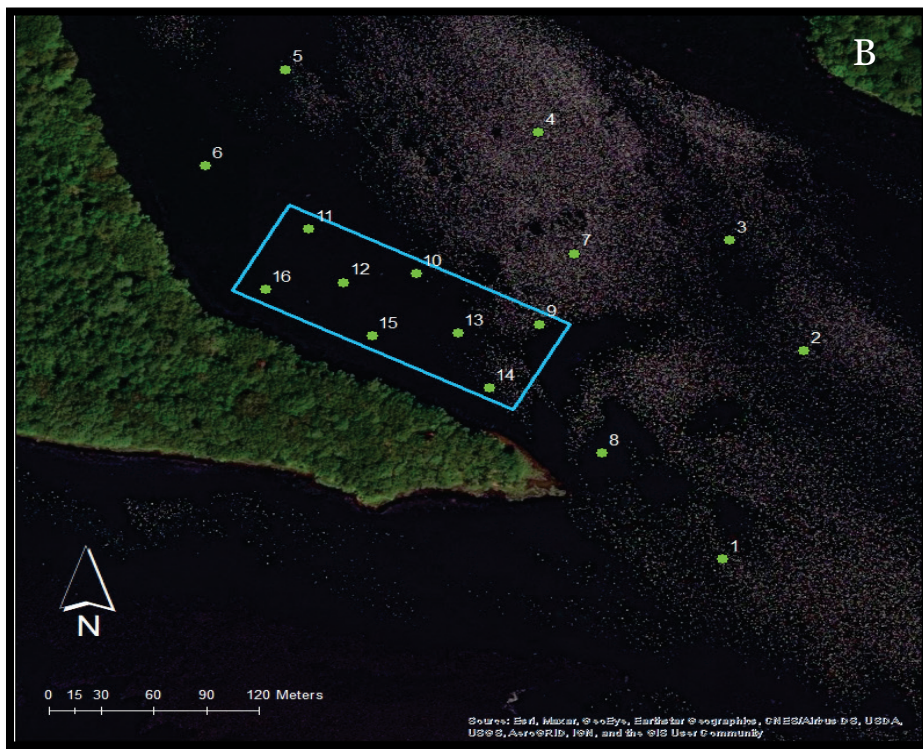
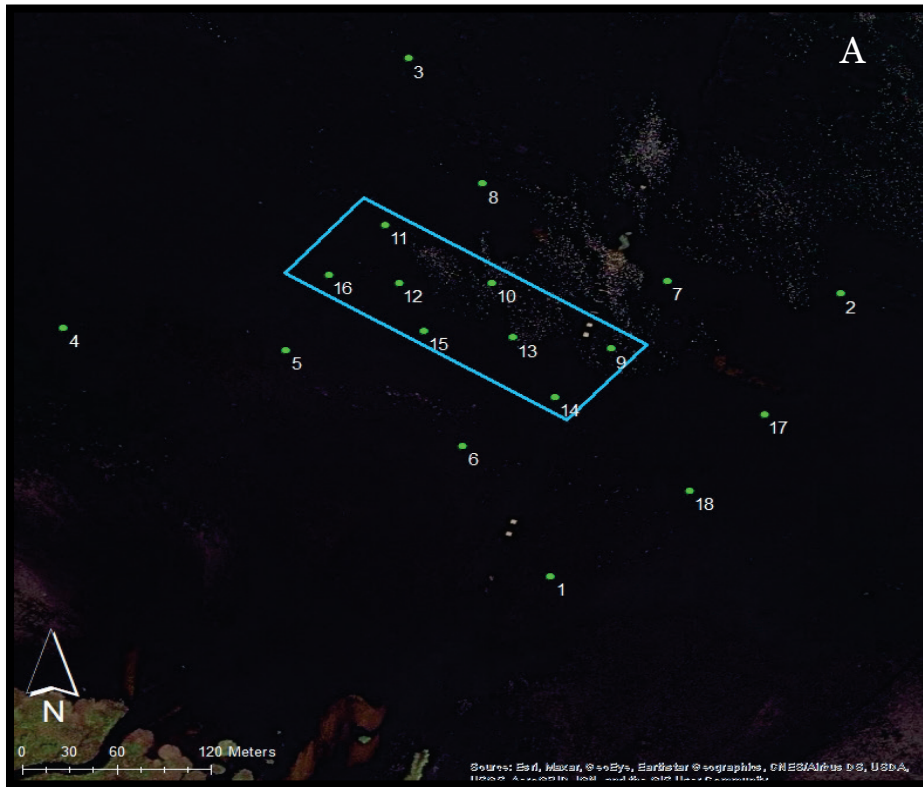
		Temperature (°C)		Dissolved O2 (% saturation)		pH	
		Sample Time					
Depth(m)		AM	PM	AM	PM	AM	PM
Plot 1							
	0.0	26.5	28.9	69.7	85.6	8.6	8.9
	0.5	26.2	28.8	70.9	87.5	8.6	8.9
	1.0	25.9	26.6	69.5	75.2	8.6	8.8
	1.5	25.3	25.3	62.2	50.0	8.4	8.6
Plot 2							
	0.0	27.6	28.8	68.7	88.4	8.6	8.6
	0.5	27.6	28.7	71.5	90.0	8.6	8.7

	1.0		27.5	28.7		72.5	90.2		8.7	8.7
	1.5		26.5	27.7		66.5	92.3		8.4	8.7
	2.0		26.5	26.6		53.2	74.7		8.2	8.5
Plot 3										
	0.0		26.05	/		41.2	/		7.4	/
	0.5		25.9	/		39.4	/		7.4	/
	1.0		25.86	/		33.2	/		7.3	/
	1.25		25.86	/		33.7	/		7.3	/
Plot 4										
	0.0		26.5	30.3		38.3	81.9		7.6	9.1
	0.5		26.4	30.1		42.6	80.7		7.6	9.1
	1.0		26.3	30.1		43.3	79.8		7.6	9.0
	1.5		26.3	28.1		42.2	78.9		7.6	8.9

2.1.2.1 Rhodamine WT dye sampling

Rhodamine WT dye was measured *in-situ* utilizing a Cyclops-7 submersible fluorometer (Turner Designs, Sunnyvale, CA) or a Manta 2 submersible fluorometer RWT probe (Eureka Water Probes, Austin, TX). Both probes were calibrated on 14 August 2020 with a three-point calibration at 0, 10, and 20 $\mu\text{g L}^{-1}$ standards created utilizing lake water. Two boats measured dye data simultaneously for the entirety of the trial. At Plots 1 and 2, RWT dye was measured at internal (i.e., inside the treatment plot) and external sampling points (i.e., outside the treatment plot) to capture dye dissipation from the targeted treatment area (Figure 3). A Lowrance chart plotter GPS was utilized for navigation to each sampling point. Vertical sampling at each point included a sample at the sediment-water interface (bottom), just below the water surface (surface), and middle of the water column (mid-depth). Dye was measured in Plot 1 at 0, 1, 3, 6, 9, and 24 hours after treatment (HAT). Dye was measured in Plot 2 at 0, 1, 3, 5, 8, and 24 HAT. For each sample, the time, relative sample depth, temperature, and RWT concentration were recorded.

Figure 3. Sampling points for RWT dye and herbicide residues at Plot 1 (A) and Plot 2 (B), Roanoke Rapids Lake.



2.1.2.2 Rhodamine WT dye data analysis

Rhodamine WT dye measurements for each plot were subjected to an exponential decay non-linear regression using SigmaPlot 14.0 statistical software (Systat Software Inc. San Jose, CA) to calculate RWT dye half-lives throughout the water column and at the surface, mid-, and bottom depths within each site. RWT dye distribution in the water column was determined by dividing the average dye concentration at each sampling depth (e.g., surface, mid-, or bottom) by the average dye concentration for all sampled depths at internal and external sampling points. RWT dye data were used to create two-dimensional (2D) illustrations of estimated RWT dye concentrations at each site and sampling interval. These were created with the Geostatistical Analyst extension, empirical Bayesian kriging (EBK) geoprocessing tool in ArcMap 10.7.1 (ESRI, Redlands, CA). Using *in situ* dye data, the EBK tool used an empirical transformation and exponential semi-variogram to predict RWT dye concentrations at unsampled locations within each plot.

2.1.2.3 Herbicide concentration sampling and data analysis

Concurrent with the RWT dye *in-situ* sampling, herbicide residue samples were collected just below the water's surface at each sampling point. These residue samples were collected by holding a syringe at elbow depth ~30 cm and retracting the plunger to pull in water at this depth. The syringe was pulled from the water column, any air bubbles were removed, and the total volume was adjusted to 20 ml. The 20 ml sample was then injected into pre-acidified and labeled amber glass bottles. Samples were collected at 1, 6, and 24 HAT and maintained on ice or refrigerated until processing and analysis.

Florpyrauxifen-benzyl is capable of converting via hydrolysis to its acid form, florpyrauxifen, which has herbicidal activity although at lower levels than that of florpyrauxifen-benzyl (Netherland and Richardson 2016; Richardson et al. 2016; Miller and Norsworthy 2018) As such, water samples were analyzed for concentrations of florpyrauxifen-benzyl and florpyrauxifen utilizing dual analytical runs of high performance liquid chromatography (HPLC) with UV detection reporting to 1 µg L. Individual analytical runs using different columns, mobile phase concentrations, wavelengths, and HPLC oven temperatures were utilized to quantify concentrations of both compounds. A minimum 6-point calibration was utilized for both molecules with linearity of calibration curves

characterized by a R^2 greater than 0.999. Each analytical sequence utilized an analytical blank injection, followed by calibration standards and laboratory fortified blanks (LFBs) at three different concentrations. LFBs were run between every 10 samples and were within 20% of the target concentration. Following analysis, herbicide concentration data were used to create 2D illustrations of florpyrauxifen-benzyl concentrations at each site and sampling interval using the same methods as those for RWT dye.

2.2 Aquatic plant surveys

2.2.1 Point intercept vegetation surveys

To evaluate herbicide efficacy, pre- and post-treatment vegetation surveys were conducted in treated and non-treated plots. Pre-treatment vegetation surveys were conducted 10-13 July 2020 and 7 August 2020. Due to unfavorable weather conditions, the originally scheduled treatment date had to be postponed due to abnormally high-water flows through the reservoir. The delay in timing between the original pre-treatment survey (July) and the rescheduled treatment date prompted the follow-up August pre-treatment survey. Post-treatment vegetation surveys were conducted 18 September 2020, four weeks after herbicide application. Within each plot, 25 points were sampled (Figure 4) following the methodology of Madsen (1999) with the modification to include species relative abundance. Vegetation assemblage and species relative abundance were assessed at each point by deploying a weighted double-sided rake and documenting the species present. Species relative abundance was rated on a 0 to 4 scale based off rake coverage with 0 = no plants, 1 = 1-25% coverage; 2 = 25-50% coverage 3 = 50-75% coverage, and 4 = 75-100% coverage.

Species relative abundance pre-treatment versus post-treatment was analyzed individually for each species within each plot using a T-test ($\alpha=0.05$). Due to a low relative abundance of several co-occurring species, statistical analysis was only performed on species that documented an average relative abundance greater than or equal to 0.20 at one of the two survey periods. Species frequency of occurrence was also calculated by averaging plant species presence across all sampling points within the plot and multiplied by 100. Data are presented as species percent frequency of occurrence.

Figure 4. Sampling points within each plot for pre- and post-treatment aquatic vegetation surveys at Roanoke Rapids Lake, NC.



3 Results and Discussion

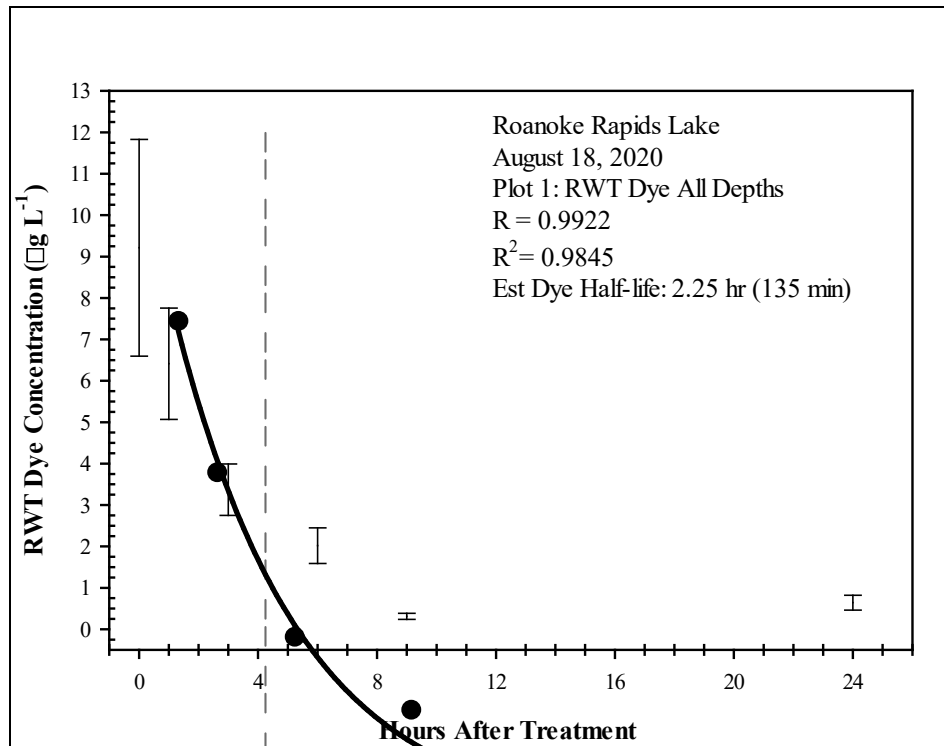
3.1 Water exchange and herbicide residue study

3.1.1 Plot 1

3.1.1.1 Rhodamine WT dye

Dye measurements collected immediately following treatment (0hr) indicated a whole plot RWT dye concentration of $9.2 \mu\text{g L}^{-1}$, throughout which was close to the target concentration of $10 \mu\text{g L}^{-1}$. The estimated RWT dye half-life within Plot 1 was very short (2 hr 15 min) and characteristic of a high-water exchange treatment site (Figure 5).

Figure 5. Mean RWT dye concentration (\pm SE), averaged across all sampling depths, at each sampling period at Roanoke Rapids Lake, Plot 1. Dye dissipation and half-life were estimated using non-linear regression [Exponential decay ($f = a \cdot \exp(-b \cdot x)$)].



Although the dye was injected directly into the water via drop hoses, it failed to distribute uniformly throughout the water column. The dense hydrilla growth throughout the plot was likely a contributor to the reduced vertical and horizontal mixing of applied dye. Sampling periods from 0 hr to 6 hr, indicated $\geq 52\%$ of the measured dye remained in the upper portion of the water column and no more than 21% was recorded at the

bottom depth (Figure 6). The average RWT dye concentration at each sampling depth also indicated limited distribution of dye throughout the water column. Dye concentration measurements at the bottom sampling depth did not exceed $1.6 \mu\text{g L}^{-1}$ (1 hr) and remained relatively constant across all sampling periods. In contrast, average dye concentrations recorded at the mid- and surface depths steadily decreased over time (Figure 7).

Figure 6. Water column distribution of RWT dye at Plot 1 Roanoke Rapids Lake, NC August 2020 (A) represents measurements within the plot and (B) represents measurements outside of the plot.

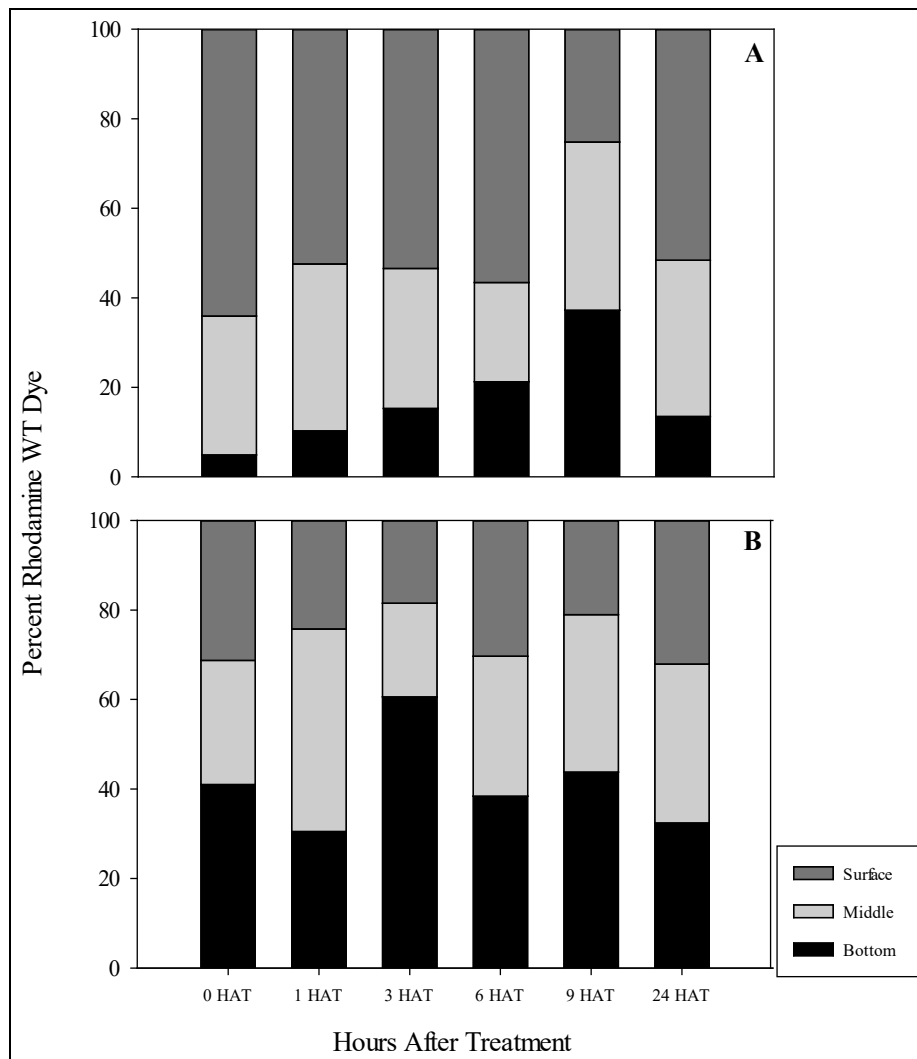
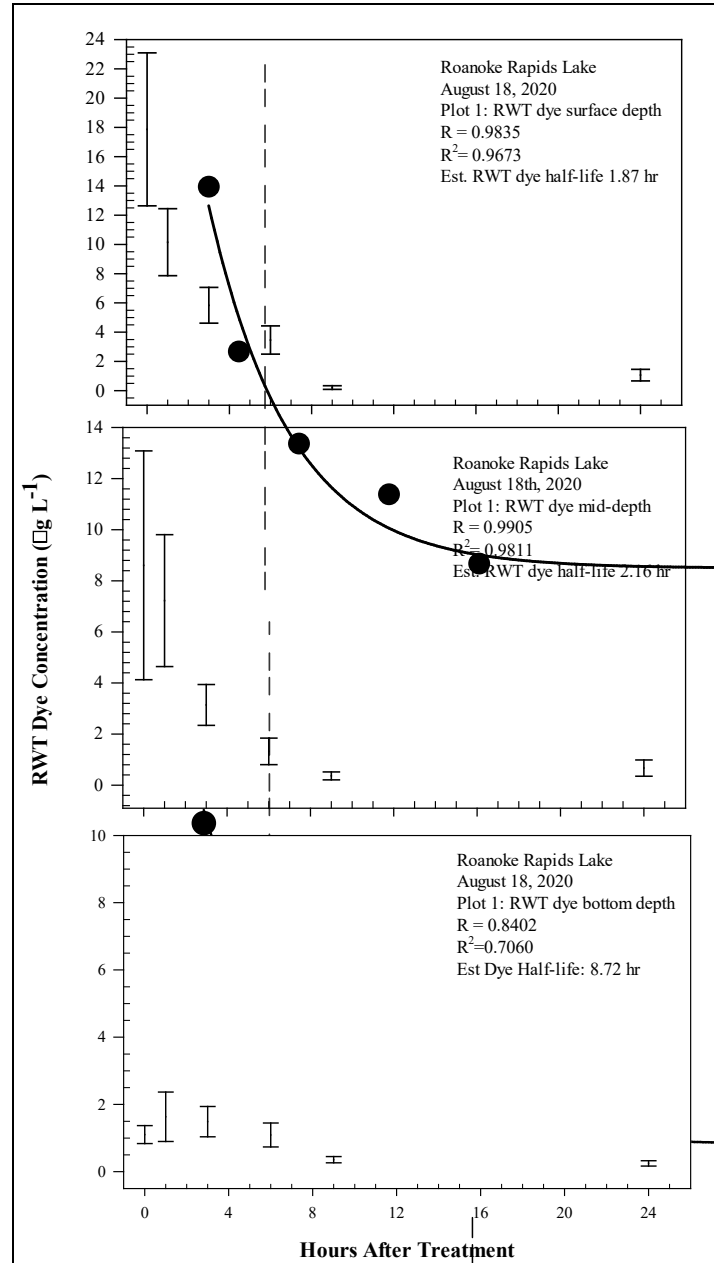


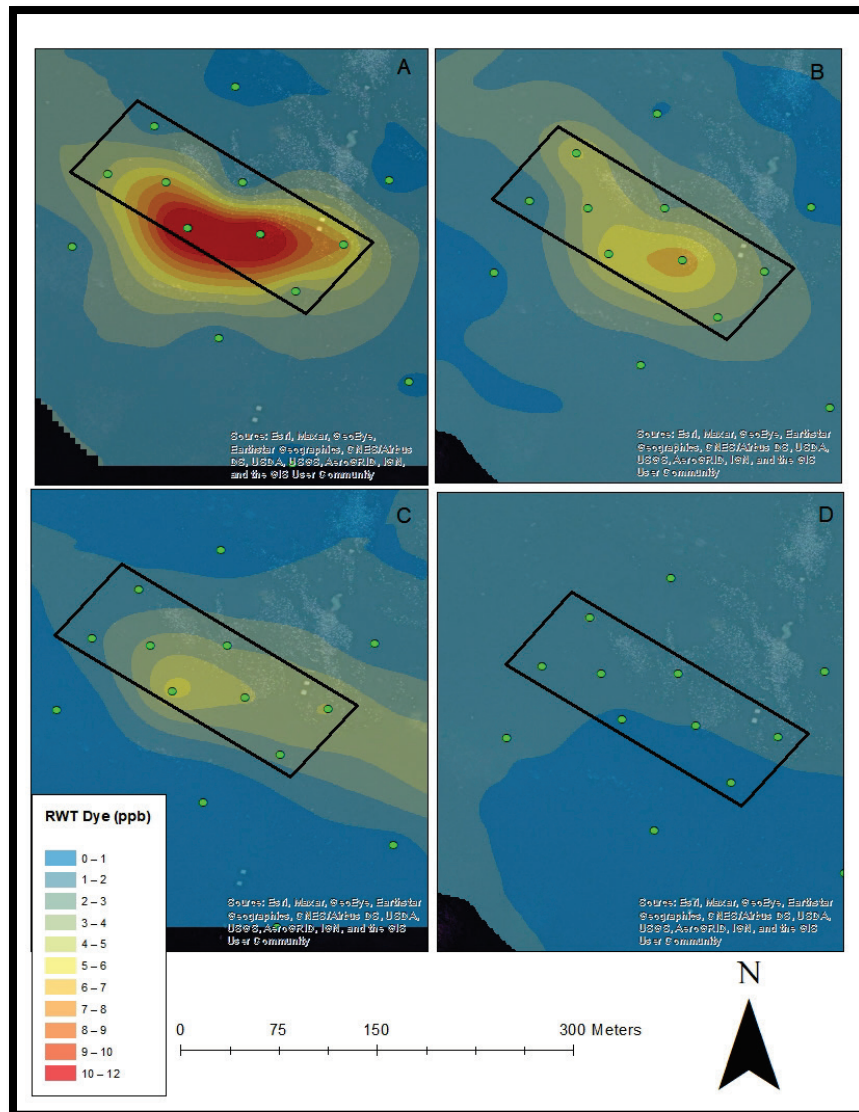
Figure 7. Mean RWT dye concentration (\pm SE) at Plot 1, recorded at the surface (top), Mid- (middle), and bottom depth (bottom) for each sampling period at Roanoke Rapids Lake. Dye dissipation and half-life were estimated using non-linear regression [Exponential decay ($f = a \cdot \exp(-b \cdot x)$)].



A time series representation of the estimated RWT dye concentrations for Plot 1 at 0, 1, 3, and 6 hr after treatment is shown in Figure 8. The highest dye concentrations ($\sim 10 \mu\text{g L}^{-1}$) at 0hr are at the plot mid-section and extend towards the southeastern plot edge. Dye concentrations 1 hr after treatment became more evenly distributed with the highest measured concentrations (~ 6.1 - $7.0 \mu\text{g L}^{-1}$) in the lower mid-section of the plot and by

3 hr after treatment dye concentrations did not exceed $\sim 6.0 \mu\text{g L}^{-1}$. The EBK model for the 3 hr after treatment data illustrates the primary dissipation direction of dye to be downstream of the plot and by 6 hr after treatment RWT dye concentrations were less than $2.0 \mu\text{g L}^{-1}$ across the internal and external plot sampling area.

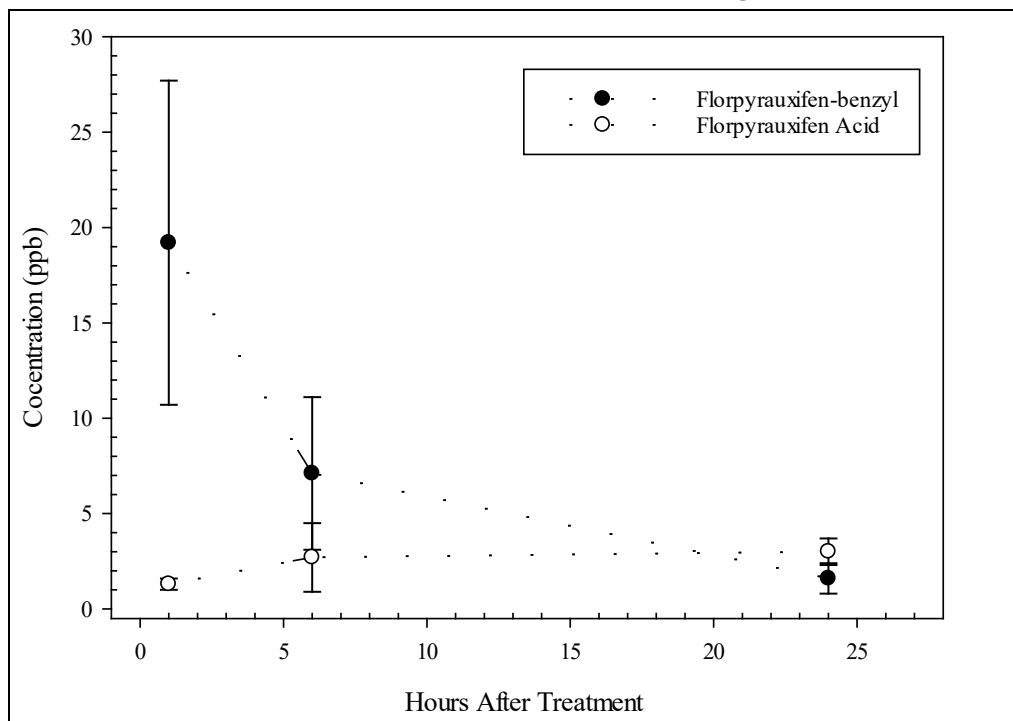
Figure 8. Estimated rhodamine wt dye concentrations at Plot 1 Roanoke Rapids Lake, NC A) 0 HAT, B) 1 HAT, C) 3 HAT, and D) 6 HAT. These data were estimated using Empirical Bayesian Kriging modeling of mean *in-situ* RWT dye measurements recorded at the top, middle, and bottom of the water column.



3.1.1.2 Herbicide residues

Aqueous herbicide levels in Plot 1 indicated rapid dissipation of herbicide out of the treatment site. Samples collected 1 hr after treatment estimated a whole plot florpyrauxifen-benzyl concentration of $19.2 \mu\text{g L}^{-1}$, which is roughly 40% of the applied target rate of $48 \mu\text{g L}^{-1}$. In addition, 1 hr samples collected at external points 1, 17, and 18 detected florpyrauxifen-benzyl concentrations of 7.2, 12.6, and $8.1 \mu\text{g L}^{-1}$ respectively, indicating rapid downstream dissipation of the applied herbicide from the treatment area. Florpyrauxifen, the parent acid of florpyrauxifen-benzyl was also detected at the 1 hr sampling period with an average surface concentration of $1.3 \mu\text{g L}^{-1}$. The 6 hr sampling interval resulted in lower levels of florpyrauxifen-benzyl and increased concentrations of florpyrauxifen, with average in plot surface concentrations of 7.1 and $2.7 \mu\text{g L}^{-1}$ respectively and by 24 hr after treatment, detectable levels of the parent acid florpyrauxifen had exceeded those of florpyrauxifen-benzyl (Figure 9).

Figure 9. Mean concentration of florpyrauxifen-benzyl and florpyrauxifen acid detected within Plot 1, Roanoke Rapids, NC August 2020.



Similar to the RWT dye measurements, the detected herbicide concentrations varied within the plot. Two of the eight samples collected 1 hr after treatment indicated non-detectable levels of florpyrauxifen-benzyl while residues at the additional sampling points varied from 1.3 to

54.6 $\mu\text{g L}^{-1}$. The kriging model at 1 hr illustrates higher concentrations of florpyrauxifen-benzyl were detected along the center and southeastern portions of the plot (Figure 10). Over-head imagery following the application (Figure 11) clearly indicates increased concentrations of dye in certain portions of the plot relative to others and coincides with the sampling points that resulted in higher detected levels of florpyrauxifen-benzyl residues.

Figure 10. Estimated florpyrauxifen-benzyl concentrations at Plot 1 Roanoke Rapids Lake, NC at 1 HAT. These data were estimated using Empirical Bayesian Kriging modeling of *in-situ* aqueous herbicide residues at the surface only.

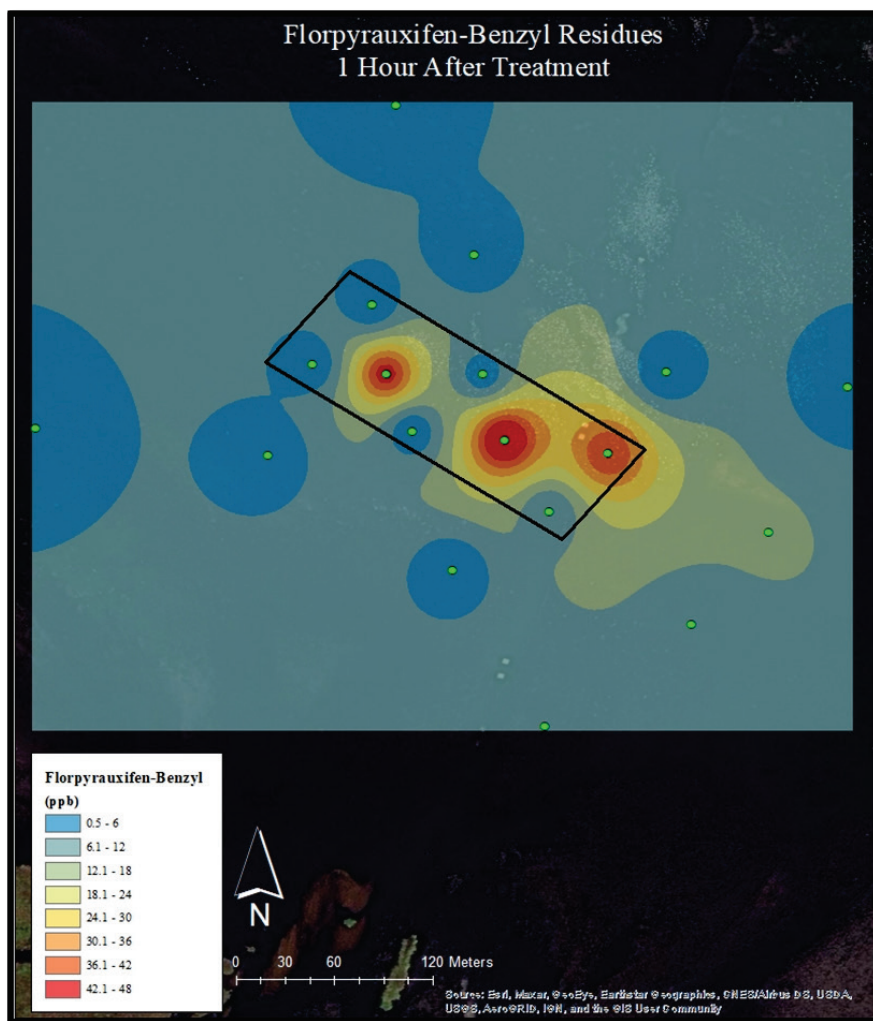


Figure 11. RWT dye and herbicide immediately after application (0 HAT) at Plot 1 Roanoke Rapids Lake, August 2020.



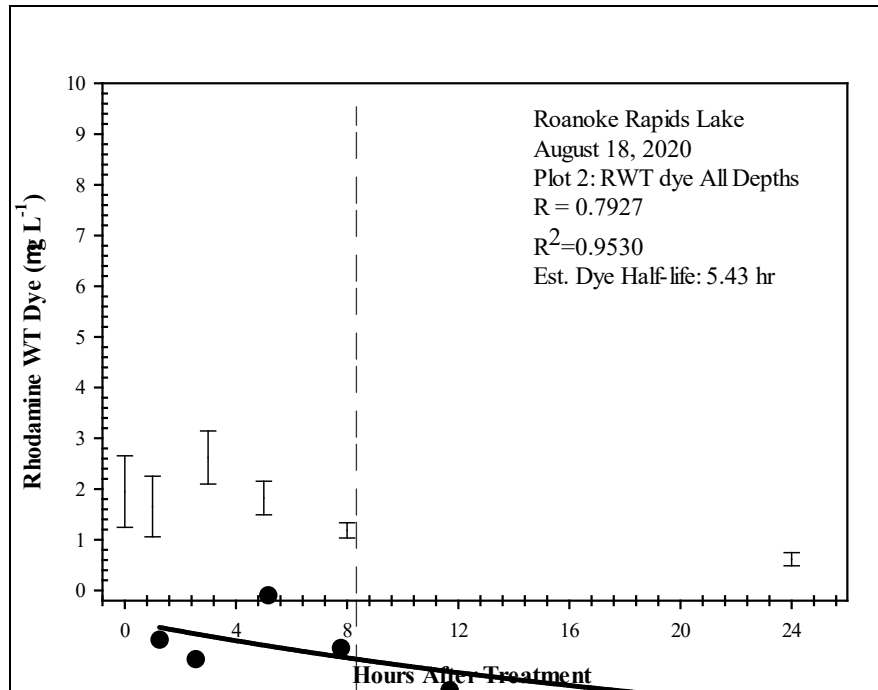
3.1.2 Plot 2

3.1.2.1 Rhodamine WT dye

Measured dye concentrations throughout the water column in Plot 2 at 0 and 1 hr sampling, indicated a whole plot dye concentration of 1.9 and 1.6 $\mu\text{g L}^{-1}$, respectively. Both were well below the target rate of 10 $\mu\text{g L}^{-1}$. The whole plot concentration at 3 hr was 2.6 $\mu\text{g L}^{-1}$ (Figure 12) and like RWT dye concentrations in Plot 1 at the 3 hr sampling period.

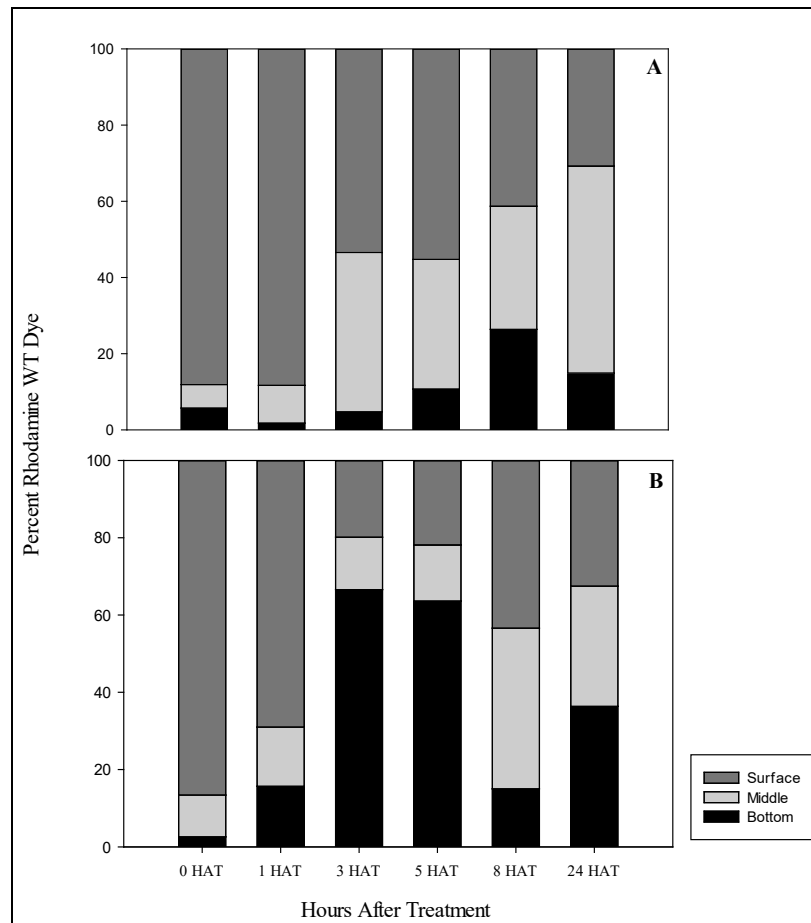
The low dye concentration recorded at the 0 hr and 1 hr sampling period is likely a result of the applied dye not being able to thoroughly mix horizontally or vertically due to the abundance of hydrilla occurring throughout the site. No dye was measured at concentrations greater than 1 $\mu\text{g L}^{-1}$ at three of eight and two of eight internal dye-sampling points at the 0 hr and 1 hr sampling periods, respectively. This likely resulted in underestimated whole plot dye concentration at the 0 hr and 1 hr sampling periods and an overestimated dye half-life of 5.4 hr.

Figure 12. Mean RWT dye concentration (\pm SE), averaged across all sampling depths, at each sampling period at Roanoke Rapids Lake, Plot 2. Dye dissipation and half-life were estimated using non-linear regression [Exponential decay ($f = a \cdot \exp(-b \cdot x)$)].



Dye data indicated poor vertical mixing of dye throughout the water column. Greater than 85% of measured dye was recorded at the surface depth at 0 and 1 hr after treatment (Figure 13). Measured concentrations at 3 and 5 hr indicated better distribution of dye throughout the water column. Dye readings at 3 hr indicated 53 and 42% of measured dye being recorded at the surface and mid-depth levels, respectively. Dye measurements at the surface and mid-depth level contributed to 55 and 34% of the dye concentration respectively at the 5 hr after treatment sampling period. Less than 10% of the measured dye concentration was recorded at the bottom depth for the 0, 1, 3, and 5 hr sampling intervals.

Figure 13. Water column distribution of RWT dye at Plot 2 Roanoke Rapids Lake, NC August 2020 (A) represents measurements within the plot and (B) represents measurements immediately outside of the plot.



Average RWT dye recorded at the surface depth throughout the sampling period indicated an estimated dye half-life of 5.8 hr (Figure 14). It should be noted however, that multiple sampling points at the 0 and 1 hr sampling period did not indicate a dye reading greater than $1.0 \mu\text{g L}^{-1}$, which was likely due the variables relative to horizontal and vertical dye mixing. Thus, the exponential decay regression analysis may have overestimated the half-life of RWT dye at the surface depth. Mid- and bottom depth dye concentrations were highly variable with limited concentrations of dye being detected across sampling periods; thus, an estimated dye half-life was unable to be calculated at these depth ranges. Due to the low percentage of dye recorded at the mid- and bottom sampling depths, a time series map illustrating the approximate dissipation of RWT dye from the upper portion of the water column at 0, 1, 3, and 5 hr sampling periods is provided in Figure 15.

Figure 14. Mean RWT dye concentration (\pm SE) at Plot 2, recorded at the surface (top), Mid- (middle), and bottom depth (bottom) for each sampling period at Roanoke Rapids Lake. Dye dissipation and half-life were estimated using non-linear regression [Exponential decay ($f = a \cdot \exp(-b \cdot x)$)].

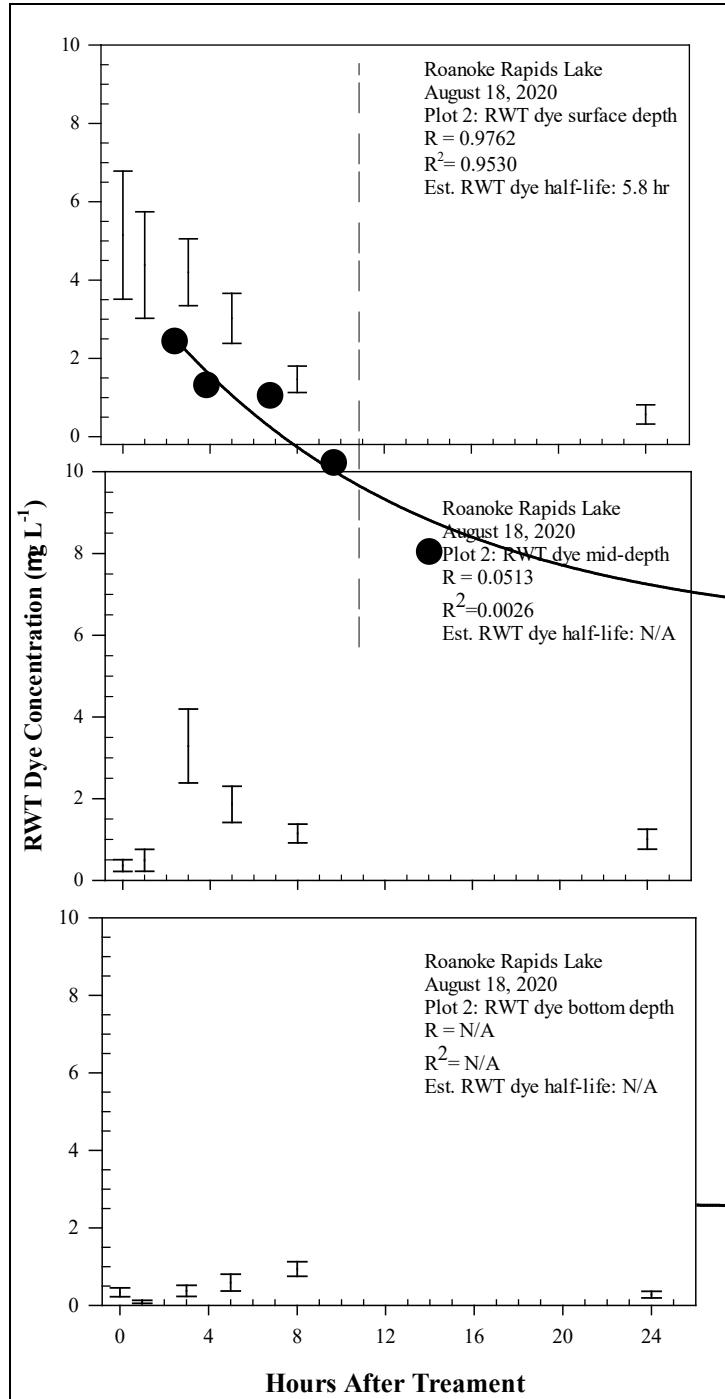
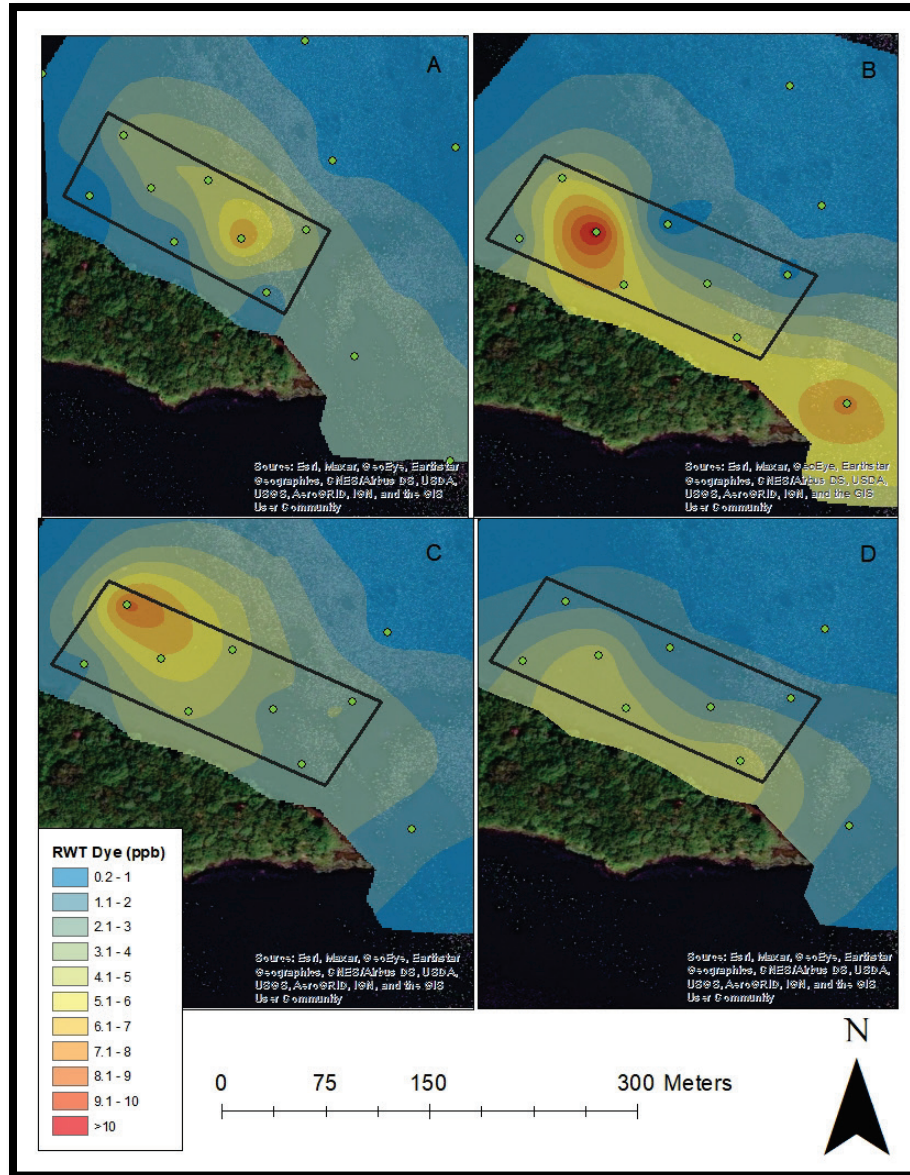


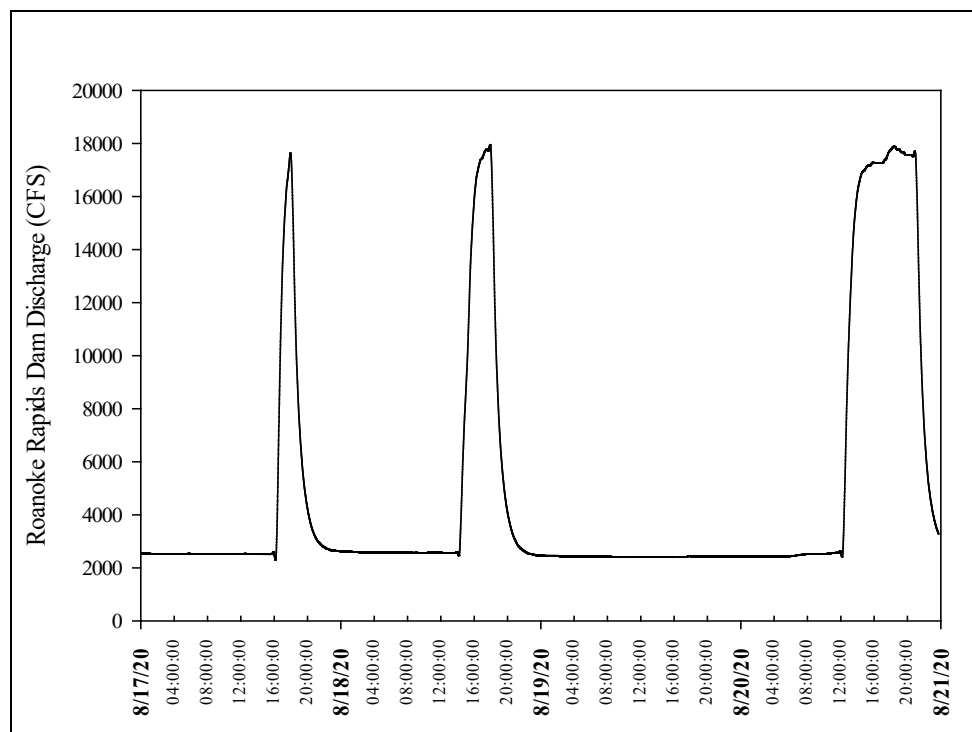
Figure 15. Estimated rhodamine wt dye concentrations at the surface sampling depth in Plot 2 Roanoke Rapids Lake, NC at 0 (A), 1 (B), 3 (C), and 5 HAT (D). These data were estimated using Empirical Bayesian Kriging modeling of *in-situ* RWT dye measurements recorded at the surface depth.



Several factors likely contributed to the rapid dissipation of RWT dye from each plot. The excessive hydrilla growth occurring within each plot was potentially a major contributor to the limited horizontal and vertical mixing of the applied dye. Consequently, this allowed for concentrations of dye to remain at or near the water surface and be more susceptible to wind driven surface currents. Light northwesterly winds (1.1 kmph) were reported during and prior to treatment but increased to 8-13 kmph at 1 to 2 hr post treatment, potentially contributing to dye movement out of each

plot. Plot size, proximity to the river channel, and dam operations may have also influenced dye dissipation. Roanoke Rapids Dam daily discharge during the week of treatment is shown in Figure 16. Dam discharge is low, 2,500 to 3,000 cubic feet per second (CFS), before increasing substantially to 18,000 CFS around 14:00 that coincides with the 3 to 6 hr dye sampling periods.

Figure 16. Roanoke Rapids Dam discharge (cubic feet per second) during the week of treatment.



3.1.2.2 Herbicide residues

Measured aqueous herbicide levels indicated a rapid movement of the applied herbicide out of treatment Plot 2. Samples collected 1 hr after treatment indicated a mean whole plot florpyrauxifen-benzyl concentration of $25.6 \mu\text{g L}^{-1}$ or recovery of approximately 54% of the applied target rate of $48 \mu\text{g L}^{-1}$. Movement of the herbicide downstream was the primary dissipation direction as indicated by the levels of florpyrauxifen-benzyl residues detected 1 HAT at the external sampling points shown in Figure 17. Samples collected at the 5 hr interval resulted in substantially less recovery of aqueous florpyrauxifen-benzyl residues with an estimated concentration of $4.2 \mu\text{g L}^{-1}$ at the surface sampling depth. In contrast, recovery of the parent acid florpyrauxifen increased from a non-detectable concentration at 1 hr to $2.4 \mu\text{g L}^{-1}$ at 5 hr

(Figure 18). Samples collected 24 HAT resulted in increased levels florpyrauxifen and corresponds to the 24 hr residue samples for Plot 1.

Figure 17. Estimated florpyrauxifen-benzyl concentrations at Plot 2 Roanoke Rapids Lake, NC at 1 HAT. These data were estimated using Empirical Bayesian Kriging modeling of *in-situ* aqueous herbicide residues at the surface only.

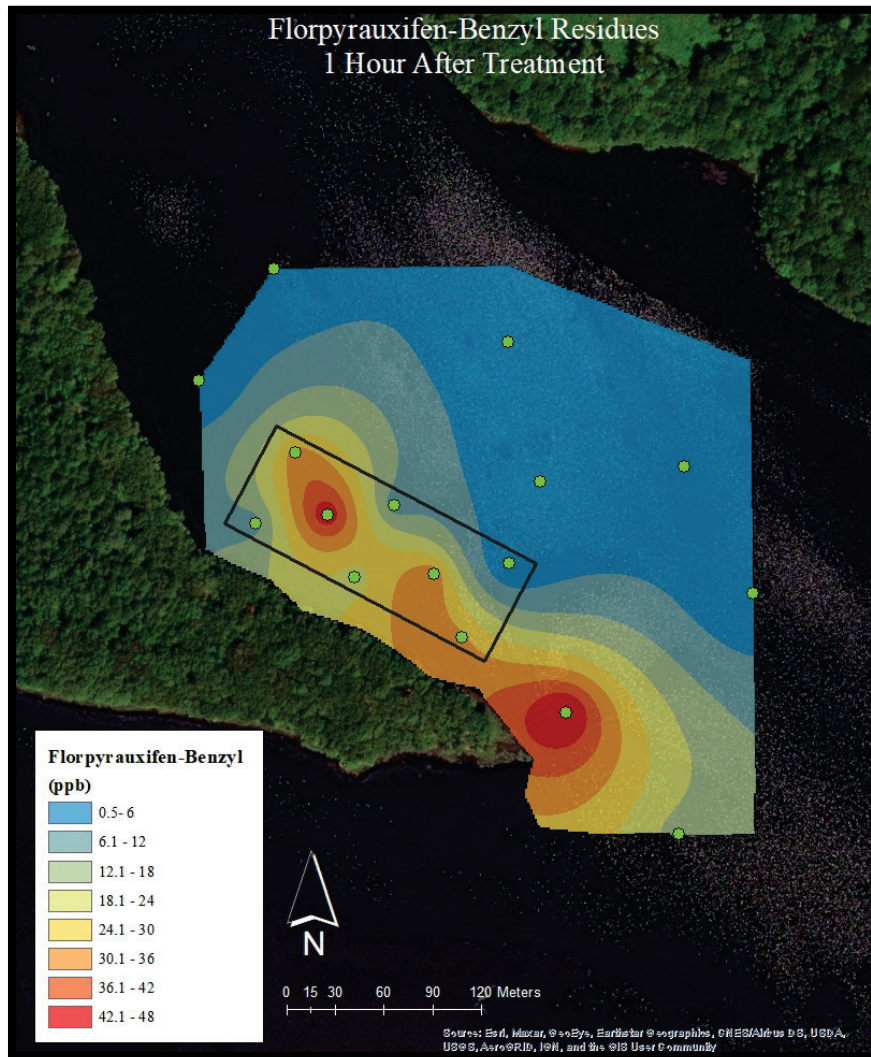
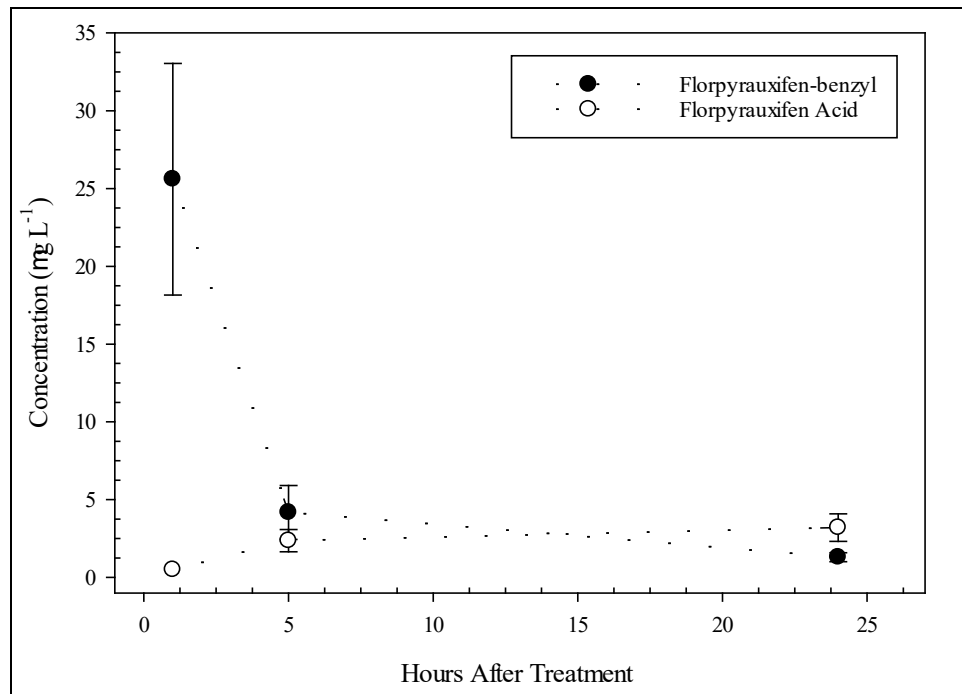


Figure 18. Mean florpyrauxifen-benzyl and florpyrauxifen acid concentrations detected within Plot 2, Roanoke Rapids, NC August 2020.



3.2 Aquatic plant surveys

Pre-treatment aquatic plant surveys indicated consistent 100% occurrence of hydrilla in all four plots (Table 2). Hydrilla was also the most abundant species with relative abundance ratings ranging from 1.92 to 2.80 (Table 3). No other species documented a mean relative abundance greater than 0.68 during pre-or post-treatment surveys. Southern naiad was the second most frequently occurring species and was reported in all four plots. Pre-treatment frequency of occurrence varied from 52% in Plot 3 to 68% in Plot 2. Mean relative abundance ranged from 0.52 to 0.68 indicating plants were sparse in all four plots. EWM was the only other species reported in all four plots and coontail occurrence was 20 and 24% for Plots 2 and 4, respectively, at pre-treatment.

Vegetation surveys 4 WAT in treatment plots did not show a decrease in the frequency of hydrilla occurrence and all plots surveyed reported hydrilla frequency of occurrence to be $\geq 92\%$. In addition, hydrilla relative abundance increased significantly in the treatment and reference plots (Table 2). Southern naiad occurrence in treatment Plot 2 was stable following treatment but decreased from 60 to 32% in treatment Plot 1. An increase or decrease in relative abundance for additional co-occurring species was not indicated in either of the two treatment plots. Coontail

relative abundance did decrease from 0.24 pre-treatment to 0.04 post-treatment in treatment Plot 2; however, a similar response was documented in reference Plot 4 where coontail abundance decreased from 0.24 pre-treatment to 0.00 4 WAT (Table 2). Overall, the frequency and abundance of occurrence for co-occurring species was sparse at all survey periods and slightly decreased over time. This was likely a result of the abundance of hydrilla initially present and significantly increasing over the monitoring period.

Table 2. Percent frequency of occurrence of plant species reported during pre- and post-treatment aquatic vegetation surveys.

	Frequency of Occurrence						
	<i>H. verticillata</i>	<i>M. spicatum</i>	<i>N. minor</i>	<i>C. caroliniana</i>	<i>C. demersum</i>	<i>Chara/Nitella</i>	<i>N. guadalupensis</i>
Plot 1	-----%						
Pre-Treatment	100	16	0	0	4	0	60
4 Weeks Post-treatment	100	4	0	0	0	0	32
Plot 2							
Pre-Treatment	100	8	0	8	20	0	68
4 Weeks Post-treatment	100	0	0	4	4	4	64
Plot 3							
Pre-Treatment	100	4	0	8	0	0	52
4 Weeks Post-treatment	100	0	0	0	0	0	48
Plot 4							
Pre-Treatment	100	4	0	0	24	0	64
4 Weeks Post-treatment	92	4	4	0	0	0	56

Table 3. Mean relative abundance (\pm SE) of species reported during pre- and post-treatment aquatic vegetation surveys at Roanoke Rapids Lake, NC. An asterisk indicates a significant difference in relative abundance between pre-and post-treatment surveys according to a T-test (0.05). Only species that documented a mean abundance rating ≥ 0.20 were included in the analysis and plant species within each plot were analyzed separately.

		Relative Abundance						
		<i>H. verticillata</i>	<i>M. spicatum</i>	<i>N. minor</i>	<i>C. caroliniana</i>	<i>C. demersum</i>	<i>Chara/Nitella</i>	<i>N. guadalupensis</i>
Plot 1								
Pre-Treatment		2.68 \pm 0.16	0.20 \pm 0.10	0.00 \pm 0.00	0.00 \pm 0.00	0.04 \pm 0.04	0.00 \pm 0.00	0.60 \pm 0.10
4 Weeks Post-treatment		3.80 \pm 0.08*	0.04 \pm 0.04	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.10	0.00 \pm 0.00	0.36 \pm 0.11
Plot 2								
Pre-Treatment		2.80 \pm 0.16	0.08 \pm 0.06	0.00 \pm 0.00	0.08 \pm 0.06	0.24 \pm 0.10	0.00 \pm 0.00	0.68 \pm 0.10
4 Weeks Post-treatment		3.88 \pm 0.00*	0.00 \pm 0.00	0.00 \pm 0.00	0.04 \pm 0.04	0.04 \pm 0.04	0.04 \pm 0.04	0.72 \pm 0.12
Plot 3								
Pre-Treatment		2.52 \pm 0.15	0.04 \pm 0.04	0.00 \pm 0.00	0.08 \pm 0.06	0.00 \pm 0.00	0.00 \pm 0.00	0.52 \pm 0.10
4 Weeks Post-treatment		3.80 \pm 0.08*	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.64 \pm 0.15
Plot 4								
Pre-Treatment		1.92 \pm 0.22	0.04 \pm 0.04	0.00 \pm 0.00	0.00 \pm 0.00	0.24 \pm 0.09	0.00 \pm 0.00	0.68 \pm 0.10
4 Weeks Post-treatment		3.40 \pm 0.24*	0.04 \pm 0.04	0.04 \pm 0.04	0.00 \pm 0.00	0.00 \pm 0.00*	0.00 \pm 0.00	0.76 \pm 0.17

Despite the rapid dissipation of florpyrauxifen-benzyl, CETs were sufficient to cause herbicide symptoms to hydrilla by 3 DAT. Symptoms consisted of epinasty and curling of leaflets, swollen nodes, and bleached brittle apical meristems. Although plants displayed rapid initial injury, hydrilla recovery and new growth was documented in both treatment plots

by 4 WAT. These results are consistent with Netherland and Richardson (2016) that reported auxin-like symptoms 1 to 2 DAT following static exposures to $>9 \mu\text{g L}^{-1}$ and Mudge et al. (2021a) that documented bleaching of dioecious hydrilla apical tips at 3 DAT and recovery 2 to 4 weeks after exposure to florpyrauxifen-benzyl at 12 to $36 \mu\text{g L}^{-1}$ for 12 to 48 hr.

Herbicide symptoms were also observed for EWM, coontail, and southern naiad 3 DAT. EWM has been shown to be susceptible to low use rates and short exposures of florpyrauxifen-benzyl (Netherland and Richardson 2016; Beets et al. 2019; Mudge et al. 2021ab). Small-scale research has reported initial coontail injury from florpyrauxifen-benzyl (Mudge et al. 2021b; however, given the low occurrence and abundance of these species within the reference and treatment plots, it is unclear if changes in abundance and frequency were a direct result of the herbicide treatment.

4 Conclusion and Recommendations

4.1 Conclusions

The following conclusions were determined based off the evaluations conducted at Roanoke Rapids Lake, NC in 2020.

- Rhodamine WT dye data indicated high water exchange was occurring in small treatment plots (< 1.2 ha).
- When applied to small plots, the CETs of florypyrauxifen-benzyl were not sufficient to achieve adequate control of monoecious hydrilla by 4 WAT.
- A more prolonged exposure of florypyrauxifen-benzyl is needed to achieve effective control of monoecious hydrilla.

4.2 Recommendations

The following recommendations are made based on the evaluations documented in this report.

- Because of complex water exchange processes in RRL, treatment plots should be increased to > 4 hectares/ 10 acres.
- To reduce the impact of hydraulic complexity and improve herbicide efficacy, treatments should coincide with minimal reservoir discharge events to extend herbicide CET relationships.
- Robust data sets linking water exchange processes, aqueous florypyrauxifen-benzyl levels, and treatment efficacy should continue to be developed.
- Evaluations should be conducted earlier in the growing season, on young, actively growing plants, to discern potential differences in efficacy due to treatment timing and phenology.
- More information on herbicide concentration and exposure time relationships for monoecious hydrilla should be developed in growth chamber and mesocosm settings to improve species selective management of monoecious hydrilla in hydrodynamic reservoirs.

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Appendix: Images of Hydrilla Occurrence and RWT Dye and Florpyauxifen-benzyl being applied at Roanoke Rapids Lake, NC

Figure 19. Hydrilla occurrence at Plot 1 prior to treatment.



Figure 20. Hydrilla documenting florpyauxifen-benzyl injury 3 days after treatment.

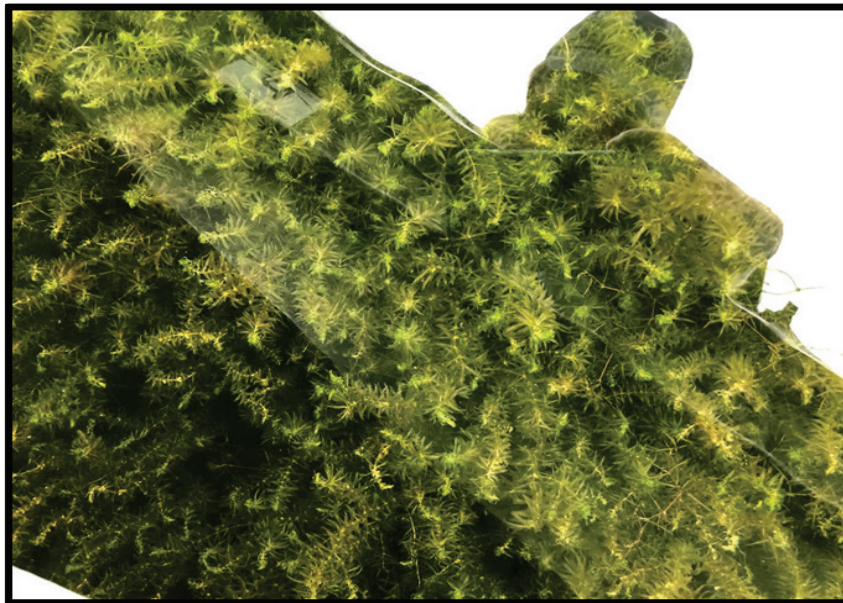


Figure 21. Hydrilla occurring in Plot 1 and RWT dye plus florpyrauxifen benzyl being applied August 2020, Roanoke Rapids Lake, NC.



Figure 22. RWT dye and herbicide immediately after application (0 HAT) at Plot 2 Roanoke Rapids Lake, NC August 2020.



Figure 23. RWT dye and herbicide 1 hr after treatment at Plot 2 Roanoke Rapids Lake, NC August 2020.



REPORT DOCUMENTATION PAGE

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14. ABSTRACT Four demonstration plots were selected at Roanoke Rapids Lake, NC to evaluate water exchange and aqueous herbicide residues in stands of submersed aquatic vegetation (SAV) following treatment with rhodamine wt dye and florpyrauxifen-benzyl to control monoecious hydrilla. Florpyrauxifen-benzyl (Procellacor™ SC) was applied in combination with Rhodamine WT (RWT) at two of the plots. Dye measurements and herbicide residue samples were collected at specific time intervals to draw comparisons between herbicide and RWT dye dissipation. The two additional plots served as reference plots to the treatment plots. Pre- and post-treatment vegetation surveys were conducted to evaluate monoecious hydrilla control and non-target species response. RWT dye and herbicide residue data indicated rapid water exchange was occurring with each treatment plot. As a result, florpyrauxifen-benzyl concentration and exposure times (CETs) towards monoecious hydrilla were not sufficient to achieve adequate control by 4 weeks after treatment (WAT). To reduce the impact of hydraulic complexity and improve herbicide efficacy, treatments should coincide with minimal reservoir discharge events to extend herbicide CET relationships. Evaluations of florpyrauxifen-benzyl on late season, mature plants may have impacted herbicide efficacy. Evaluations should be conducted earlier in the growing season, on young, actively growing plants, to discern potential differences in efficacy due to treatment timing and phenology. More information on herbicide concentration and exposure time relationships for monoecious hydrilla should be developed in growth chamber and mesocosm settings to improve species selective management of monoecious hydrilla in hydrodynamic reservoirs.						
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