

Observations of a submersed field application of florypyrauxifen-benzyl suppressing hydrilla in a small lake in central Florida

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ABSTRACT

In August 2018, a submersed injection of florypyrauxifen-benzyl (FPB) was administered at $48 \mu\text{g L}^{-1}$ to suppress a dominant infestation of dioecious hydrilla [*Hydrilla verticillata* (L.f.) Royle] in a 20-ha littoral section of Fish Lake (92 ha) in Osceola County, FL. This treatment was extensively monitored out to 289 d after treatment (DAT; May 2019) to evaluate suppression efficacy on hydrilla and selectivity to common nontarget plant species. Prior to treatment, hydrilla was the most frequent and abundant species throughout the entire littoral zone, with an average biovolume (BV) of all submersed aquatic vegetation (SAV) of 64%. Dissipation of FPB in the treatment area was rapid and concentrations were nondetectable by 168 h after treatment. However, concentrations of its parent acid, florypyrauxifen, were detected out to 336 h after treatment. Regardless, BV in some treated areas was near zero for up to 133 d. By 289 DAT, average BV in treated areas increased to 52%. Conversely, BV in the nontreated littoral area steadily increased to 99% with monotypic hydrilla niches advancing from moderate to dense over 289 DAT. American eelgrass (*Vallisneria americana* Michx.), and spatterdock [*Nuphar advena* (Aiton) W. T. Aiton] were the most frequent SAV and emergent species, respectively, but still minor representatives of the plant community compared to hydrilla. While typical auxin-like symptoms were observed on spatterdock, there was no apparent herbicide suppression in relative abundance for either of these species compared to the nontreated littoral area. American lotus (*Nelumbo lutea* Willd.), and fragrant waterlily (*Nymphaea odorata* Ait.) were also observed with similar acute symptoms but again were only present in low frequencies and not distinguishable otherwise between treated and nontreated areas. The reduction in hydrilla abundance showed a positive response in aquatic plant community balance promoting diversity. This study supports further evaluation of FPB for selective management of hydrilla in aquatic plant community restoration.

Key words: injection, herbicide dissipation, non-target injury.

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INTRODUCTION

Hydrilla [*Hydrilla verticillata* (L.f.) Royle], commonly referred to as the “perfect aquatic weed,” is the most difficult to control and aggressive invasive plant species in the state of Florida (Langeland 1996). In 2019, hydrilla management efforts treated 9.7k ha allocating close to 60% of the entire Florida Fish and Wildlife Conservation Commission's (FWC's) invasive plant management budget (FWC 2019). Up until the 1980s, the aquatic herbicides copper, diquat, and endothall were commonly used for small, localized treatments of hydrilla (Langeland 1996). These herbicides were fast acting but required concentrations on the scale of milligrams per liter (mg L^{-1}) to be effective and only offered short-term control. The registration of fluridone (a slow-acting, systemic herbicide) for aquatic use in the mid-1980s offered a new standard for whole-lake treatments providing selective long-term hydrilla control at much lower concentrations $< 15 \mu\text{g L}^{-1}$. However, the overreliance on fluridone as a highly effective and economical treatment eventually selected for fluridone-resistant hydrilla (FRH) in the late 1990s (MacDonald et al 2001, Michel et al. 2004, Arias et al. 2005, Dayan and Netherland 2005, Richardson 2008).

Today, populations of FRH are distributed widely throughout peninsular Florida (Netherland and Jones 2015a). From 2008 to 2014, several additional herbicides were registered for aquatic use as tools to mitigate FRH and future selection pressure (Getsinger et al. 2008). Penoxsulam, bispyribac-sodium, flumioxazin, and imazamox have shown to selectively control hydrilla (Mudge 2007, Netherland 2011). However, these herbicides were not completely adopted by resource managers. Instead, public agencies reverted back to endothall by developing new use patterns for larger-scale, partial lake treatments. This move towards endothall was critical to maintaining hydrilla management statewide and has been the standard for the last two decades (Netherland and Jones 2012, 2015a). Consequently, overreliance on a single, effective mode of action, without rotation, has led to suspected endothall resistance in some hydrilla populations in central Florida (Berger et al. 2011, Giannotti et al. 2014). To date, there have been very few alternative herbicides adopted with similar exposure time requirements as endothall to make a practical rotation. Herbicide resistance of hydrilla to fluridone and potentially endothall has been the stimulus for coordinating pest management industries with regulatory agencies to strategically continue pursuing aquatic use registration of

alternative modes of action in order to sustain hydrilla management in Florida (Netherland et al. 2005, Getsinger et al. 2008).

In February 2018, floryrauxifen-benzyl (FPB) was registered for aquatic use under the trade name Procellacor^{®1} in both emulsifiable and soluble concentrates. This new herbicide is classified as having an auxin-mimic mode of action (Weed Science Society of America Group 4) in the arylpicolinate herbicide family and categorized as a reduced-risk pesticide by the U.S. Environmental Protection Agency (Epp et al. 2016). FPB's half-life in water is relatively short (1-2 d at pH values > 9) as it primarily degrades via photolysis (Taylor et al. 2014). Additionally, FPB is highly lipophilic and is believed to rapidly absorb into plant tissue (solubility = 0.011 mg L⁻¹, K_{oc} = 32,308) (Lewis et al. 2016). In mesocosms, FPB was shown to be highly active on hydrilla at 24 µg L⁻¹ (labeled for use at 19 to 48 µg L⁻¹) with results similar to endothall at 3,000 µg L⁻¹ following a 24-h exposure period (Beets and Netherland 2018).

Additionally, FPB has been shown to be selective towards desirable native submersed species American eelgrass (*Vallisneria americana* Michx.) and Illinois pondweed (*Potamogeton illinoensis* Morong) at concentrations up to 27 µg L⁻¹ with 24-h exposure (Beets et al. 2019). Based on FPB attributes of systemic activity at low use rates and short exposure times, including species selectivity, this newly registered herbicide mode of action offers a potential rotation complement to standard endothall lake treatments (Netherland and Richardson 2016, Richardson et al. 2016, Beets and Netherland 2018).

Here, we monitor an in-lake submersed FPB treatment at operational scale on a littoral infestation of dioecious hydrilla on a small, shallow lake in central Florida. The objectives of this study were to 1) measure FPB and parent acid floryrauxifen (FPA) dissipation of a partial lake treatment, 2) monitor acute nontarget sensitivity and influence on species composition, and 3) evaluate the consequent level of FBP suppression on hydrilla.

MATERIALS AND METHODS

Site and treatment description

Fish Lake is a small, eutrophic lake with an elliptical basin, approximately 92 ha, with an average depth of 1.5 m and maximum depth > 5 m, for a total estimated volume of 1.5 millions m³. It is located between East and West Lake Tohopekaliga in Osceola County, FL, (28°16'7"N; 81°20'39"W) and channeled to the north cove of West Lake Tohopekaliga by the Partin Canal. The immediate surrounding watershed consists of privately owned residential and agricultural properties. Hydrilla was first recorded in this lake in 1983 and was managed to low detectable levels with a dense population of triploid grass carp (*Ctenopharyngodon idella* Val.) introduced in 1994 (Hanlon et al. 2000). In 2016, a dramatic increase in hydrilla growth and dominance in the littoral zone was observed (FWC 2019). There were no prior records of herbicide treatments to control hydrilla on this lake.

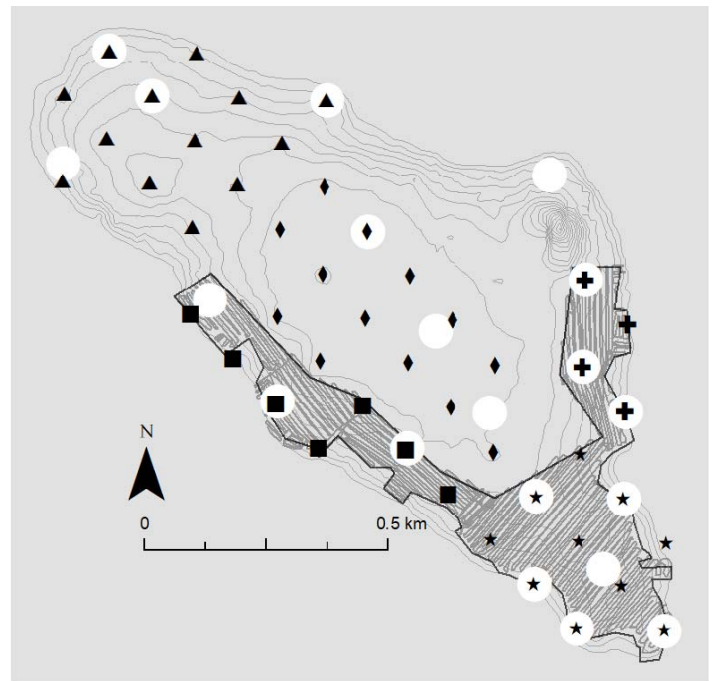


Figure 1. Map of Fish Lake with ~ 0.3-m contours. Black polygon denotes the treatment area. Water grab sample (large white dots) and vegetation point-intercept grids (black symbols) representing nontreated (triangle), basin (diamond), cove treatment (star), southwestern shoreline treatment (square), and eastern shoreline treatment (plus). Application tracks displayed within the treatment plot (grey).

On 14 August 2018, FPB was applied to a 20-ha littoral section up to the shoreline, at a target concentration of 48 µg L⁻¹ (maximum single application rate) (Figure 1). The average depth of the littoral section was 1.4 m with the distal edge of the littoral section reaching depths to 2.1 m. Plot widths varied from < 50 m to > 450 m from the adjacent shoreline, creating a small area to perimeter ratio of 47.2 m. The treatment was applied with trailing hoses submersed approximately 30 cm below the surface from two airboats running parallel transects spaced 6 m apart, traveling at 2 m s⁻¹, calibrated to deliver 187 L ha⁻¹ (Figure 1). No precipitation was recorded on the day of treatment, with southeasterly winds averaging 1.8 m s⁻¹ over the 24-h period and gusts up to 4.6 m s⁻¹ several hours after the treatment (data not shown). The average wind speed increased up to 3.2 m s⁻¹ over the next 48 h posttreatment and was again out of the southeast, with gusts up to 10.8 m s⁻¹. Mean air temperature for the day was 26.6 C with a midafternoon high of 31.5 C. Water temperature and pH measured 30.4 C and 7.4, respectively, in the treatment plot with a sonde⁵ at 50 cm below the surface during treatment.

Data collection

Water grab samples were collected at 12 georeferenced stations in the treatment area and eight sites in nontreated portions of the lake at 0, 6, 24, 48, 72, 168, and 336 h after treatment (HAT) to quantify FPB and FPA concentrations (Figure 1). Samples were collected at 30 cm below the surface into 60-ml amber glass vials that were acidified with

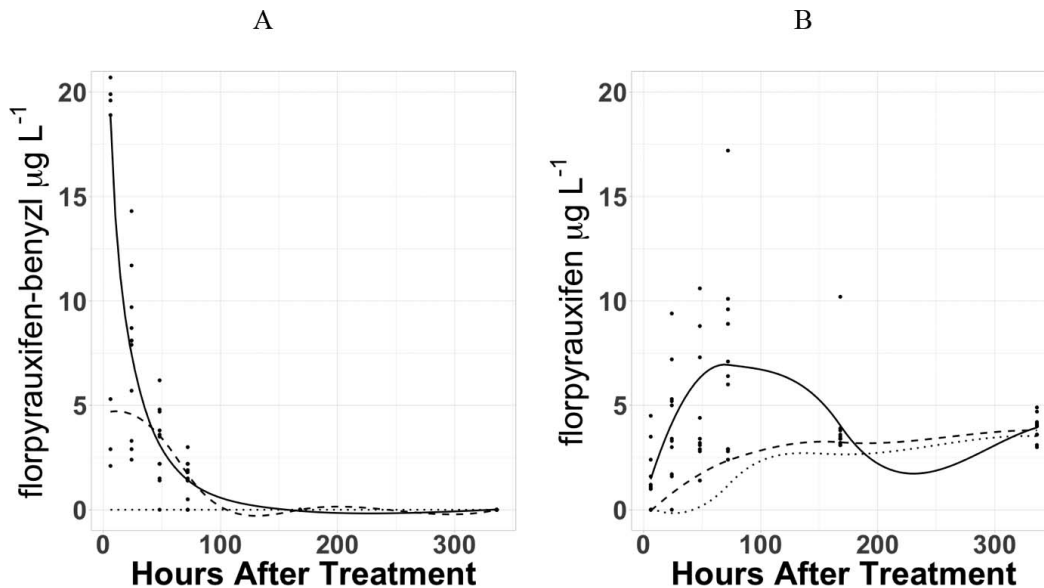


Figure 2. (A) Florpyrauxifen-benzyl and (B) florpyrauxifen concentrations from Fish Lake, Florida, in treated zones (solid), nontreated basin (dashed), and nontreated littoral areas (dotted) at 6, 24, 48, 72, 168, and 336 h after treatment with florpyrauxifen-benzyl.

50 μl of 23.6 M formic acid to prevent microbial degradation and preserved with methanol (5% of sample volume) to disassociate the active ingredient from the glass interior. Samples were placed on ice and shipped within 24 h for analytical service.⁶ Concentrations of FPB and parent acid were quantified with high-performance liquid chromatography against a known standard titration with a detection limit of 1 $\mu\text{g L}^{-1}$.

Aquatic plant surveys were performed by boat on the entire lake from August 2018 to May 2019, starting 1 wk prior to treatment and repeated on 27, 56, 93, 125, 155, 192, 247 and 289 d after treatment (DAT). Transects were spaced 127 m apart traversing the longitudinal axis at speeds $\leq 2.2 \text{ m s}^{-1}$.

The biovolume (BV) of submersed aquatic vegetation (SAV) was passively assessed from hydroacoustic data recorded with a Lowrance HDS7³ Gen 2 logger integrated with a 20° beam transducer (model: HST-WSBL) transmitting a 200-kHz signal at 10 pings s^{-1} and an external wide-area augmentation system global positioning system (GPS) antenna recording coordinate positions every second, consistent with manufacturer recommendations (Navico 2014). The GPS antenna (horizontal error $\pm 5 \text{ m}$) was mounted at the helm within 2 m of the transom-mounted transducer, which was also $\sim 30 \text{ cm}$ below the water surface. Scan log data files (.sl2) recorded on the logger were submitted to BioBase² for cloud-based data processing to create depth, aquatic plant height, and BV (i.e., plant height over depth) attributes for each georeferenced point in the transect survey. Geostatistical krigging algorithms in BioBase were used to create a spatial grid of equidistant points (14-m spacing) for interpolating BV values in the nonsampled spaces encompassed between neighboring transects.

Aquatic plant species identification, frequency, and abundance were determined by a point-intercept grid method deployed simultaneously with the hydroacoustic

surveys as described in previous studies (Madsen 1999, Hauxwell 2010, Netherland and Jones 2015b, Valley et al. 2015). The equidistant points (i.e., 127 m apart, $n = 58$) were made congruent with the hydroacoustic transects (Figure 1). Each point was recorded for presence and abundance of all aquatic species (Valley et al. 2015). Submersed aquatic vegetation was collected with a modified four-tine rake head (i.e., 10 cm galvanized, threaded bolts radially mounted to a polyvinyl chloride cap) attached to a telescopic pole that could extend to depths $> 3.5 \text{ m}$. A sample was collected by deploying the rake head to the lake bottom and twisting or rotating two full revolutions. Relative abundance was visually and manually estimated and ranked on a 0 to 3 scale based on “rake fullness” index of 0 to 3 adopted from Hauxwell et al. (2010): 0 = no plants present, 1 = sparse (small strands of a plant species), 2 = moderate (multiple strands of fresh biomass collected, tines visibly exposed), 3 = abundant (large collection of biomass with no visible tines). Rankings were assigned for emergent plants and floating leaf plants based on visual estimates of percentage of cover of a full circular scan around the boat out to 5 m: 0 = 0%, 1 = $< 10\%$, 2 = $< 50\%$, 3 = $> 50\%$. Dissolved oxygen was recorded on several occasions out to 42 DAT. During this period, dissolved oxygen levels were constant within a normal range between 4.0-7.6 mg L^{-1} (data not shown).

Geostatistical analysis

Water grab sample, vegetation point-intercept, exported BV point grids were projected to Universal Transverse Mercator North American Datum 83 Zone 17N in ArcGIS v. 10.3.1.⁴ The exported BV grids were rasterized into 15-m pixel surfaces with the Point Statistic tool in Spatial Analyst calculating the mean BV from points within a 56.419-m search radius (i.e., 1-ha area) as an attribute value for each pixel. The mean BV values of the matching pixels were

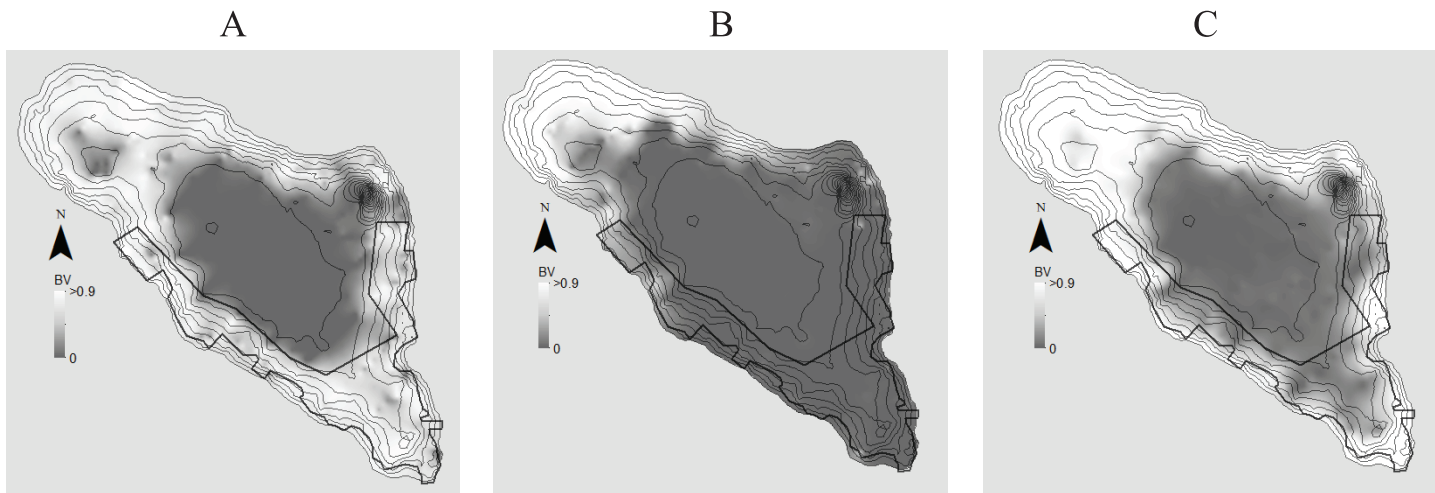


Figure 3. Maps depicting submerged vegetation biovolumes (BV) on Fish Lake (A) 8 d prior to treatment, (B) 133 d after treatment, and (C) 289 d after treatment with florypyrauxifen-benzyl. Black polygon denotes the treatment area. Average BV in treated and nontreated areas for A, B, and C were 64% and 63%, 4% and 54%, and 43% and 84%, respectively.

extracted to the water grab sample and vegetation point-intercept points for each survey date for further presentation on the localized effects of the measured herbicide concentrations and hydrilla abundances, respectively.

Plant community structure was assessed for each grid point based on relative abundance rankings (described above) that were log-transformed to better estimate rake fullness as a nonlinear assessment of the 0 to 3 scale. With richness (S) being the total number of species present, species diversity was determined by Simpson's Index (D) (Simpson 1949):

$$D = \frac{1}{\sum_{i=1}^s p_i^2} \quad (1)$$

where p_i is the proportional abundance of an individual species in the community. Evenness was then calculated as the ratio of Simpson's Index (D) over D_{\max} (equivalent to species richness [S]):

$$E = D/s \quad (2)$$

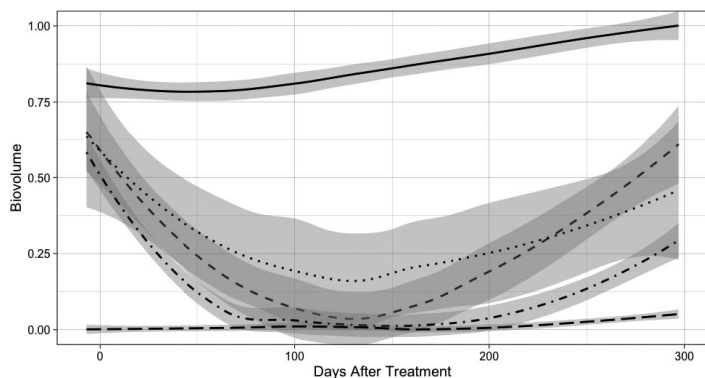


Figure 4. Submersed aquatic plant biovolume over time after treatment with florypyrauxifen-benzyl from treated cove (dashed), treated southwest (dotted), treated east (dot-dash), nontreated basin (long dash), and nontreated littoral (solid) zones in Fish Lake, Florida.

Water grab samples and vegetation point-intercept grid points were categorized into zones: nontreated (littoral areas with depth < 2.1 m), basin (nontreated area with depth > 2.1 m), treated (entire 20-ha treatment area), treated southwest (treated littoral flank on the southwest shoreline), treated east (treated littoral flank on the eastern shoreline), and treated cove (treated cove the southern shoreline) (Figure 1). Data presented over time were analyzed using nonparametric local regression fitted with a 90% confidence band in R⁷ (Cleveland 1979, Wickham 2016).

RESULTS AND DISCUSSION

The FPB treatment was applied to the entire 20-ha area in approximately 3.5 h, suggesting an efficient, uniform application that achieved the target concentration (Figure 1). Mean FPB concentrations within the treated area rapidly declined to $18.8 \mu\text{g L}^{-1}$ at 6 HAT, $7.5 \mu\text{g L}^{-1}$ at 24 HAT, $3.1 \mu\text{g L}^{-1}$ at 48 HAT, and $1.5 \mu\text{g L}^{-1}$ at 72 HAT, and were below detectable levels at 1 wk after treatment (Figure 2). There were no detectable FPB concentrations in the nontreated littoral zone during this monitoring period. However, the nontreated basin adjacent to the littoral treatment registered a mean concentration of $4.4 \mu\text{g L}^{-1}$ at 6 HAT, which peaked to $5.5 \mu\text{g L}^{-1}$ at 24 HAT and equilibrated with the treated area at 48 and 72 HAT. Concentrations of FPA in treated zones registered a mean concentration of $1.5 \mu\text{g L}^{-1}$ at 6 HAT and steadily increased until peaking at approximately $7 \mu\text{g L}^{-1}$ 69 HAT at which point concentrations rapidly declined. By 336 HAT, mean florypyrauxifen concentrations in treated areas were reduced to $3.9 \mu\text{g L}^{-1}$. Meanwhile, florypyrauxifen concentrations in nontreated areas had steadily increased across all sampling points reaching equilibrium with the treated area at 336 HAT.

FPB has been categorized as a proherbicide that will convert to alternate active forms via enzymatic hydrolysis (Jeschke 2015, Epp et al. 2016). Therefore, some initial FPB metabolites, such as FPA, exhibit herbicidal activity, albeit, less than FPB (Netherlands and Richardson 2016, Richardson

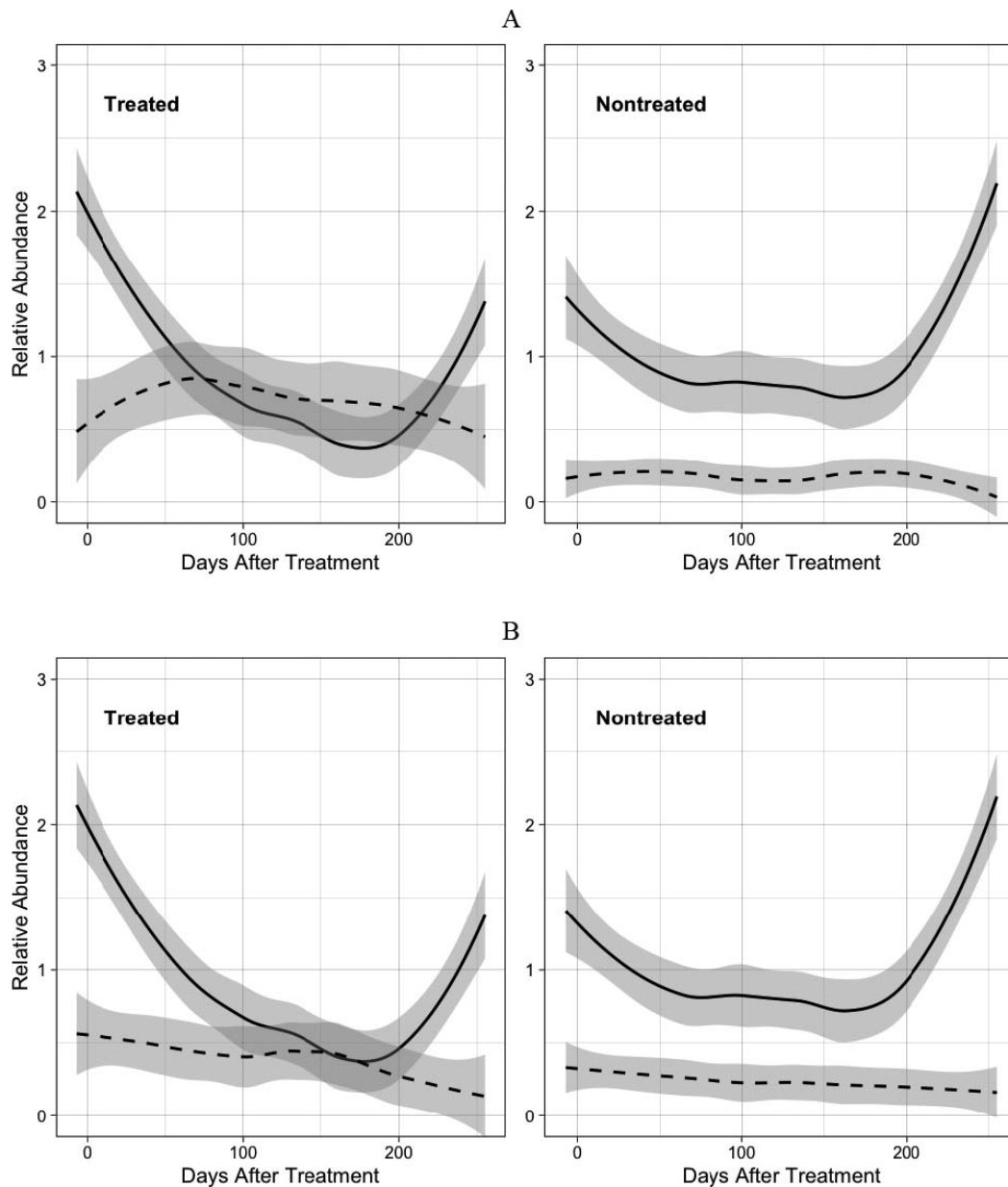


Figure 5. Hydrilla (solid) and nontargets (dashed) (A) American eelgrass or (B) spatterdock abundance over time in florypyrauxifen-benzyl treated and nontreated areas of Fish Lake from 0 to 289 d after treatment.

et al. 2016, Miller and Norsworthy 2018). However, according to the dose-response model of Netherland and Richardson (2016) the sustained florypyrauxifen concentrations (i.e., $> 5 \mu\text{g L}^{-1}$ for 48 h) measured in the treatment plot was high enough to result in low-level activity on hydrilla.

There were several factors likely contributing to the rapid dissipation of FPB from the littoral treatment area. Light winds (mean 1.8 m s^{-1}) were constant out of the south-southeast direction during the 48-h period after treatment (data not shown). Even light wind exposure will create turbulence and currents on the lake surface, which can affect herbicide dispersion and migration (Getsinger et al 1990, Wüest and Lorke 2003). Furthermore, the volume of the nontreated basin was ~ 2.7 times larger than the littoral

treatment volume directly adjacent, creating a strong concentration gradient driving herbicide diffusion. Here we observed the herbicide migrate north and east into the deepest part of the lake. This was particularly noticeable in the southwestern edge of the treatment plot where FPB concentrations dropped to $< 6 \mu\text{g L}^{-1}$ by 6 HAT. Lastly, photolysis of the benzyl ester compound is rapid in natural water systems with half-lives less than 5 h (Taylor et al. 2014). This is possibly another major contributor to the rapid decline measured in the treatment plot.

The entire littoral zone recorded consistently high BV at 8 d prior to treatment with means from sample points for treated and nontreated areas at 64 and 81%, respectively (Figure 4). In contrast, minimal BV was recorded in the

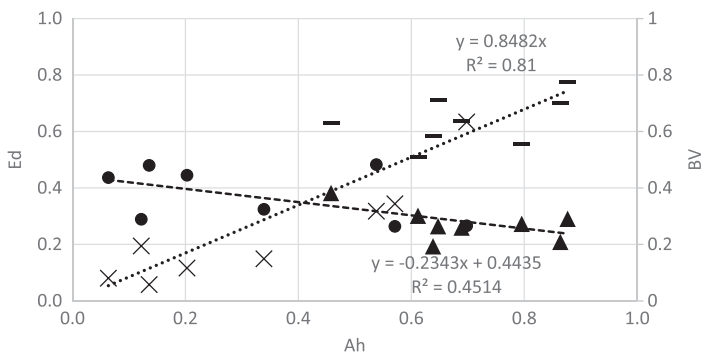


Figure 6. Mean evenness (E_d , circle and triangle) and biovolume (BV, X and dash) values from treated (circle and X, $n = 21$) and nontreated (triangle and dash, $n = 13$) littoral areas plotted against the relative abundance of hydrilla (A_h). Each of the mean values were derived from corresponding vegetation point-intercept grid points for each sample date from 8 d prior to treatment to 247 d after treatment ($n = 8$).

basin of the lake throughout the monitoring period, likely due to a lack of light penetration discouraging SAV growth (Figure 3) (Van et al. 1977, Bowes et al. 1979). The nontreated littoral area continued to measure BV increase over time until reaching a mean 99% in late May of 2019 (289 DAT) (Figure 4). In the treated cove zone, mean BV was minimized to levels close to zero from 89 to 168 DAT but increased with warming temperatures and longer days increasing to 52% at the end of May (289 DAT). Among the treated zones, BV reduction on the southwestern shoreline was less than the other treatment zones, which could be attributable to the lower herbicide exposure described above. The eastern shoreline treatment zone sustained BV near zero from 72 to 205 DAT, which was the longest duration of all zones.

Injury was observed in spatterdock [*Nuphar advena* (Ait.) W. T. Aiton], fragrant waterlily (*Nymphaea odorata* Ait.) and American lotus (*Nelumbo lutea* Willd.) outside of the treatment area with stem epinasty and leaf-curling symptoms typical of an auxin-mimic herbicide. Spatterdock was observed to recover from these acute symptoms with new leaves, and shoots emerged without symptoms at the end of the monitoring period. However, a similar recovery was not observed for fragrant waterlily and American lotus during this short observation period. Overall, frequencies of these species were very low prior to treatment (< 0.08 average abundance) and not distinguishable between treated and nontreated areas (data not shown). Abundance ranks for spatterdock (i.e., the most frequent floating leaf species) were sparse (< 1) during the entire monitoring period and slightly decreased over time in both treated and nontreated areas (Figure 5). American eelgrass was unresponsive to the herbicide treatment, but in contrast was observed to have an apparent response to the reduction in hydrilla abundance (Figure 5). The apparent tolerance of American eelgrass exhibited in this operational treatment is supported by Beets et al. (2019) showing FPB tolerance in mesocosm trials.

The relative abundance of hydrilla determined by rake fullness showed a positive linear trend with the interpolated BV values (Figure 6). This corroborates previous research showing the congruency between the two methods of

estimation (Valley et al. 2015) and further supports the majority of the estimated BV being hydrilla-dominated in littoral zones. There is also an inherently clear segregation of the plotted points between the treated and nontreated sections as a cross-validation proving FPB suppressed hydrilla. This is key to increasing aquatic native plant diversity and abundance (Getsinger et al. 1997). Removing dominance of one species can shift community composition by allowing underrepresented species to exploit the opportunity and flourish in a new environment, even with some reduction in species richness (Netherland and Jones 2015b). Here, we show a negative linear trend of plant community evenness with hydrilla abundance, where again plotted points are segregated between treated and nontreated sections clearly showing the treated section to have greater evenness with a more balanced representation of the other minority species. This was an acute response to a single treatment that was likely transient and quickly reverting back to hydrilla dominance. Nonetheless, this outcome has offered insight to large-scale strategies suited for auxin-mimic herbicides with reduced concentrations, shorter exposures, and greater selectivity (Netherland and Jones 2015b, Nault et al. 2017).

CONCLUSION

These data suggest single late-summer applications of FPB can provide temporary control of dense hydrilla infestations in central Florida lakes. Concentrations of FPB were below detectable levels by 7 DAT, while the parent acid persisted 14 DAT (~ 3 to $5 \mu\text{g L}^{-1}$). American eelgrass was tolerant to FPB treatment and no reductions in abundance were observed. However, substantial injury to nontarget emergent and floating leaf species was observed in some areas, especially inside the treatment zone. Spatterdock was able to recover from injury. Meanwhile, American lotus and fragrant waterlily were not observed to recover within this short observation period. Future research should continue to document and assess FPB effectiveness, the potential carryover effects of the parent acid, and nontarget plant response in other subtropical lake systems. Comparable treatments should be tested at different times of year with monitoring periods covering at least 1 yr. Furthermore, future work should investigate FPB retreatment and herbicide combinations for prolonged hydrilla suppression.

SOURCES OF MATERIALS

- ¹ProcellaCOR™ SC, SePRO Corp., Carmel, IN 46032.
- ²BioBase 5.2, Navico Inc., Egersund, Norway.
- ³Lowrance HDS, Navico Inc., Egersund, Norway.
- ⁴ArGIS v.10.3.1, ESRI, Redlands, CA 92373.
- ⁵YSI 556 MPS, YSI Inc., Yellow Spring, OH 45387.
- ⁶SePRO Research & Technology Campus, Whitakers, NC 27891.
- ⁷R version 3.6.1, R Foundation for Statistical Computing, Vienna, Austria.

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