INTRODUCTION

The Big Sand Lake Property Owners Association (BSLPOA) successfully applied for an Aquatic Invasive Species (AIS) Control Grant in February of 2010 to control Eurasian water milfoil (EWM) in 2010 and 2011. This report discusses the second and final year of treatment under this grant-funded project. Additional information regarding the treatment completed in 2010 can be found in the 2010 treatment report.

Following the 2010 peak-biomass survey, a conditional treatment permit map was created proposing 16.5 acres of treatment (Map 1). The large-scale treatment conducted in 2010 was shown to be extremely successful, with EWM decreasing in occurrence by over 90% within the lake. To continue the success of reducing EWM on Big Sand Lake, spot-treatment of remaining EWM colonies was proposed for 2011. The 2011 treatment was proposed to be completed using a liquid formulation of 2,4-D at a concentration of 2.5 ppm a.e. On May 25, 2011, Onterra staff visited Big Sand Lake to survey the proposed treatment areas and refine their boundaries as appropriate. Unable to locate any EWM within treatment site C-11 in the spring, this treatment site was removed, reducing the total treatment acreage to 12.6 acres (Map 1). It is believed that the EWM within site C-11 was injured from the 2010 treatment and did not survive the winter. During the survey, a temperature, dissolved oxygen, and pH profile was collected in approximately 30 feet of water due south of the boat landing. The surface water temperature was around 61°F with a pH of 8.3 (Figure 1).

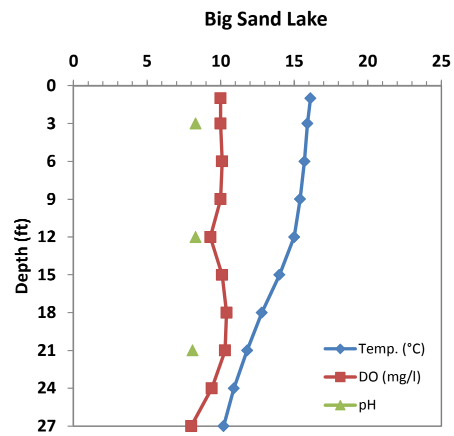


Figure 1. Temperature, dissolved oxygen, and pH profile on Big Sand Lake. May 25, 2011.

On June 3, 2011, the final treatment areas were treated with liquid 2,4-D by Bonestroo (now Stantec and previously Northern Environmental). They reported air temperatures of 60-65°F and 0-5 mph winds out of the southwest.

2011 TREATMENT MONITORING

The goal of herbicide treatments is to maximize target species (EWM) mortality while minimizing impacts to valuable native aquatic plant species. Monitoring herbicide treatments and defining their success incorporates both quantitative and qualitative methods. As the name suggests, quantitative monitoring involves comparing number data (or quantities) such as plant frequency of occurrence before and after the control strategy is implemented. Qualitative monitoring is completed by comparing visual data such as EWM colony density ratings before and after the treatments.

On most lakes with spot-treatments of EWM, like the one conducted on Big Sand Lake in 2011, quantitative data are collected from point-intercept sub-sample locations situated within the treatment areas and spaced 20 meters apart. However, on Big Sand Lake, because the large-scale treatment in 2010 was intended to disperse herbicide throughout the entire lake, quantitative evaluation was made through the collection of point-intercept data at a whole-lake level following methodologies from the Wisconsin Department of Natural Resources (WDNR). This same survey was completed in the summer of 2011 to assess the effectiveness of the 2010 treatment and determine if the native plant population incurred any negative impacts one year following a large-scale treatment. This method will allow for quantitative comparisons of aquatic plant species' occurrences between 2010 and 2011 on a lake-wide level, but does not allow for a quantitative evaluation of their occurrences specifically within the 2011 treatment areas.

The whole-lake aquatic plant point-intercept surveys conducted on Big Sand Lake in 2010 and 2011 included 902 sampling points spaced 80 meters apart. However, only the sampling points that were less than or equal to the depth of maximum plant growth were included in the analysis. Aquatic plants were found growing to a depth of 18 feet in 2010 and 20 feet in 2011, yielding 540 and 606 sampling locations falling within the maximum depth of plant growth, respectively. At these locations, EWM and native aquatic plant species presence and rake-fullness were documented along with water depth and substrate type. Specifically, these surveys aim to determine if significant differences in frequencies of occurrence of EWM and native species occur following the herbicide application. Although it is never the intent of the treatments to impact native species, it is important to remember that while 2,4-D is thought to be selective towards broad-leaf (dicot) species at the concentrations and exposure times of the treatments in 2010 and 2011 on Big Sand Lake, emerging data from the WDNR and USACE suggests that some narrow-leaf (non-dicot) species may also be impacted by this herbicide.

Spatial data reflecting EWM locations were collected using a sub-meter Global Positioning System (GPS) during the late summers of 2010 and 2011, when this plant is assumed to be at its peak biomass or growth stage. Comparisons of the survey results are used to qualitatively evaluate the 2011 herbicide treatment on Big Sand Lake. Qualitatively, a successful treatment on a particular site would include a reduction of EWM density as demonstrated by a decrease in density rating (e.g. highly dominant to dominant). In terms of a treatment as a whole (lake-wide), at least 75% of the acreage treated that year would decrease by one level of density as described above for an individual site.

2011 TREATMENT RESULTS

Post treatment surveys were completed by Onterra on August 17, 2011. Map 2 shows the results of the mid-August 2011 peak-biomass survey. No EWM could be located within the 2011 treatment areas, exceeding the treatment-wide qualitative success criteria (75% of acreage treated). However, EWM was found to have rebounded since the 2010 treatment in other areas of the lake, and were comprised of small dominant and highly dominant colonies (Map 2).

During the summer of 2010, 0.9% of the point-intercept locations contained EWM compared to 1.2% in 2011. Though there was a slight increase in occurrence, this change was not statistically valid (Table 1). The low occurrence of EWM in the 2011 survey indicates that the large-scale treatment conducted in 2010 was very effective and that EWM had not rebounded greatly within the lake during this timeframe. Data concerning native aquatic plant species were also collected during these surveys. Table 2 shows that while some native aquatic plant species saw statistically valid decreases in occurrence from 2010 to 2011, these declines were relatively small. Similarly, some native species had statistically valid increases in occurrence over this period (Table 2).

Of particular interest, is the increase in naiad species from 2010 to 2011. During the 2011 survey, two species of naiad, slender naiad (*Najas flexilis*) and southern naiad (*Najas guadalupensis*) were located. Southern naiad was found at approximately 17% of the point-intercept locations in 2011, while it was not located at all in a 2006 survey by WDNR. In 2006 and 2011, slender naiad was located at approximately 6% and 13% of the point-intercept locations, respectively. Both slender naiad and southern naiad are morphologically similar, and distinguishing between the two is often difficult. It is believed the occurrence of slender naiad in 2010 is likely a combination of both slender and southern naiad occurrences due to misidentification in the field. For this reason, the occurrences of both naiad species were combined for analysis (Table 1). However, the fact no southern naiad was located in 2006 indicates this plant may be increasing rapidly in Big Sand Lake.

Emerging research is indicating that hybrids between southern naiad subspecies exist and are often observed acting aggressively and growing to nuisance levels (Les et al. 2010). Southern naiad was found throughout littoral areas of Big Sand Lake in 2011, but was most prevalent in the southern, shallow portion of the lake where it was observed forming large beds which matted on the water's surface.

Table 1. Statistical comparison of aquatic plant frequency data from 2010 and 2011 whole-lake point-intercept surveys. Only species with littoral frequency of occurrence greater than 5.0% in at least one of the two surveys are applicable for analysis.

	Scientific Name	Common Name	2010 LFOO	2011 LFOO	Percent Change	Direction	Chi-square Analysis	
							Statistically Valid	p-value
D	*Myriophyllum spicatum*	Eurasian water milfoil	0.9	1.2	24.8	▲ (not valid)	No	0.704
D	*Ceratophyllum demersum*	Coontail	16.9	17.3	2.8	▲ (not valid)	No	0.831
Non-dicots	*Potamogeton robbinsii*	Fern pondweed	58.3	50.5	-13.4	▼	Yes	0.008
Non-dicots	*Elodea canadensis*	Common waterweed	26.7	34.0	27.5	▲	Yes	0.007
Non-dicots	*Potamogeton praelongus*	White-stem pondweed	24.6	17.5	-29.0	▼	Yes	0.003
Non-dicots	*Najas spp.*	Naiad spp.	13.1	23.3	77.0	▲	Yes	0.000
Non-dicots	*Isoetes spp.*	Quilwort species	6.1	1.8	-70.3	▼	Yes	0.000
Non-dicots	*Juncus pelocarpus*	Brown-fruited rush	5.7	2.1	-62.6	▼	Yes	0.002
Non-dicots	*Chara spp.*	Muskgrasses	3.5	6.8	92.3	▲	Yes	0.014
Non-dicots	*Potamogeton zosteriformis*	Flat-stem pondweed	7.6	7.8	2.1	▲ (not valid)	No	0.918
Non-dicots	*Potamogeton gramineus*	Variable pondweed	7.4	9.2	24.8	▲ (not valid)	No	0.263
Non-dicots	*Vallisneria americana*	Wild celery	7.0	7.9	12.6	▲ (not valid)	No	0.571
Non-dicots	*Potamogeton illinoensis*	Illinois pondweed	5.0	5.0	-1.0	▼ (not valid)	No	0.969

2010 N = 540, 2011 N = 606
D = Dicots; LFOO = Littoral Frequency of Occurrence
▲ or ▼ = Change Statistically Valid (Chi-square; α = 0.05)
▲ or ▼ (grey) = Change Not Statistically Valid (Chi-square; α = 0.05)

Big Sand Lake was again selected to participate in a residual herbicide monitoring research project being conducted by the WDNR and US Army Corps of Engineers (USACE). Water samples were collected by a Big Sand Lake volunteer from sites located both within each of the three final herbicide application areas. The water samples were properly fixed and sent to the USACE laboratory for analysis. The preliminary data show that herbicide concentrations were considerably higher within Site A-11, the largest treatment site. Appendix A contains the USACE draft report with more detail regarding the residual sampling study on Big Sand Lake.

2011 TREATMENT STRATEGY

The 2011 EWM treatment on Big Sand Lake was met with success; no EWM could be located within the 2011 treatment areas. The point-intercept survey also showed that the 2010 large-scale treatment remains successful, with the lake-wide occurrence of EWM remaining very low. The native plant community saw statistically valid increases and decreases of some species, yet none of the changes were substantial.

Herbicides that target submersed plant species are directly applied to the water, either as a liquid or an encapsulated granular formulation. Factors such as water depth, water flow, treatment area size, and plant density work to dilute herbicide concentration within aquatic systems. Understanding concentration-exposure times are important considerations for aquatic herbicides. Successful control of the target plant is achieved when it is exposed to a lethal concentration of the herbicide for a specific duration of time. Much information has been gathered in recent years, largely as a result of a joint research project between the WDNR and the USACE. Based on their preliminary findings, lake managers have adopted two main treatment strategies; 1) whole-lake treatments, and 2). spot treatments.

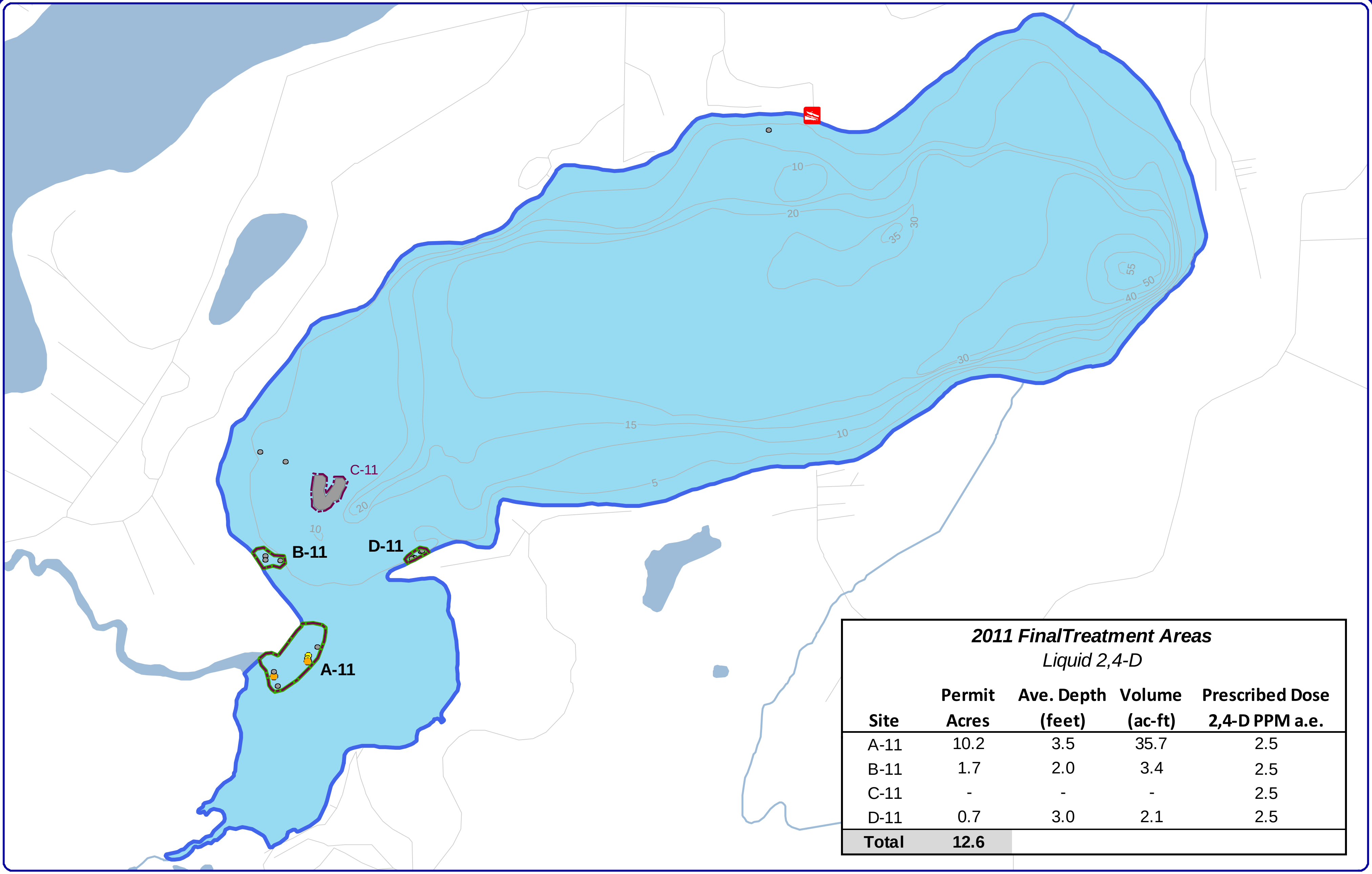
As discussed above, a whole-lake treatment was implemented in 2009 and 2010. Whole-lake treatments are those where the herbicide is applied to specific sites, but when the herbicide reaches equilibrium within the entire volume of water (of the lake, lake basin, or within the epilimnion of the lake or lake basin); it is at a concentration that is sufficient to cause mortality to the target plant within that entire lake or basin. The application rate of whole-lake treatments is dictated by the volume of water which the herbicide will reach equilibrium within. The target herbicide concentration is typically between 0.225 and 0.325 ppm a.e. when exposed to the target plants for 7-14 days or longer. However, these same rates have been shown to impact some native plant species, particularly dicot species, some thin-leaved pondweeds, and naiad species.

Spot treatments are a type of treatment strategy where the herbicide is applied to a specific area (treatment site) such that when it dilutes from that area, its concentrations are insufficient to cause significant affects outside of that area. This is the strategy implemented in 2011 on Big Sand Lake. Spot treatments typically rely on a short exposure time (often hours) to cause mortality and therefore are applied at a much higher herbicide concentration than whole-lake treatments. For Eurasian water milfoil, 2,4-D is typically applied between 2.25 and 3.0 ppm acid equivalent (a.e.). in spot treatment scenarios. A newly adopted term, micro-treatments are small spot treatments (working definition is less than 5 acres) and because of their small size, are extremely difficult to predict if they will be effective because of the rapid dilution of the herbicide. Larger treatment areas tend to be able to hold effective concentrations for a longer time.

Some rebound of EWM following the 2010 large-scale treatment was expected, and this is what was observed in 2011 (Map 2). Huge strides were made following the 2010 and 2011 treatments, and now only a few small colonies of EWM remain. Map 2 displays the proposed 9.8 acres of EWM treatment for 2012. While the 2011 treatment utilizing liquid 2,4-D at 2.5

ppm a.e. was very successful, a slightly modified strategy is proposed for 2012 due to the small size of the proposed treatment sites. The 2012 proposed treatment strategy for Big Sand Lake includes an expanded buffer (40-foot) around the EWM colonies as well as a higher proposed application rate of herbicide (Map 2).

This report marks the end of the current AIS Established Population Control (AIS EPC) Grant-funded project. Because herbicide application costs for the 2011 treatment were likely significantly less than budgeted for within this grant, the BSLPOA may want to pursue options of extending the timeframe and scope of the project to include cost-coverage for the 2012 EWM treatment and associated monitoring costs. The BSLPOA may also want to discuss applying for an additional AIS EPC grant during the subsequent funding cycle (February 1, 2011 or August 1, 2012) to continue their control project.



2011 FinalTreatment Areas

Liquid 2,4-D

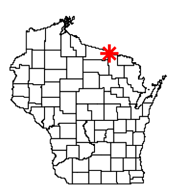
Site	Permit Acres	Ave. Depth (feet)	Volume (ac-ft)	Prescribed Dose 2,4-D PPM a.e.
A-11	10.2	3.5	35.7	2.5
B-11	1.7	2.0	3.4	2.5
C-11	-	-	-	2.5
D-11	0.7	3.0	2.1	2.5
Total	**12.6**			

1,750

Feet

Onterra LLC
Lake Management Planning
135 South Broadway Suite C
De Pere, WI 54115
920.338.8860
www.onterra-eco.com

Sources:
Hydro and Roads: WDNR
Aquatic Plant Survey: Onterra, 2010
Map Date: December 20, 2011
Filename: Map1_BigSand_EWM_2010PB_T2011.mxd



Project Location in Wisconsin

Legend

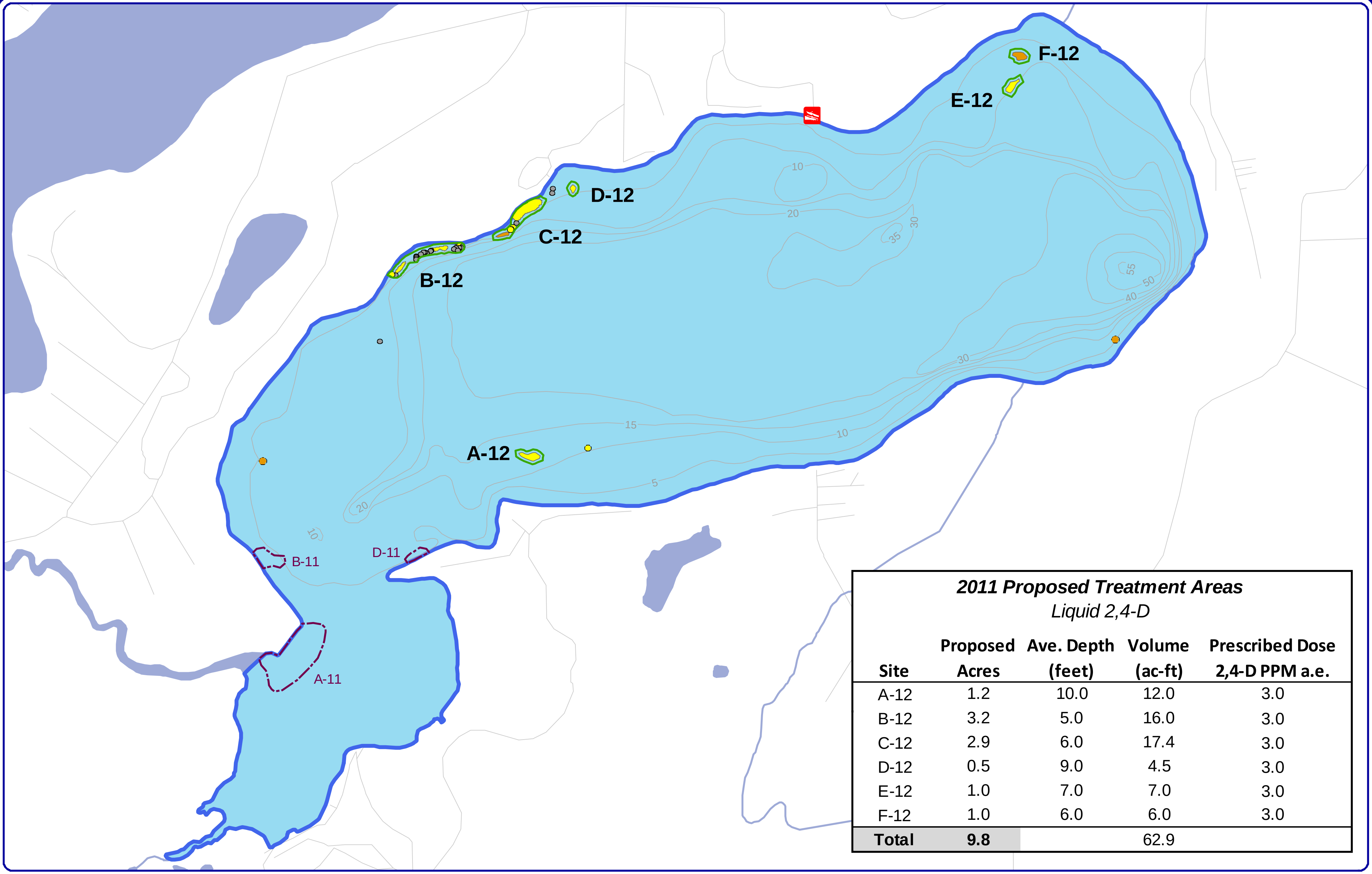
- Highly Scattered *(None)*
- Scattered
- Dominant *(None)*
- Highly Dominant *(None)*
- Surface Matting *(None)*
- Single or Few Plants
- Clumps of Plants
- Small Plant Colony
- 2011 Conditional Treatment Area
- 2011 Final Treatment Area

Map 1

Big Sand Lake

Vilas County, Wisconsin

2010 EWM Locations & 2011 Treatment Areas



2011 Proposed Treatment Areas

Liquid 2,4-D

Site	Proposed Acres	Ave. Depth (feet)	Volume (ac-ft)	Prescribed Dose 2,4-D PPM a.e.
A-12	1.2	10.0	12.0	3.0
B-12	3.2	5.0	16.0	3.0
C-12	2.9	6.0	17.4	3.0
D-12	0.5	9.0	4.5	3.0
E-12	1.0	7.0	7.0	3.0
F-12	1.0	6.0	6.0	3.0
Total	**9.8**		62.9	

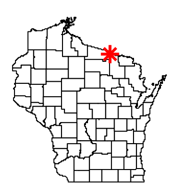
N W E S

1,750

Feet

Onterra LLC
Lake Management Planning
135 South Broadway Suite C
De Pere, WI 54115
920.338.8860
www.onterra-eco.com

Sources:
Hydro and Roads: WDNR
Aquatic Plant Survey: Onterra, 2011
Map Date: December 20, 2011
Filename: *Map2_BigSand_EWM_T2012_Cond1.mxd*



Project Location in Wisconsin

Legend

- Highly Scattered *(None)*
- Scattered *(None)*
- Dominant
- Highly Dominant
- Surface Matting *(None)*
- Single or Few Plants
- Clumps of Plants
- Small Plant Colony
- 2011 Final Treatment Area
- 2012 Proposed Treatment Area

Map 2

Big Sand Lake

Vilas County, Wisconsin

2011 EWM Locations & 2012 Proposed Treatment Areas v.1

Draft: Big Sand Lake, Vilas County, Herbicide Residual Summary, 2011
5 November 2011

John Skogerboe
US Army Engineer Research and Development Center (ERDC)

A liquid formulation of 2,4-D was applied to three sites on Big Sand Lake infested with Eurasian watermilfoil (Figure 1). The herbicide was applied on 3 June 2011 at an application rate of 2500 ug/L ae (Table 1).

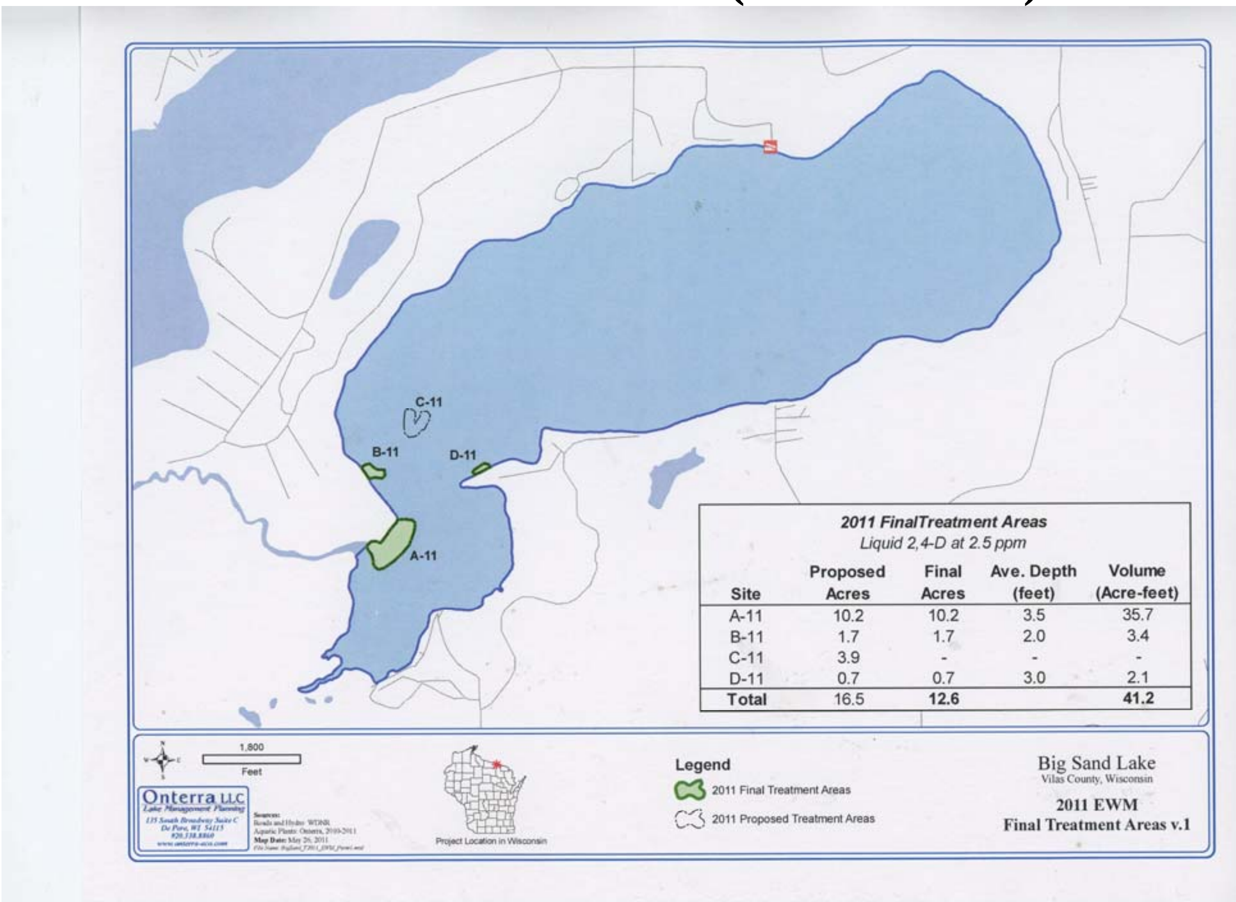
A herbicide residual sample location was located in each of the treated sites (Figure 2). Treatment site areas ranged from 0.70 to 10.2 acres. Water residual samples were collected by volunteers using an integrated water sampler to collect water from the entire water column. Samples were collected at 1, 3, 6, 24, 48, 72, 120, and 168 hours after treatment (HAT). Following completion of each sample interval, 2-3 drops of muriatic acid were added to all samples to fix the herbicide. Samples were then stored in a refrigerator until shipped to the ERDC laboratory for analysis.

Residual 2,4-D concentrations ranged from 72 to 1630 ug/L ae at 1 HAT compared to a target concentration of 2500 ug/L (Figure 3 and Figure 4). Concentrations were less than the irrigation restriction level of 100 ug/L ae at 3 HAT in the smaller sites (B and D), but remained higher in the larger site (D) until 120 HAT. The maximum mean concentration was 996 ug/L ae at 1 HAT, and was less than the irrigation restriction level at 120 HAT (Figure 5).

Table 1 Summary of Big Sand Lake, 2011 Herbicide Treatments Monitored for Herbicide Residuals

	Area	Depth	Application Rate
Site	acres	feet	ug/L ae
A	10.2	3.5	2500
B	1.7	2	2500
D	0.7	3	2500

Figure 1. Big Sand Lake, 2011 herbicide treatment sites (Onterra LLC)



2011 FinalTreatment Areas
Liquid 2,4-D at 2.5 ppm

Site	Proposed Acres	Final Acres	Ave. Depth (feet)	Volume (Acre-feet)
A-11	10.2	10.2	3.5	35.7
B-11	1.7	1.7	2.0	3.4
C-11	3.9	-	-	-
D-11	0.7	0.7	3.0	2.1
Total	16.5	**12.6**		**41.2**

Figure 2. Big Sand Lake, 2011 herbicide residual sample locations

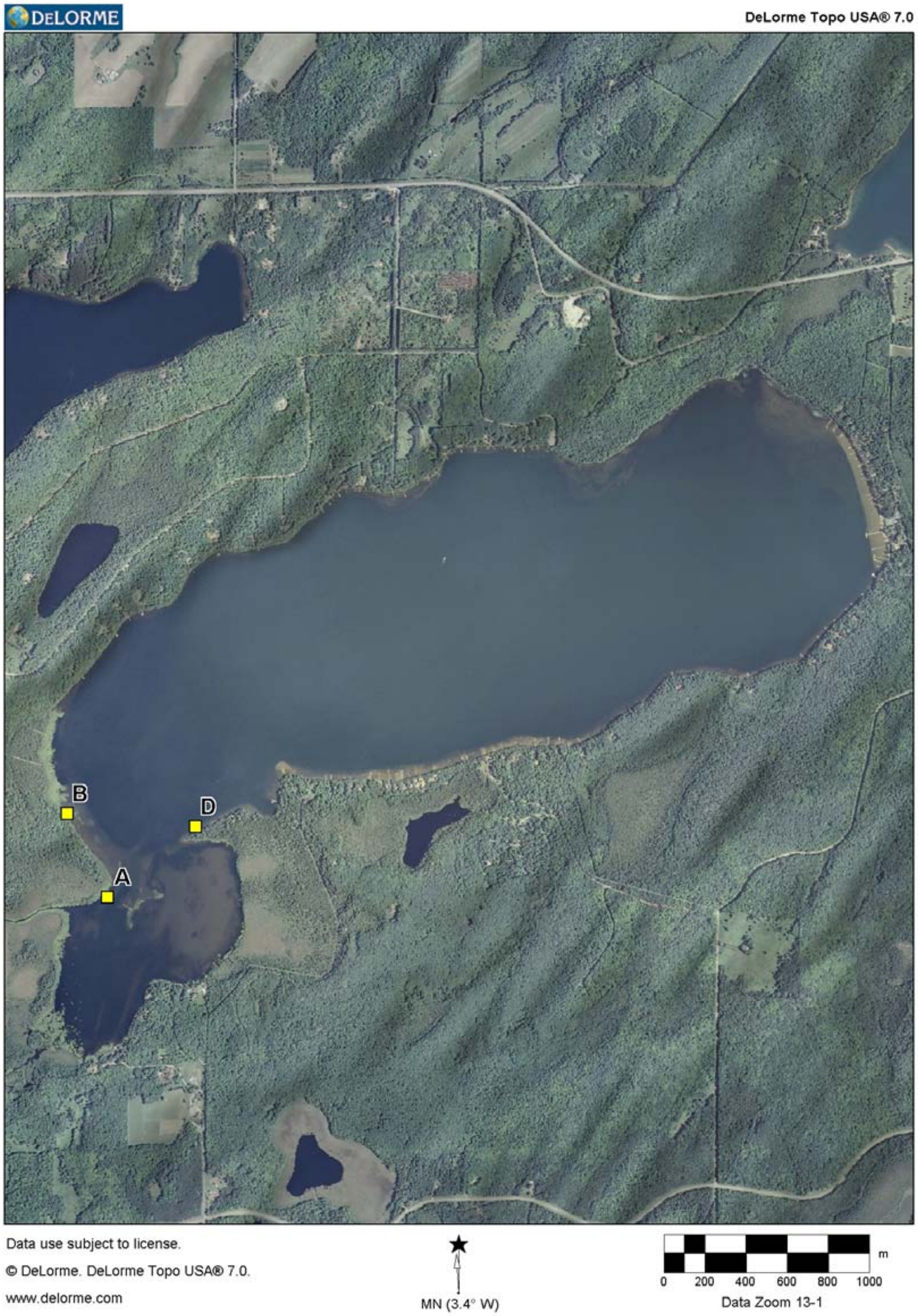


Figure 3.

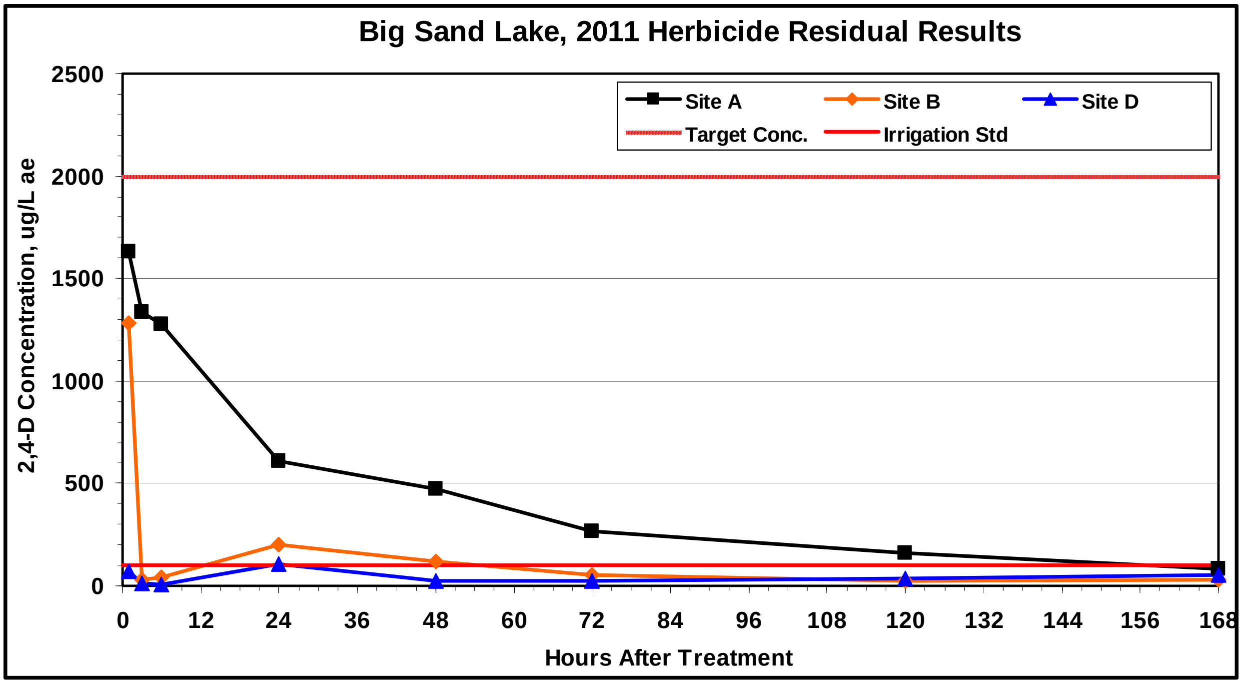


Figure 4.

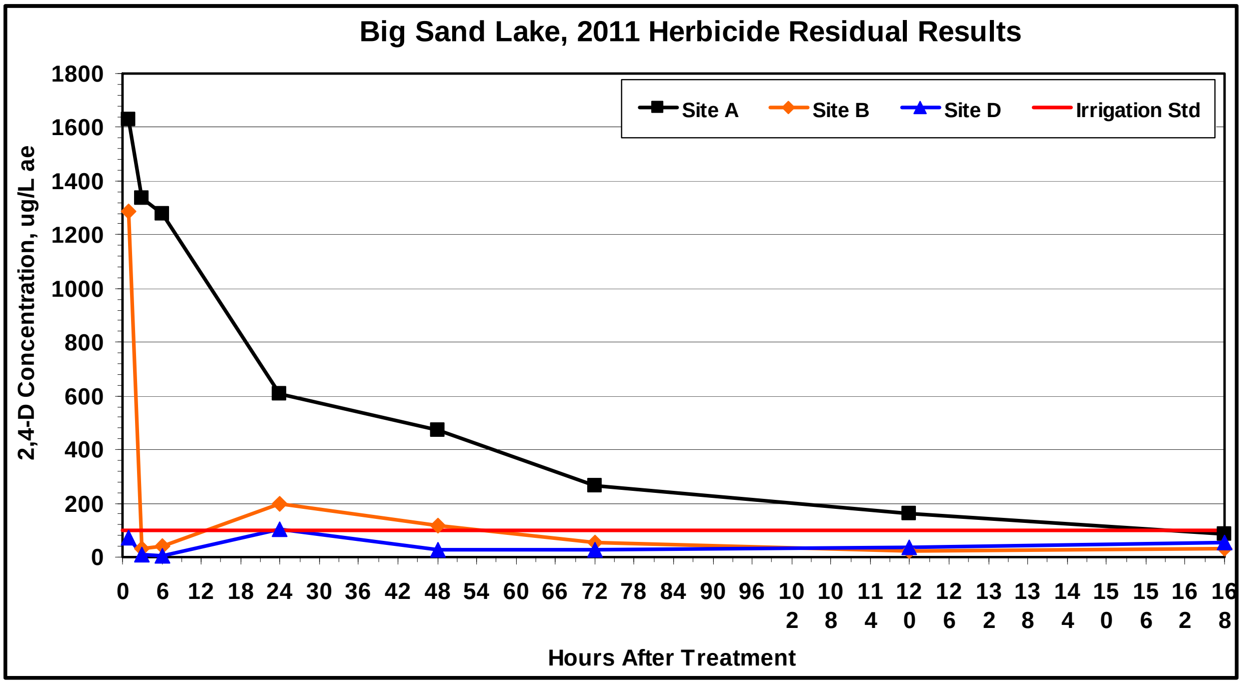
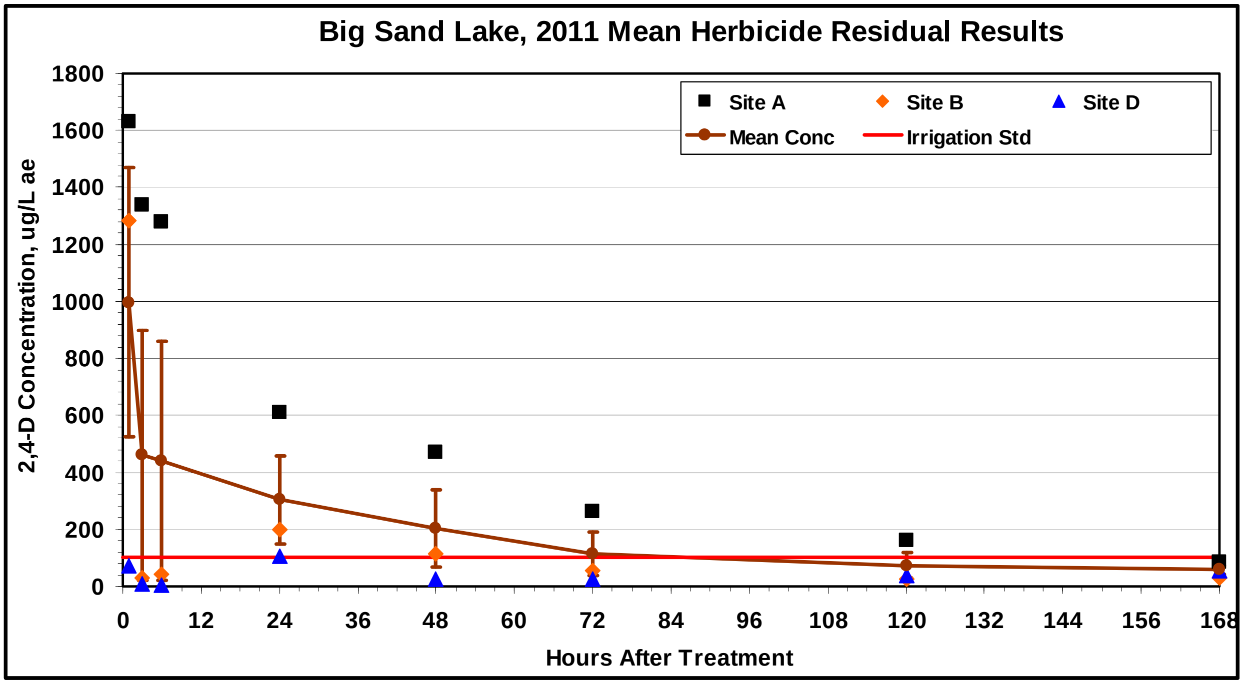


Figure 5


Big Sand Lake, 2011 Mean Herbicide Residual Results
Site A
Site B
Site D
Mean Conc
Irrigation Std
2,4-D Concentration, ug/L ae
Hours After Treatment
1800
1600
1400
1200
1000
800
600
400
200
0
0
12
24
36
48
60
72
84
96
108
120
132
144
156
168