Integrating genetics and herbicide studies to improve watermilfoil management outcomes

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Premise of the Research Program

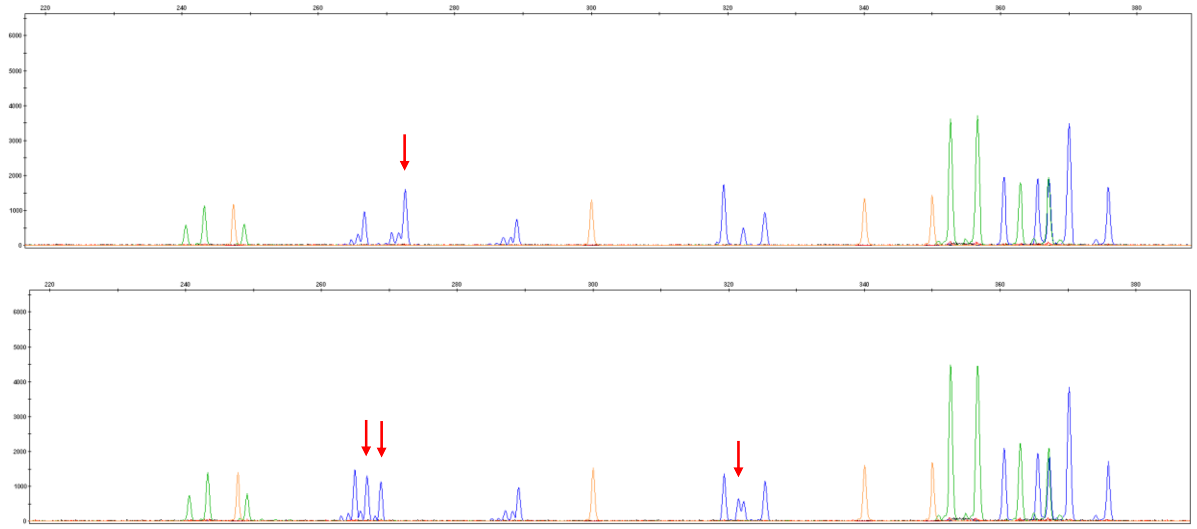
- Genetic variation can influence management outcomes

- Genetic information can be used to predict management response, and thus improve management outcomes.

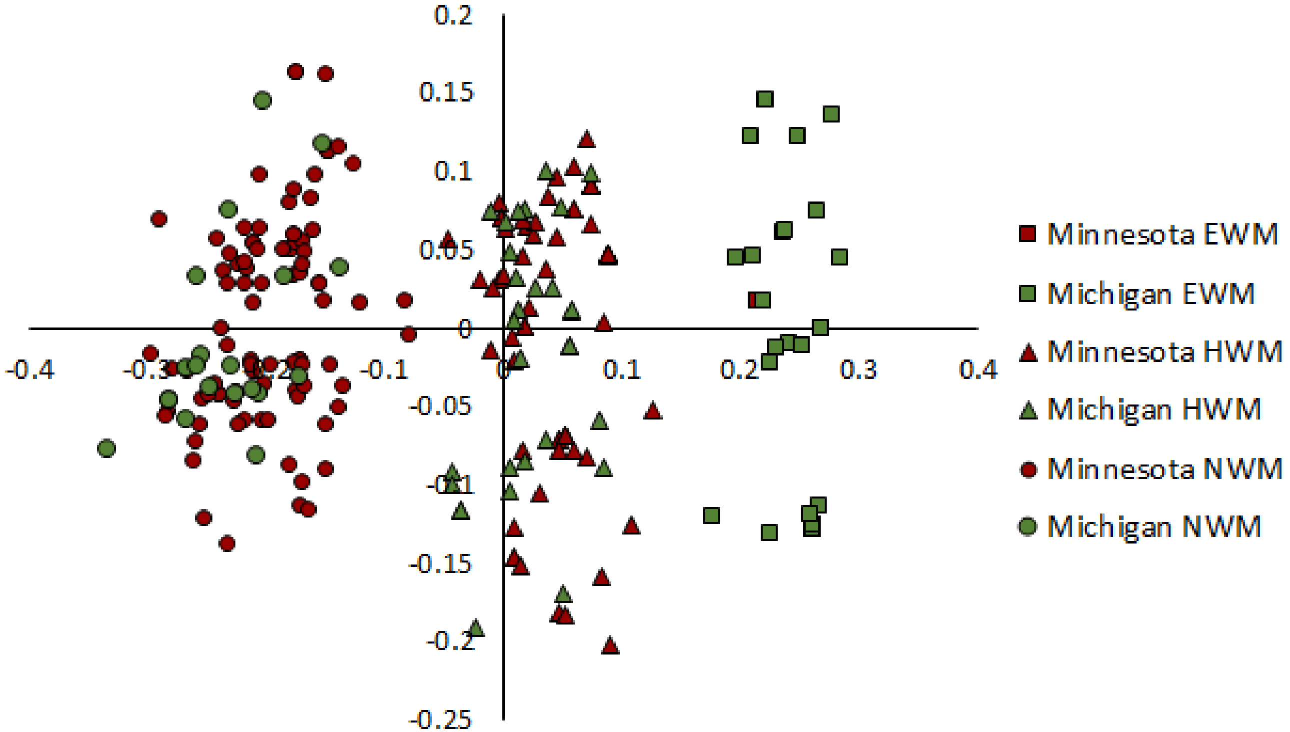
1. Health predisposition (how your genes influence your chance of having certain health conditions)
2. Carrier status (are you a carrier for certain inherited conditions)

Genetic Variation in "Eurasian" Watermilfoil

- Aquatic plant stakeholders increasingly recognize that Eurasian watermilfoil (including hybrids) is genetically diverse



DNA "Fingerprints"



Thum et al. (2020)
Invasive Plant Science & Management

Genetic Variation in "Eurasian" Watermilfoil

- Strains can differ in their growth, spread, impacts, and herbicide response.

		Diquat 0.37 mg/L		Endothall 1.5 mg/L		2,4-D 0.5 mg/L				
Taxon	Source	8 h	24 h	8 h	24 h	8 h	24 h	48 h	96 h	144 h
1 WAT										
Eurasian	Minnetonka	7 b	0 b	92	71 b	23 c	41 c	0 c	0 b	0
Hybrid	Townline	137 a	39 a	96	64 b	64 b	57 b	0 c	0 b	0
Hybrid	Frog	4 b	0 b	90	59 b	54 b	33 c	20 b	26 a	0
Hybrid	English	3 b	0 b	86	82 a	71 a	77 a	75 a	28 a	0

Netherland and Willey (2017)
J. Aquatic Plant Mgmt.

Short- and long- term approaches to using genetics to inform best management practices

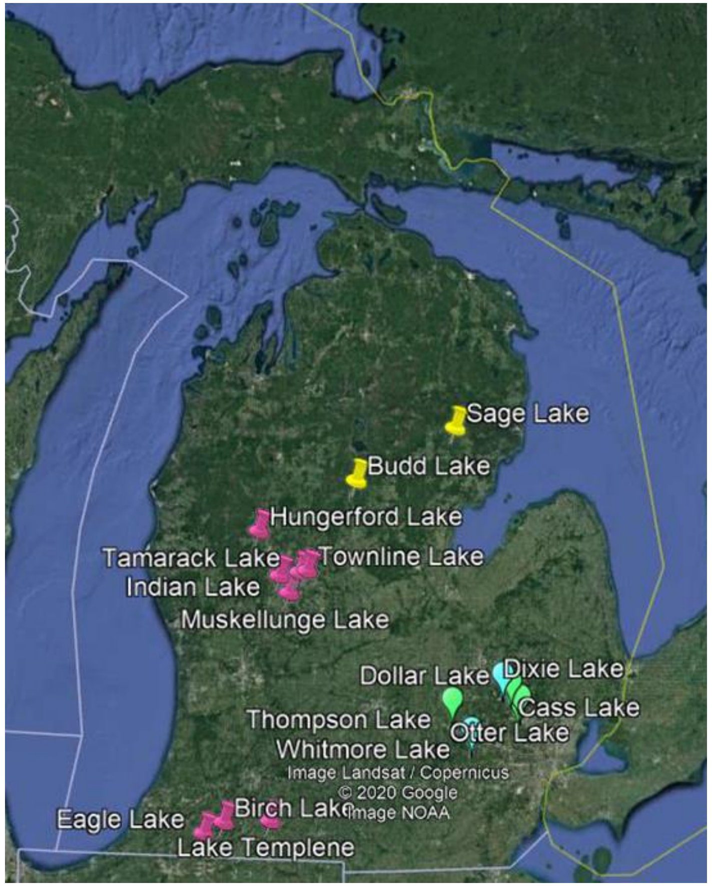
- SHORT TERM: Construct a catalog of herbicide responses for prioritized strains

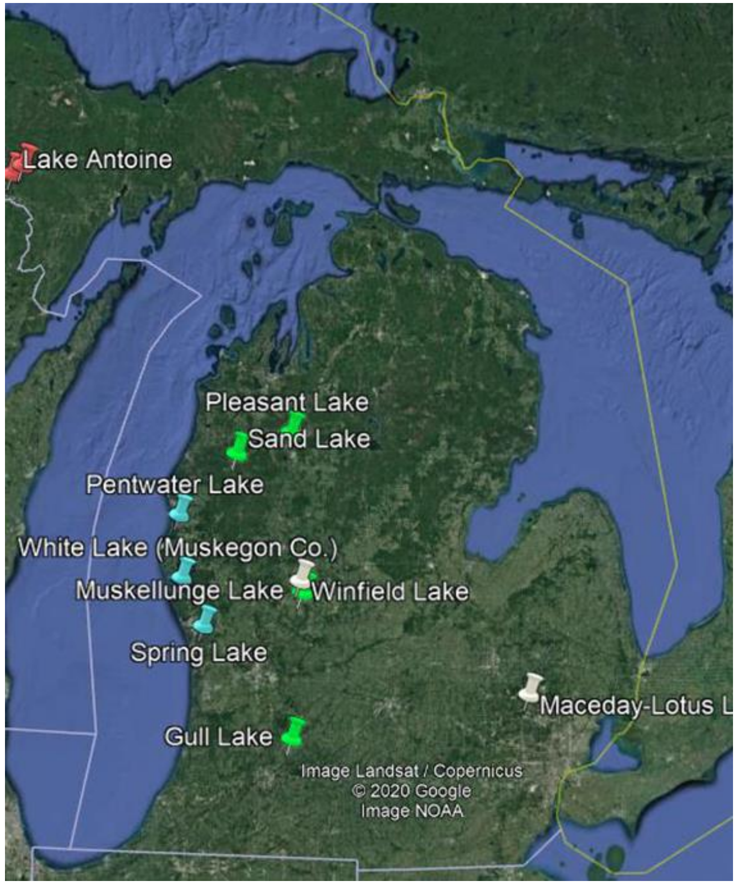
- LONG TERM: Identifying genes involved in herbicide response in order to develop specific genetic assays to predict herbicide response

Example: Identification of fluridone resistant vs sensitive strains in several Michigan lakes

- Genetic surveys reveal that the same strains can be found in more than one lake.

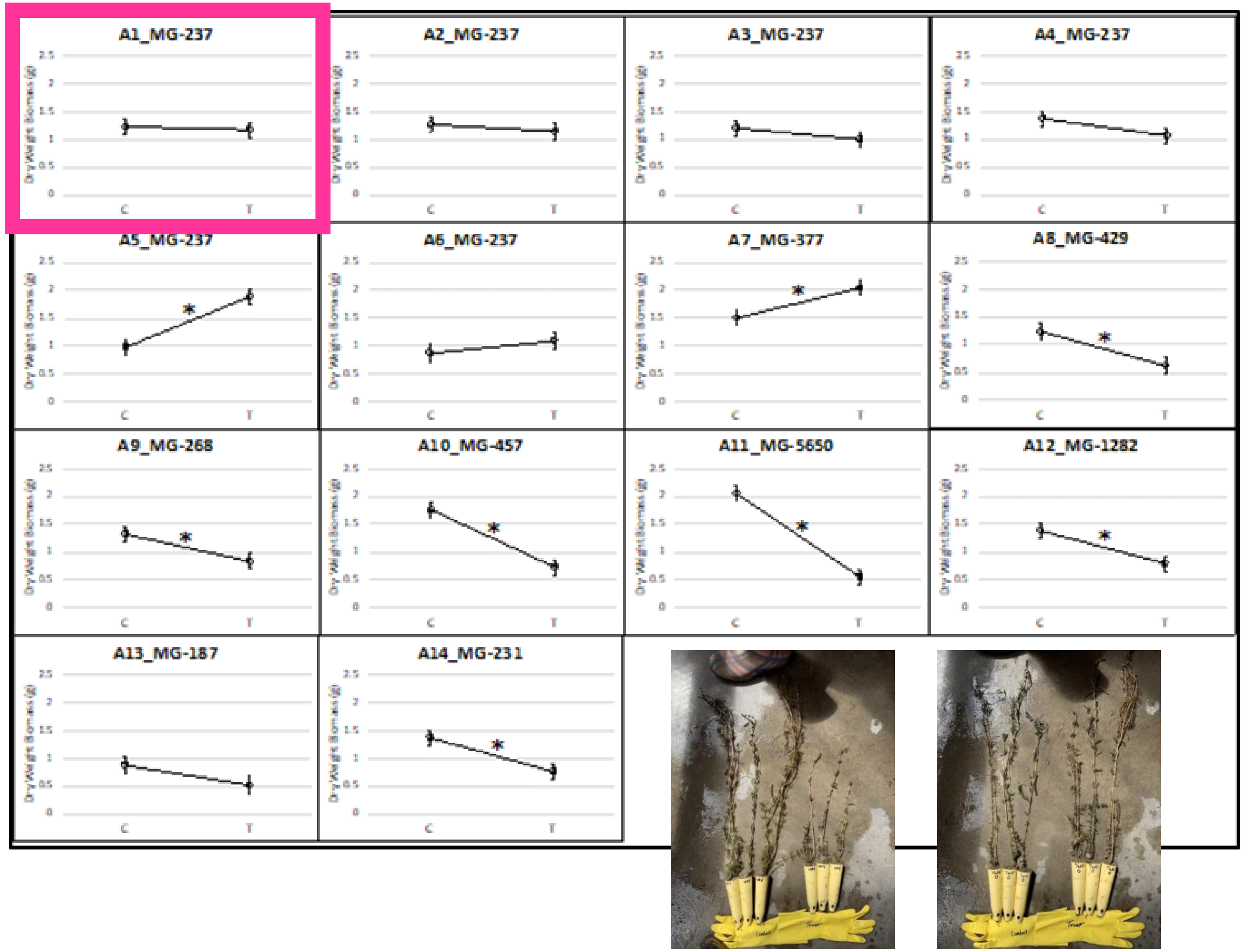
- Strain information can facilitate communication among stakeholders regarding management.



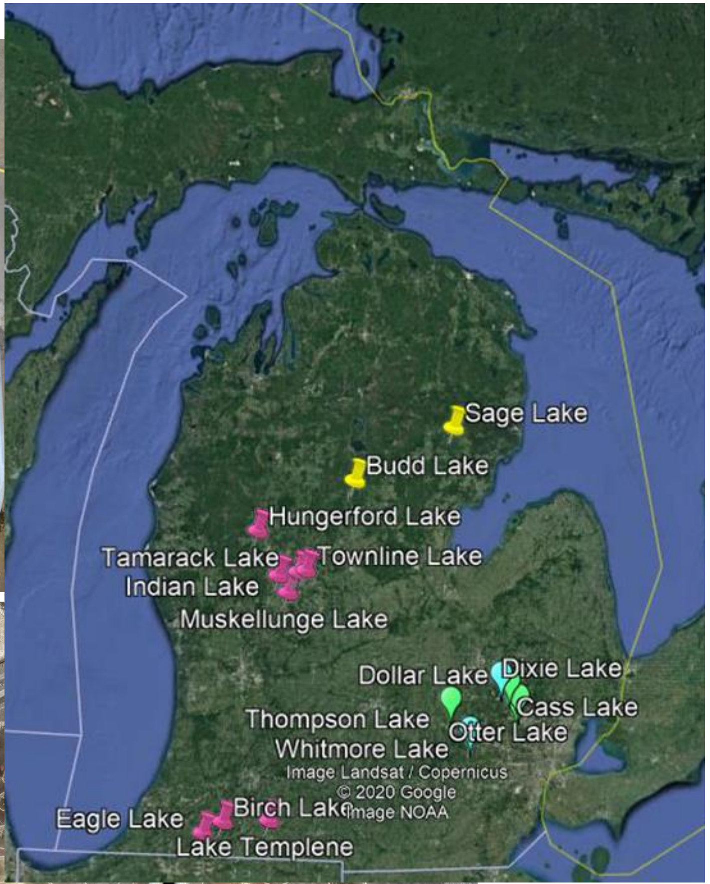


Redrawn from Thum et al. (2020)
Invasive Plant Science & Management

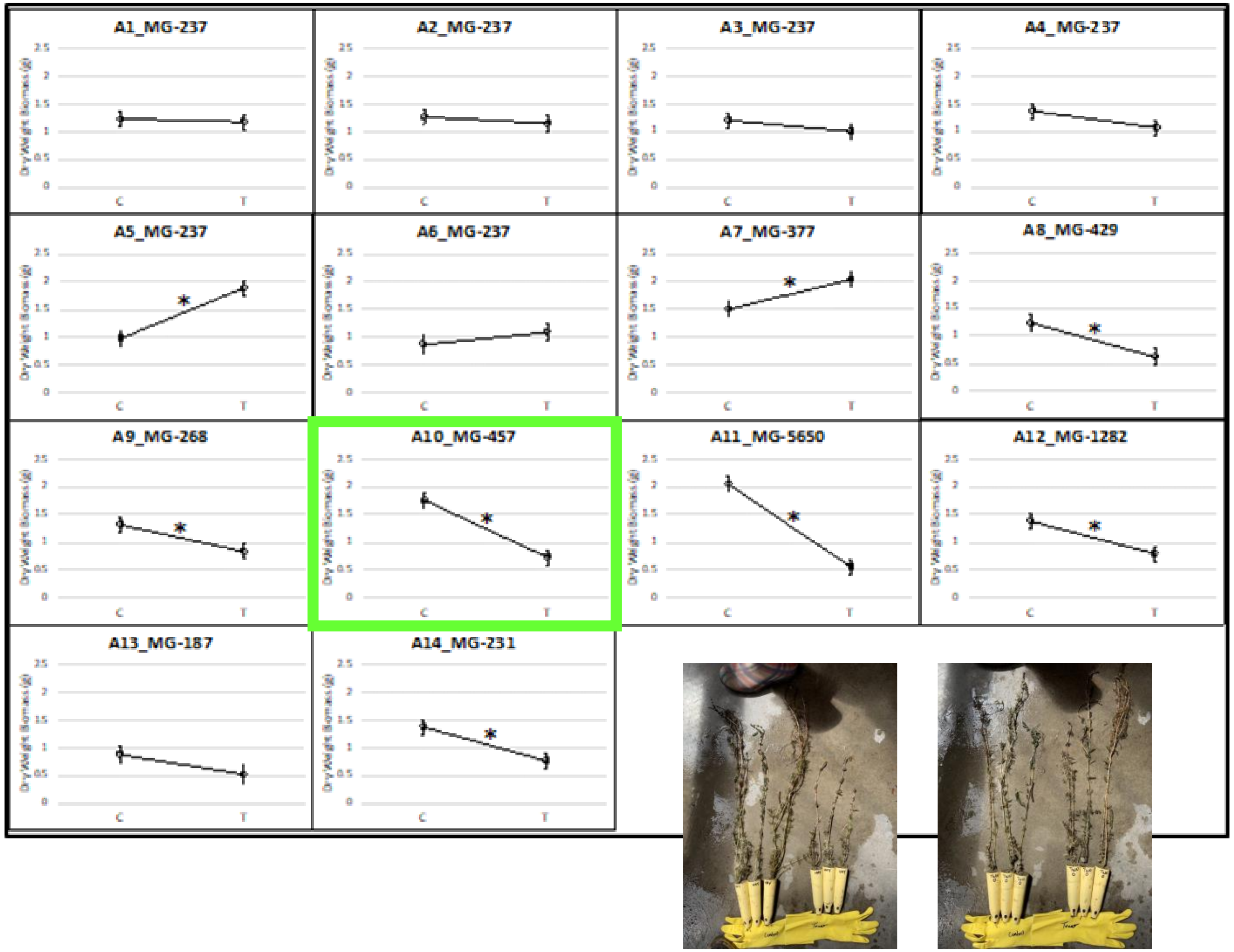
Example: Identification of fluridone resistant vs sensitive strains in several Michigan lakes



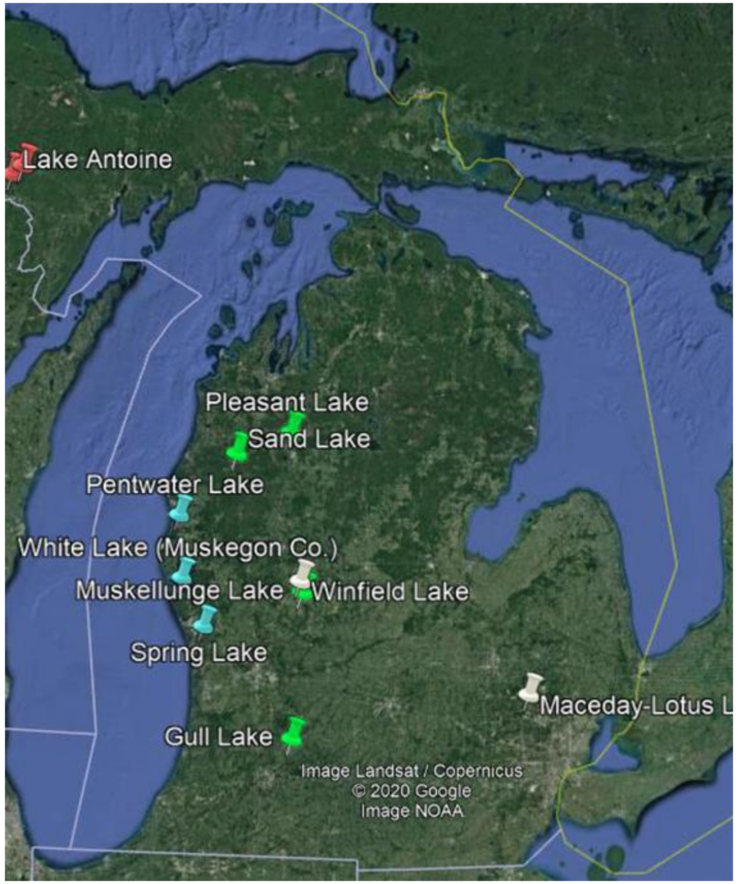
Chorak and Thum (2020)
Invasive Plant Science & Management



Example: Identification of fluridone resistant vs sensitive strains in several Michigan lakes



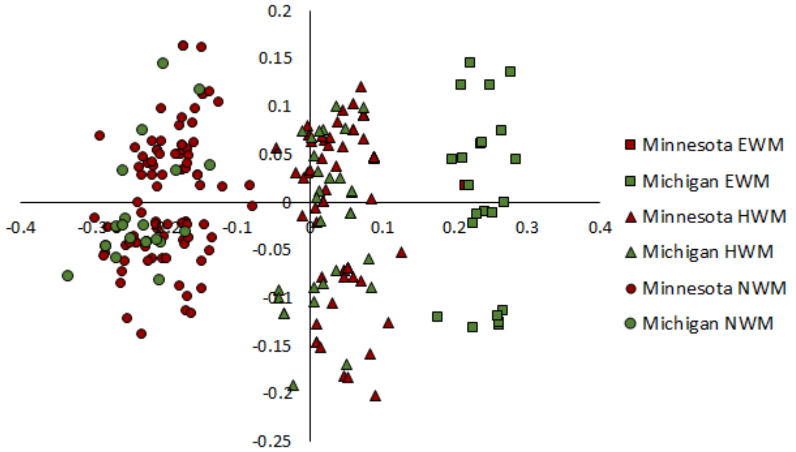
Chorak and Thum (2020)
Invasive Plant Science & Management

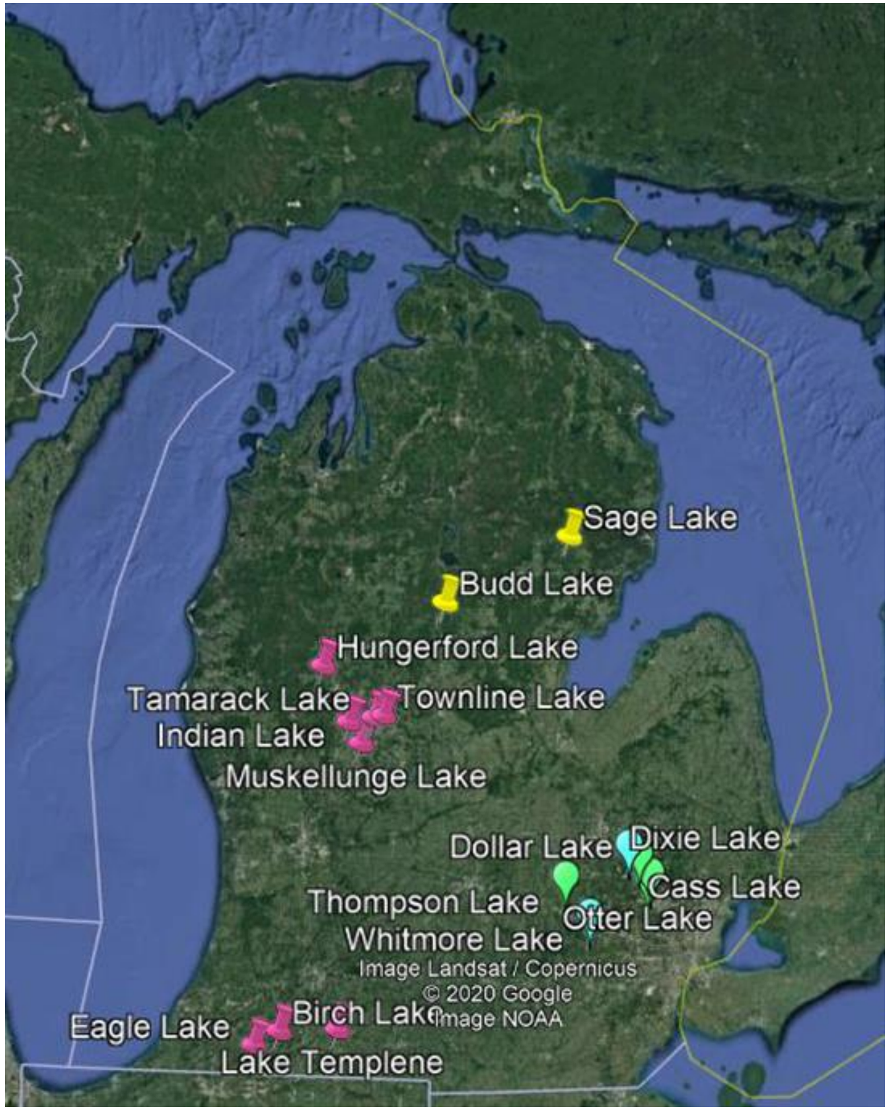


SHORT TERM: Construct a catalog of herbicide responses for prioritized strains

- Prioritizing strains for characterization
- Characterizing herbicide response
- Molecular characterization of strains
- Building and curating a database

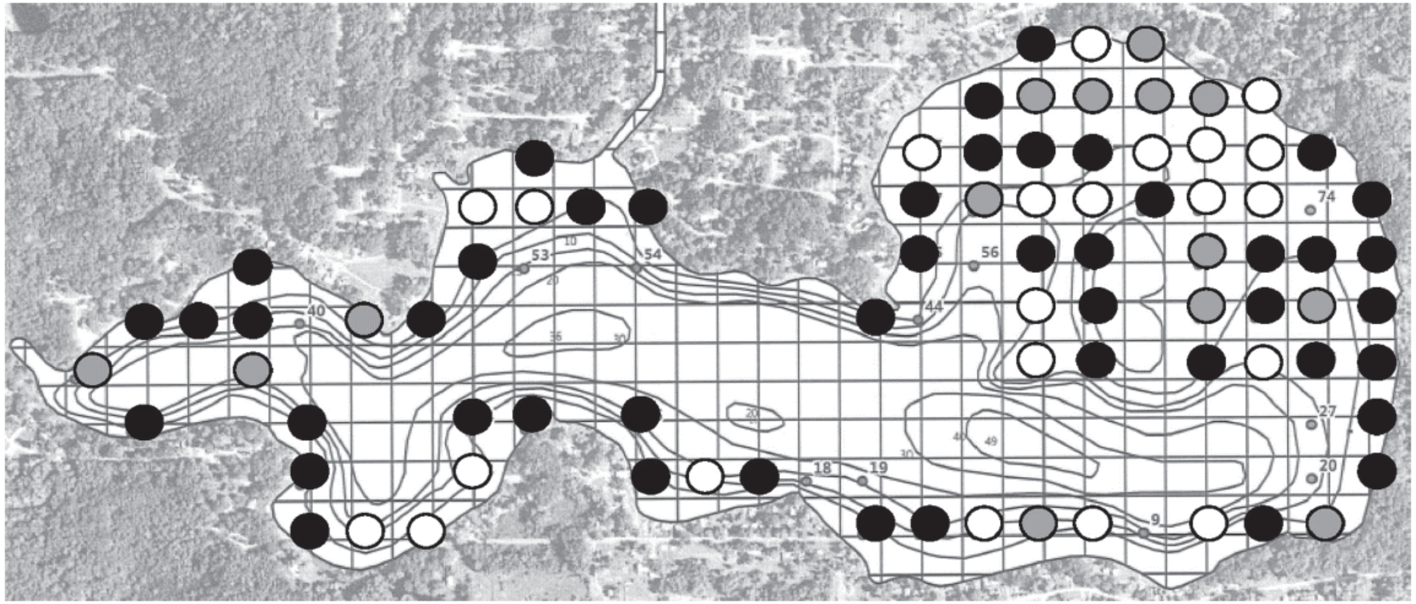
How Do We Prioritize Strains?



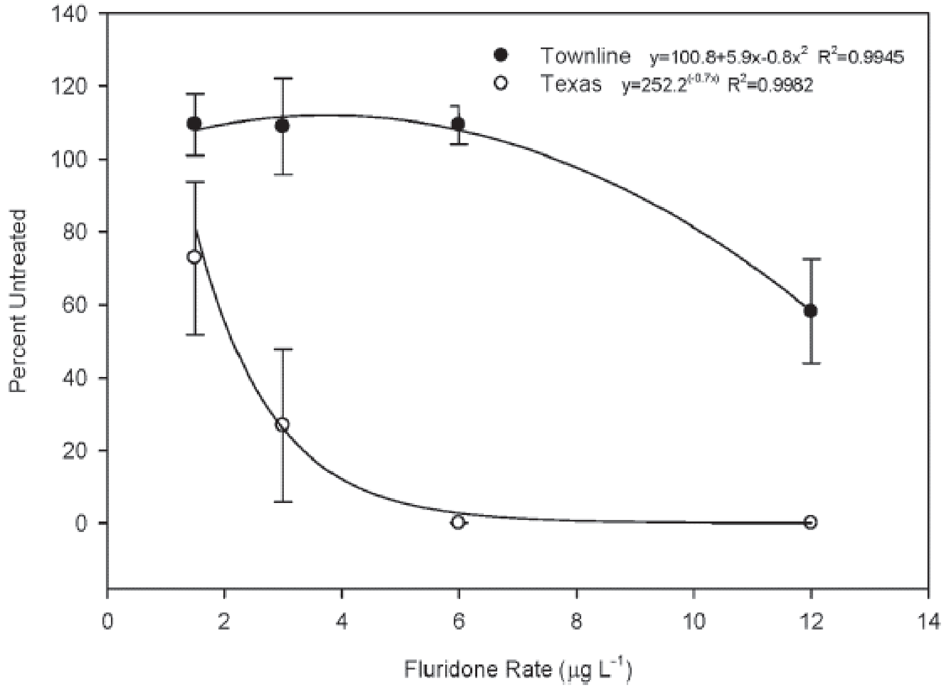


- Widespread strains identified through surveys
- Quantitative field estimates of strain-specific herbicide efficacy
- Credible manager accounts

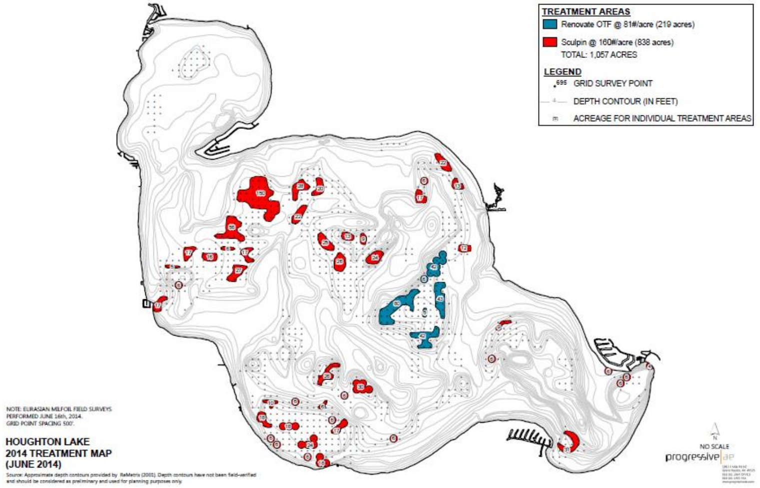
Quantitative Vegetation Mapping in a Lake with a Single Strain

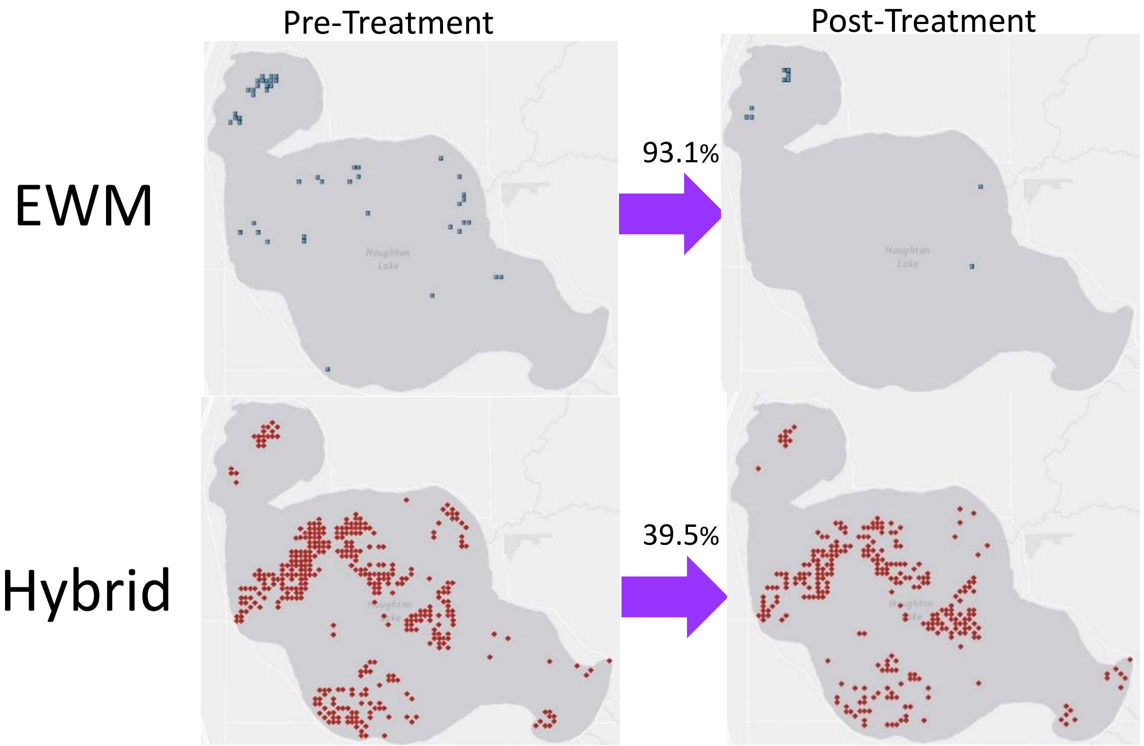


Thum et al. (2012)
J. Aquatic Plant Mgmt.



Quantitative Vegetation Mapping in a Lake with Multiple Strains





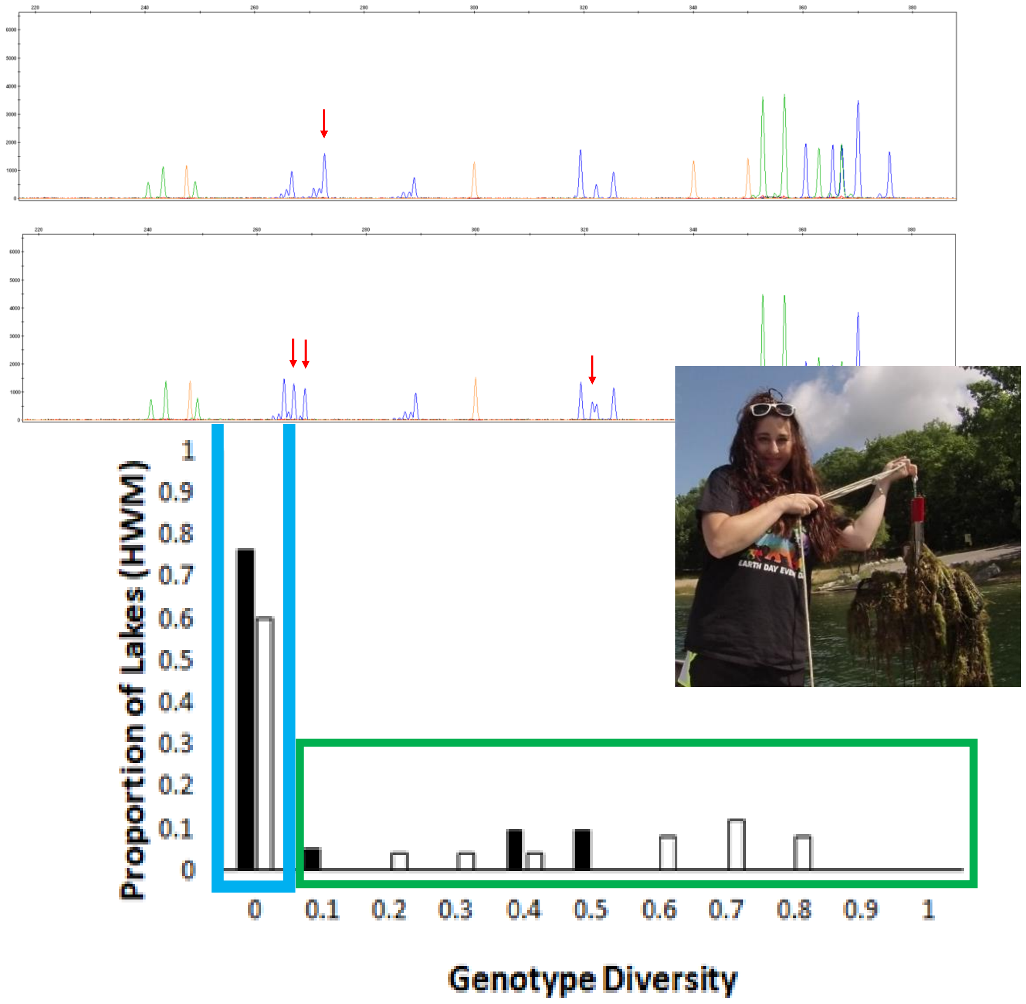
- 43% reduction of total watermilfoil
- Good EWM control, but poor HWM control
  - Zelen's test of odds ratio $p<0.001$

Parks et al. (2016)
Lake & Reservoir Management

Some Considerations

- **Number of strains**
- Initial survey (~20-50 plants) to characterize lakes
    - Some lakes have only a **single strain**
    - Other lakes have **multiple strains**
- Best to initially focus on lakes with one or a few strains
    - ability to detect differences depends on sample size for the strain

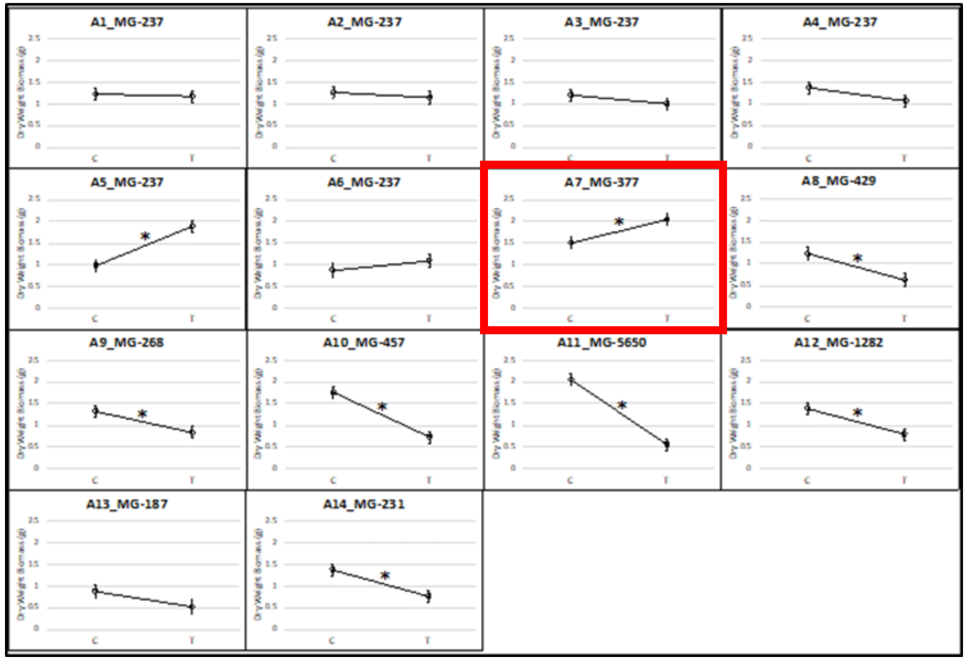
- **Treatment type**
- Easiest to focus on whole-lake treatments
    - Less confounded by dilution/dissipation



Thum et al. (2020)
Invasive Plant Science & Management

How Do We Prioritize strains?

- **Credible manager accounts**



Chorak and Thum (2020)
Invasive Plant Science & Management

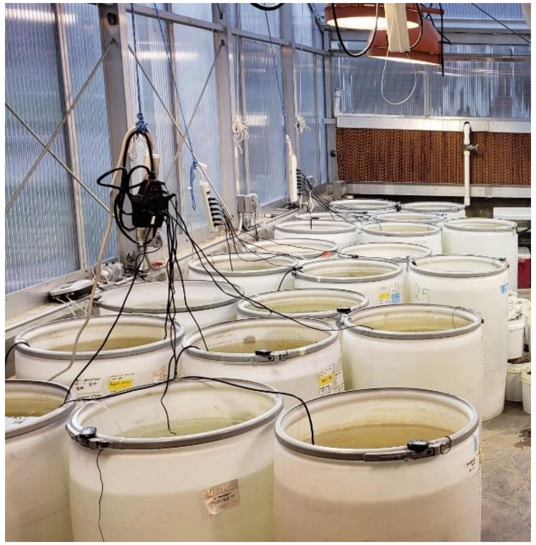
- Manager suspected resistance to 6 ppb based on field observation
- **Laboratory study at 6 ppb confirmed resistance**

SHORT TERM: Construct a catalog of herbicide responses for prioritized strains

- Prioritizing strains for characterization
- **Characterizing herbicide response**
- Molecular characterization of strains (“DNA Fingerprinting”)
- Building and curating a database

Characterizing herbicide response

- **Time and space constraints**
  - Faster assays
  - Increase concentration-exposure time (CET) combinations
  - Better in-field methods for estimating strain-specific response




SHORT TERM: Construct a catalog of herbicide responses for prioritized strains

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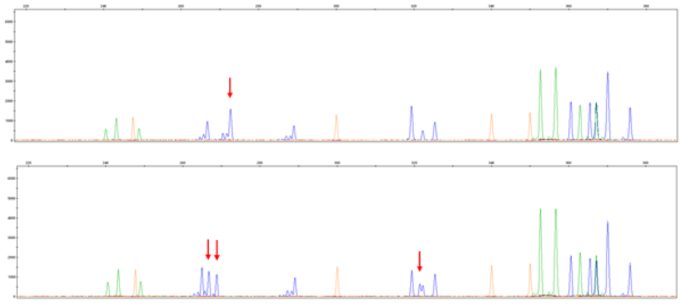
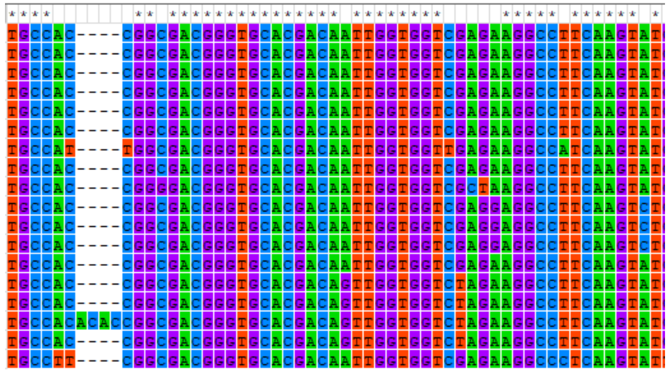
Molecular characterization of strains

- Replace fragment analysis methods with next-gen-sequencing-based methods
  - Data are less ambiguous
  - Error rates are lower
  - Easy to directly compare sequences from different laboratories because DNA sequences do not change whereas molecular marker scoring can



DNA "Fingerprints"



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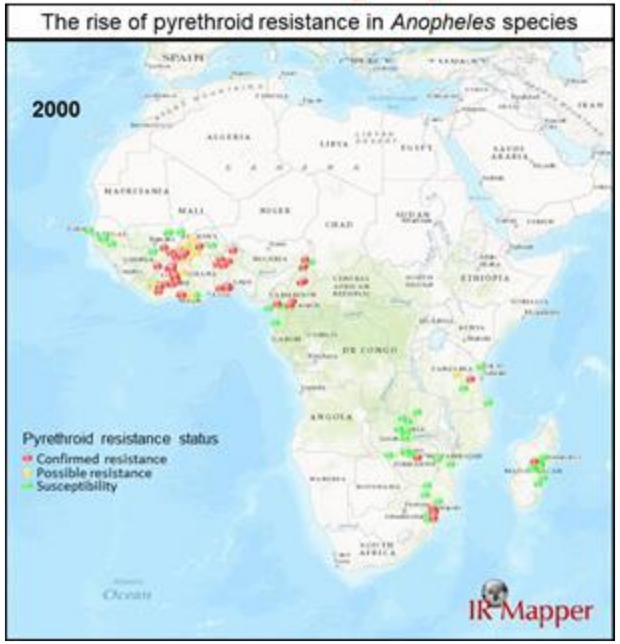
Building and curating a database

- Must be ‘living’ – able to be modified in real time
- Must be able to handle multiple types of data
  - Blast genetic data against it
  - Pull up visual data
  - Quantitative and qualitative data

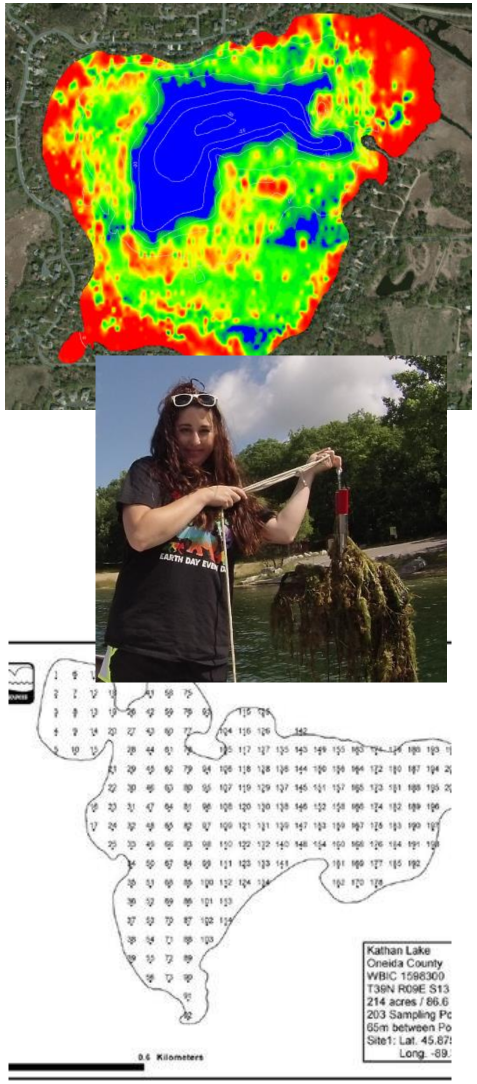
IR Mapper



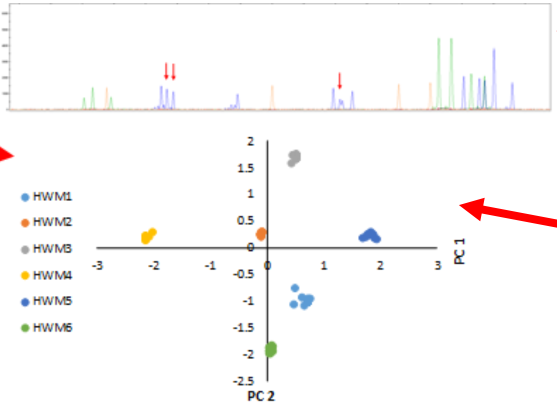
Nextstrain

Real-time tracking of pathogen evolution

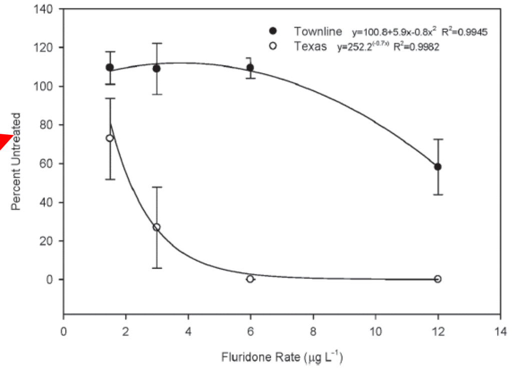
Short-term: Build a Dynamic 'Catalog' of Herbicide Response for a Prioritized Set of Milfoil Strains

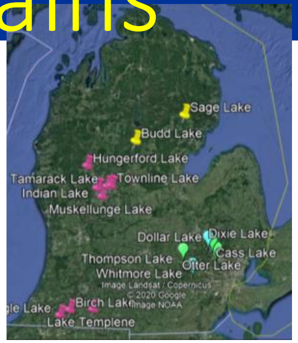


Centralized database of strain information



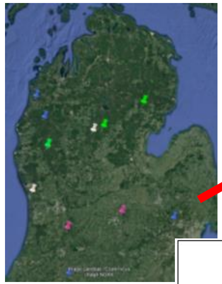
Herbicide response data available for strain(s)





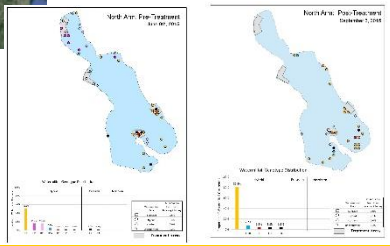
Influence control options and permitting.

Herbicide response data NOT available for strain(s)



Prioritize for characterization

- **Widespread**
- **Quantitative Field Data on Response**
- **Credible Manager Account**



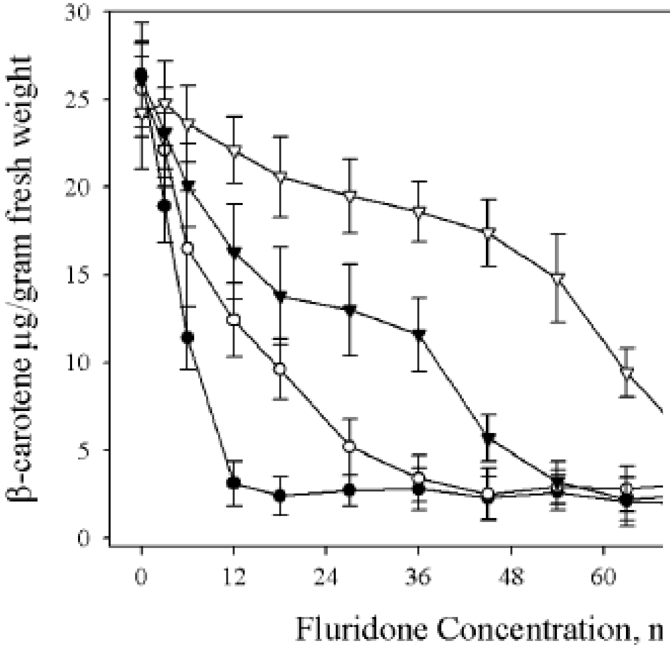
Short- and long- term approaches to using genetic markers to inform best management practices

- SHORT TERM: Construct a catalog of herbicide responses for prioritized strains

- **LONG TERM: Identifying genes involved in herbicide response in order to develop specific genetic assays to predict herbicide response**

Example: Genetic Prediction of Fluridone Susceptibility vs Resistance in Hydrilla

http://www.northeastans.org/online-guide/files/9wpCqMpA/F4-Hydrilla-verticillata.jpg



Molecular Ecology (2004) 13, 3229–3237 doi: 10.1111/j.1365-294X.2004.02280.x

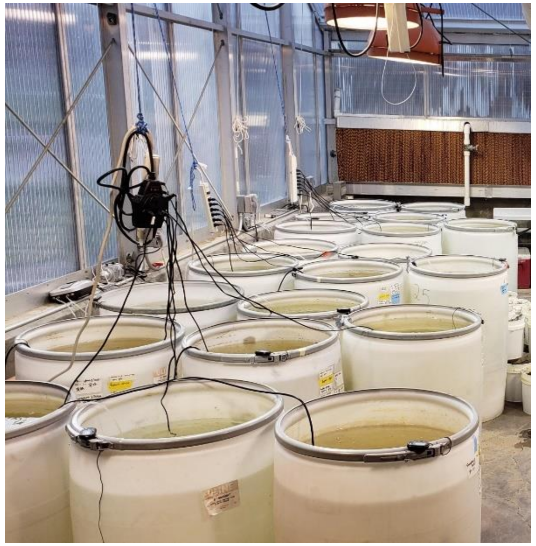
Somatic mutation-mediated evolution of herbicide resistance in the nonindigenous invasive plant hydrilla (*Hydrilla verticillata*)

ALBRECHT MICHEL,* RENEE S. ARIAS,* BRIAN E. SCHEFFLER,* STEPHEN O. DUKE,* MICHAEL NETHERLAND† and FRANCK E. DAYAN*

Location		
Rainbow River	cDNA/genomic DNA	ATTGCCTTAAAC**CGT**TTCCTTCAGGAA
	Amino acid	-I--A--L--N--**R**--F--L--Q--E-
Lulu	cDNA/genomic DNA	ATTGCCTTAAAC**AGT**TTCCTTCAGGAA
	Amino acid	-I--A--L--N--**S**--F--L--Q--E-
Pierce	cDNA/genomic DNA	ATTGCCTTAAAC**TGT**TTCCTTCAGGAA
	Amino acid	-I--A--L--N--**C**--F--L--Q--E-
Okahumpka	cDNA/genomic DNA	ATTGCCTTAAAC**CAT**TTCCTTCAGGAA
	Amino acid	-I--A--L--N--**H**--F--L--Q--E-

Controlled crosses to create "genetic mapping populations" that vary in herbicide sensitivity

- Cross sensitive strain to resistant strain
- Cross F_1 progeny to produce F_2 population that segregates (varies) for resistance
- Genotype all progeny and expose to herbicide

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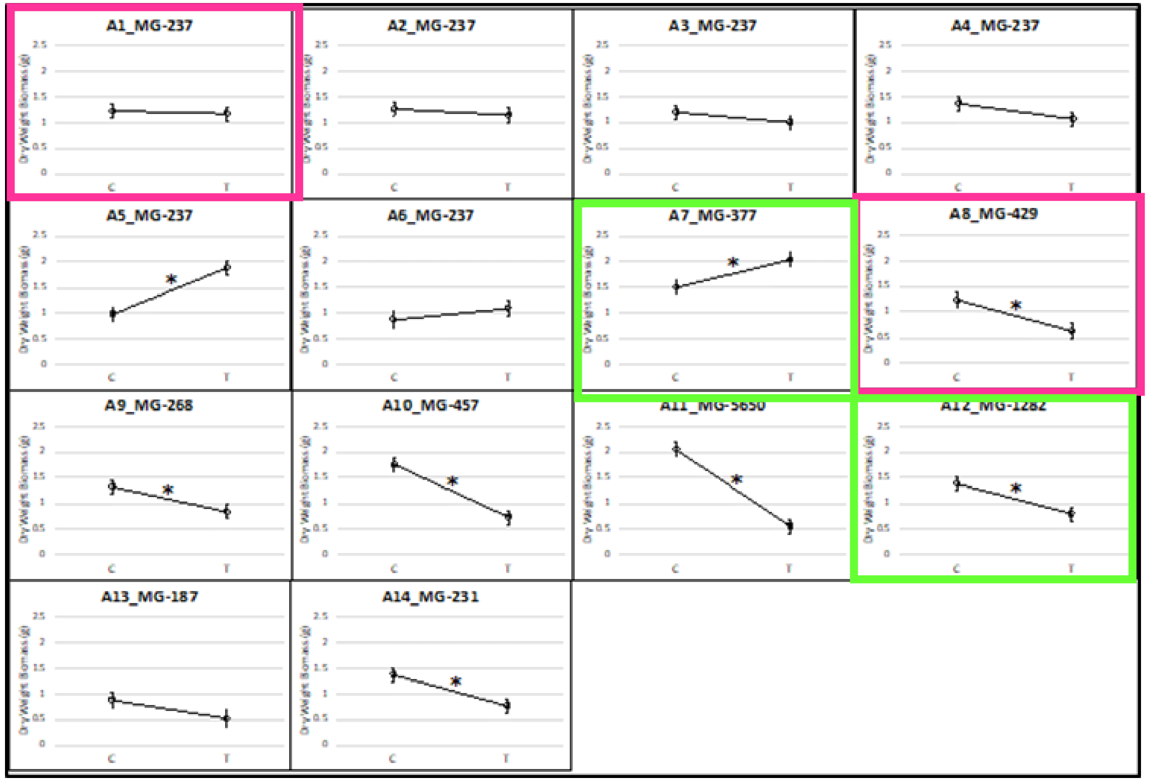
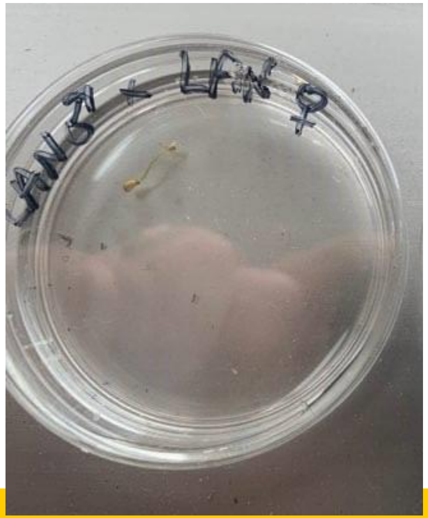
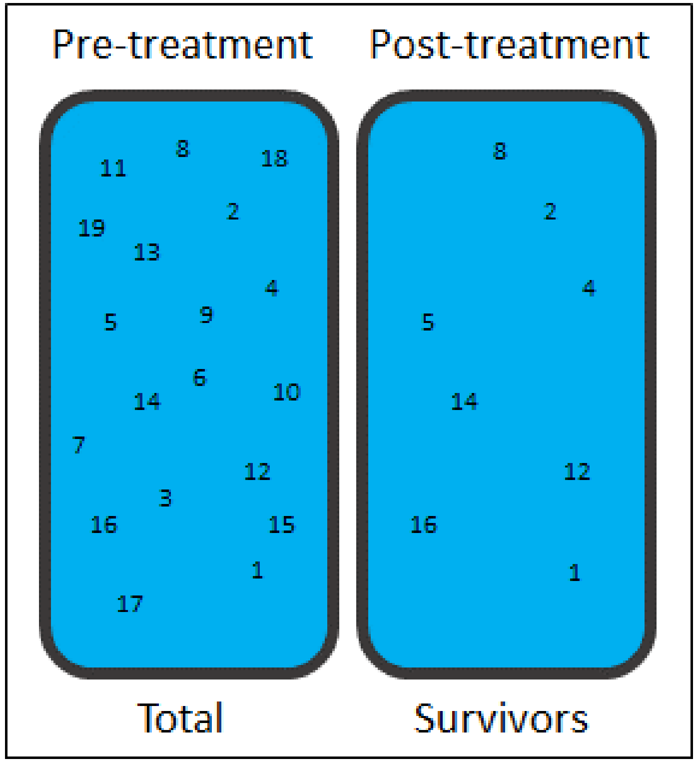


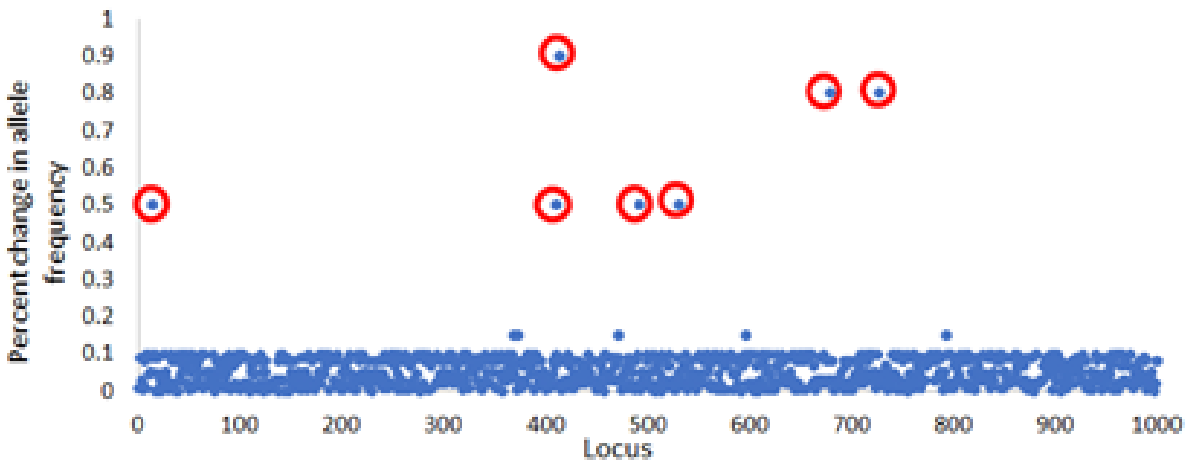
Chorak and Thum (2020)
Invasive Plant Science & Management



Herbicide selection experiments



- For each gene, compare allele frequencies pre- vs post-treatment
- Expect resistance genes to increase in frequency post-treatment



Acknowledgements

- Greg Chorak, Ray Newman, Jo Latimore, Erick Elgin, Syndell Parks, James McNair
- Mark Heilman, Paul Hausler, Rick Buteyn, Pam Tyning, Emma Rice, Hannah Hoff, Katie Gannon, Leah Simantel, Anna French, Jasmine Eltawely, Eric Fieldseth

Invasive Watermilfoil Biology and Management Strategies

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Minnesota State University, Mankato

[2]Research Biologist
USDA-ARS
Davis, California

USACE Invasive Species Webinars – November 12, 2020

Eurasian Watermilfoil

- *Myriophyllum spicatum*
- Submersed evergreen perennial introduced from Europe
- Spreads by root crowns, runners, and fragments
- Grows in 2 to 15 feet of water, forms surface canopy
- Leaves are in whorls of 4 around stem
- *ID Characteristics:* Flat leaf ends, Flat apical meristem, > 12 leaflet pairs

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Eurasian Watermilfoil

Use more than one characteristic to consistently identify watermilfoil species. Reliance on only one characteristic may lead to false identification and misplacement of management techniques

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Hybrid Watermilfoils

- Hybrid milfoil (*Myriophyllum spicatum* X *Myriophyllum sibiricum*) is becoming more and more dominant in Midwestern and western lakes given the effective management of Eurasian watermilfoil (*Myriophyllum spicatum*)

- Studies have indicated that hybrid milfoil is tolerant to 2,4-D, fluridone, norflurozon, and topramazone (Berger et al. 2012, Thum et al. 2012, LaRue 2013, Berger et al. 2015)

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Morphological variation: differences between *M. spicatum*, *M. sibiricum* and hybrid *M. spicatum* x *M. sibiricum*

M. spicatum **hybrid** ***M. sibiricum***

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Slide Credit: Michael Moody

Variable-leaf Watermilfoil

- *Myriophyllum heterophyllum*
- Native to Quebec and Ontario in Canada, and North Dakota and south to New Mexico and Florida in the United States (Godfrey and Wooten 1981)
- Introduced into Connecticut, Maine, Massachusetts, and New Hampshire
- Canopy forming submersed aquatic plant

Photo Credit: Amy Smagula

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Parrotfeather

- *Myriophyllum aquaticum*
- Non-native aquatic plant from South America
- Brought to U.S. in the late 1800's likely as an ornamental plant
- Has been, and continues to be, spread by the aquarium and water garden industries
- Plant providers in San Francisco used to plant parrotfeather in the drainage canals behind their stores to have readily available plants

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- Parrotfeather is heterophyllous
- Plants can grow emergent leaves and/or submersed leaves
- May have implications for selection of control techniques

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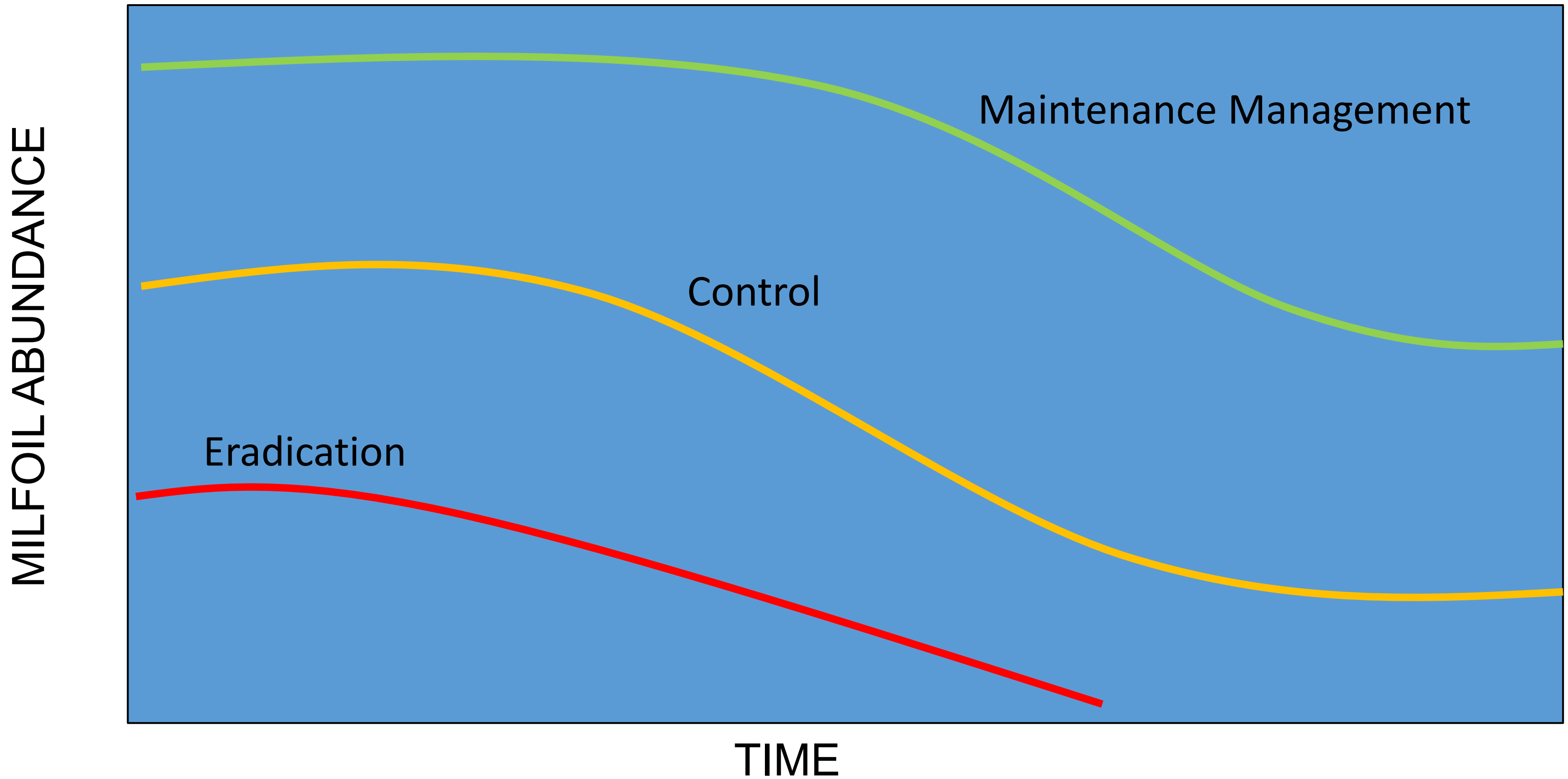
Should We Manage?

- Are the environmental impacts of invasive plants a threat to our resource?

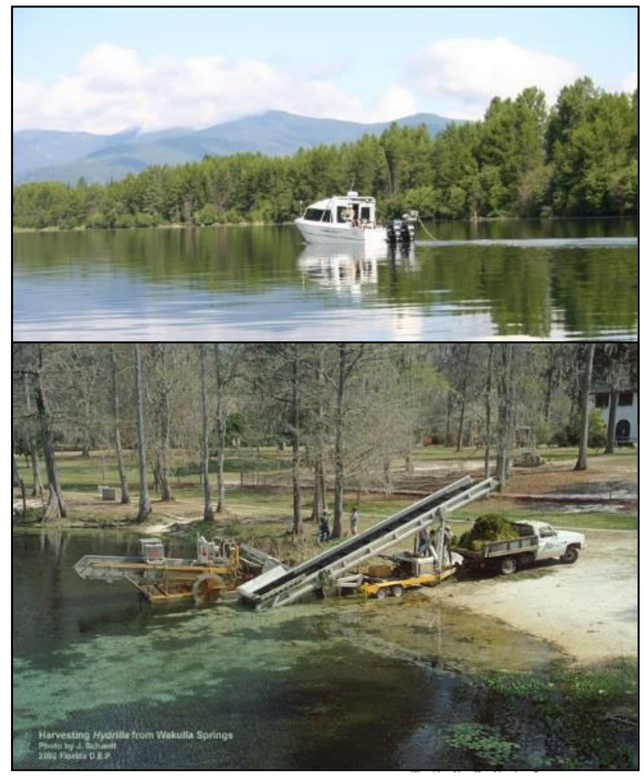
- What is the real impact of management techniques relative to no management, or to each other?

What are Reasonable Expectations?



Management Strategies

- Goal: Long-term management to cause population decline
  - Target points: carbohydrate storage, root crowns, growing points, and times of year to control invasive watermilfoils
- Manage in a way that is:
  - Cost-effective
  - Minimizes environmental damage
  - Complies with laws and regulations
  - Acceptable to stakeholders
  - Technically feasible and defensible



Seasonal Resource Allocation in Plants

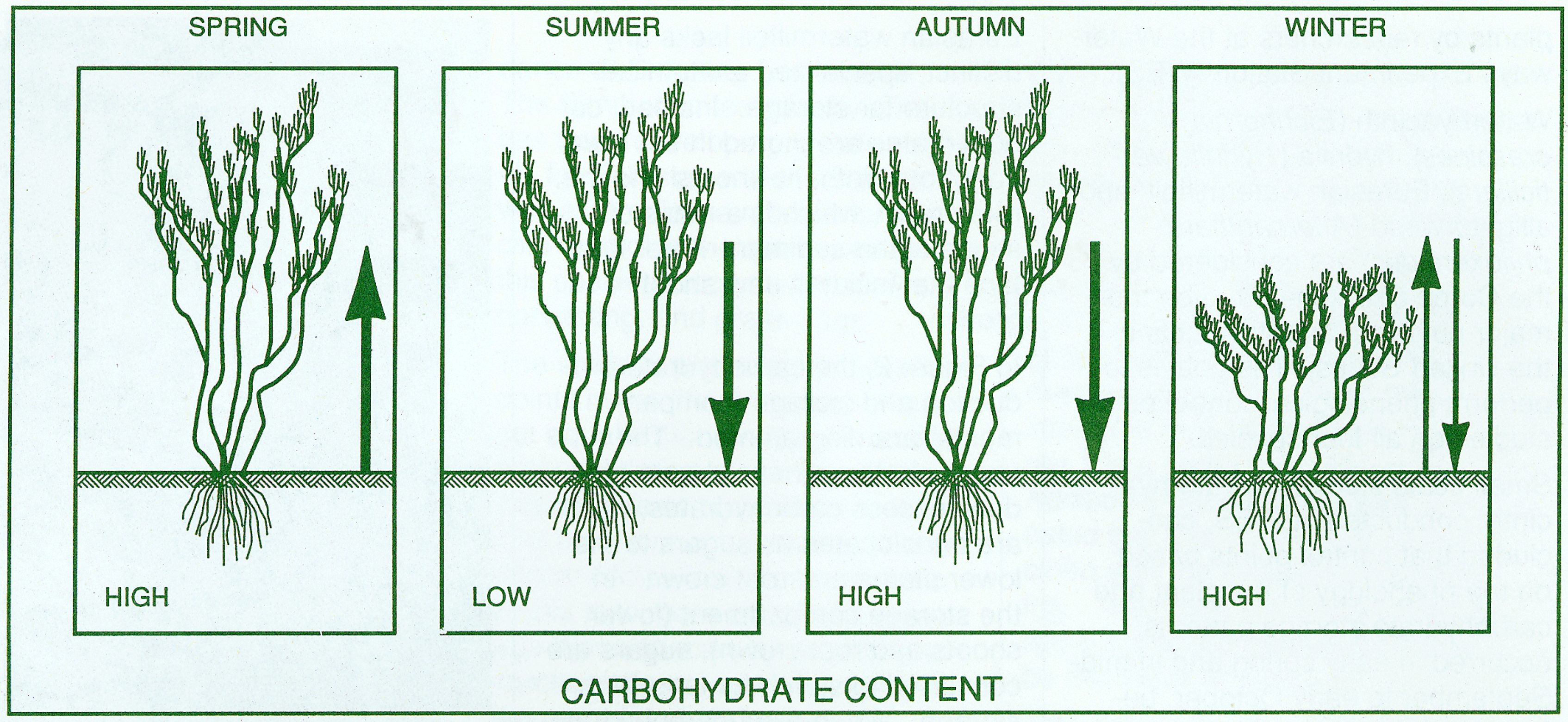


Figure 3. Seasonal cycle of carbohydrate usage and storage in Eurasian watermilfoil

Madsen 1993

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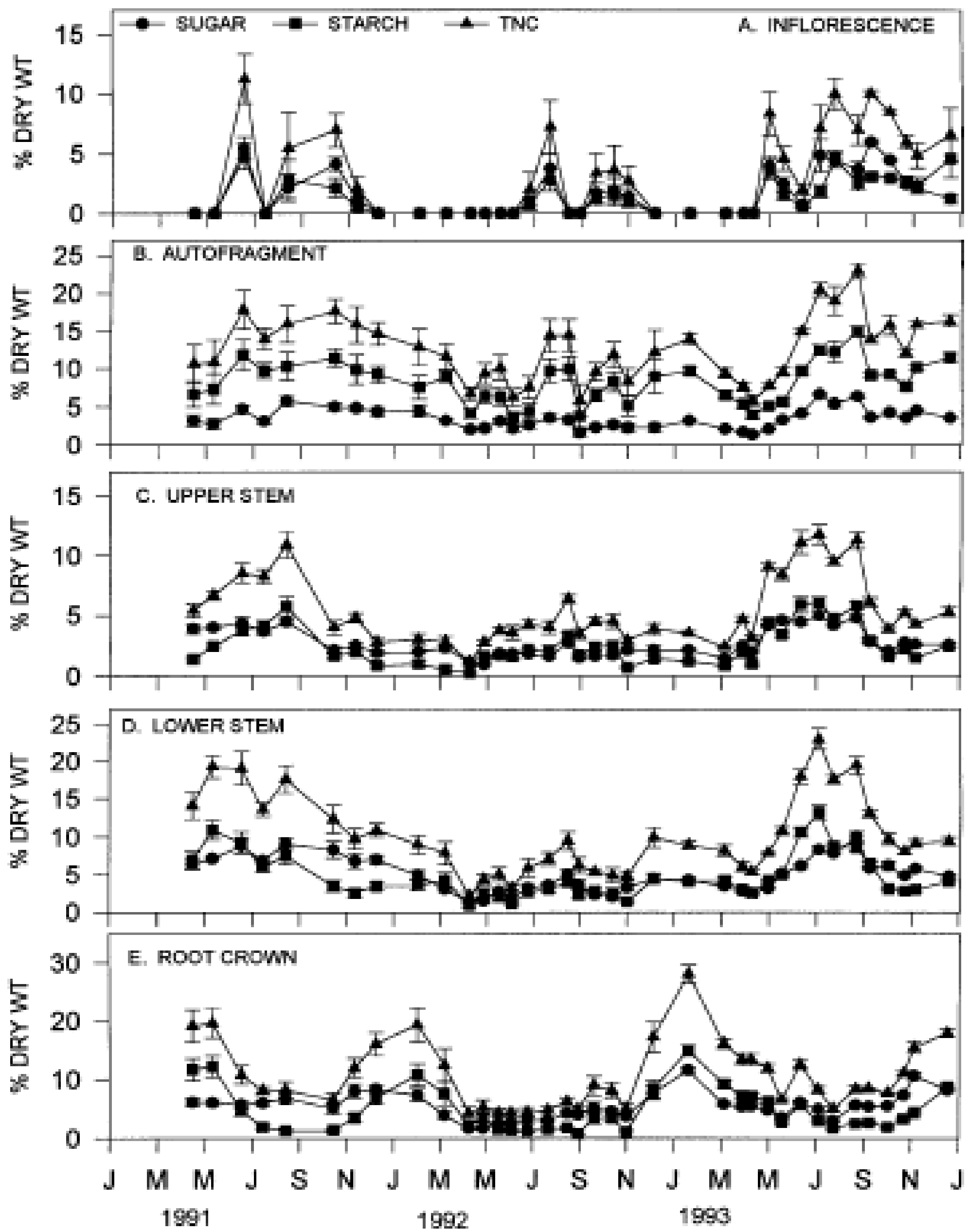
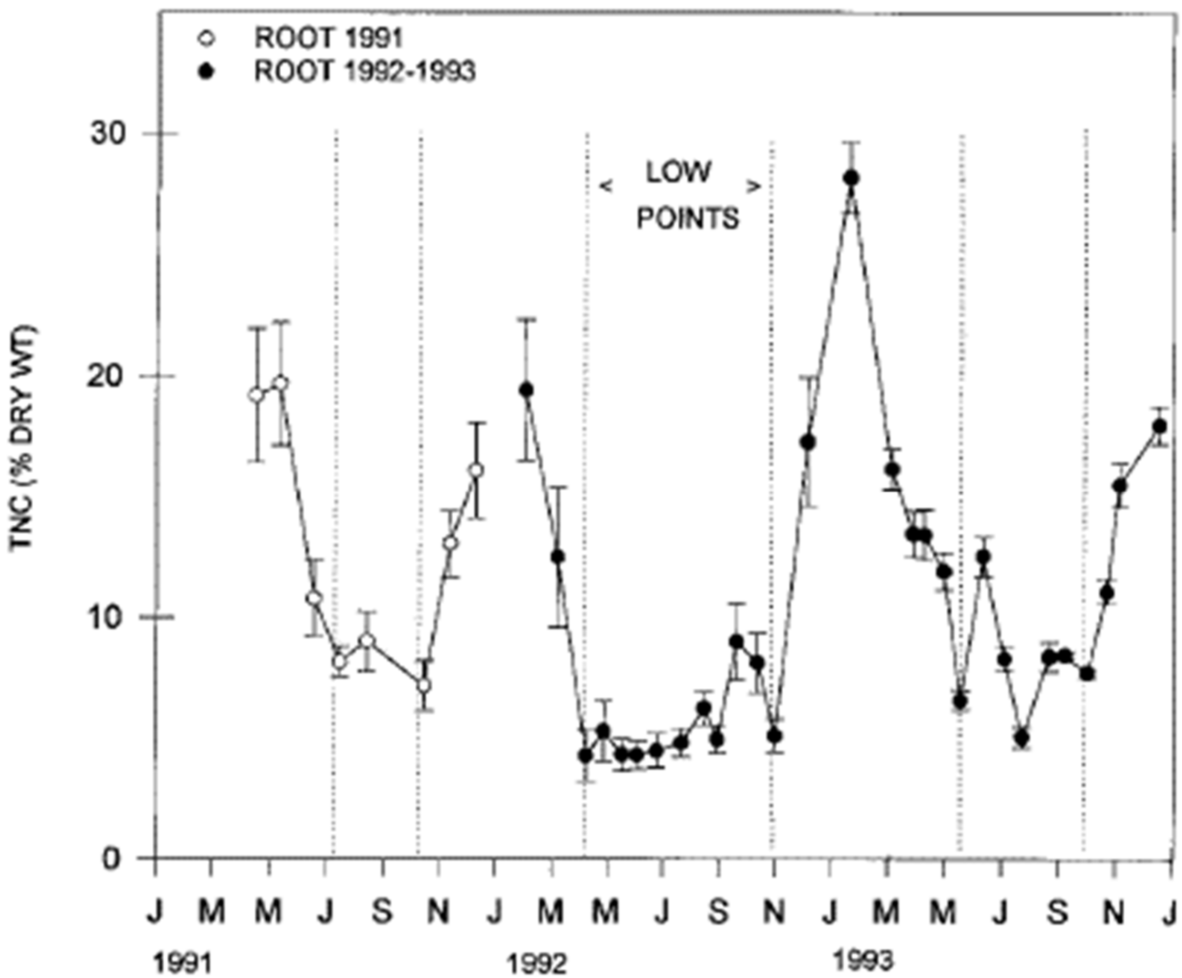
Eurasian watermilfoil



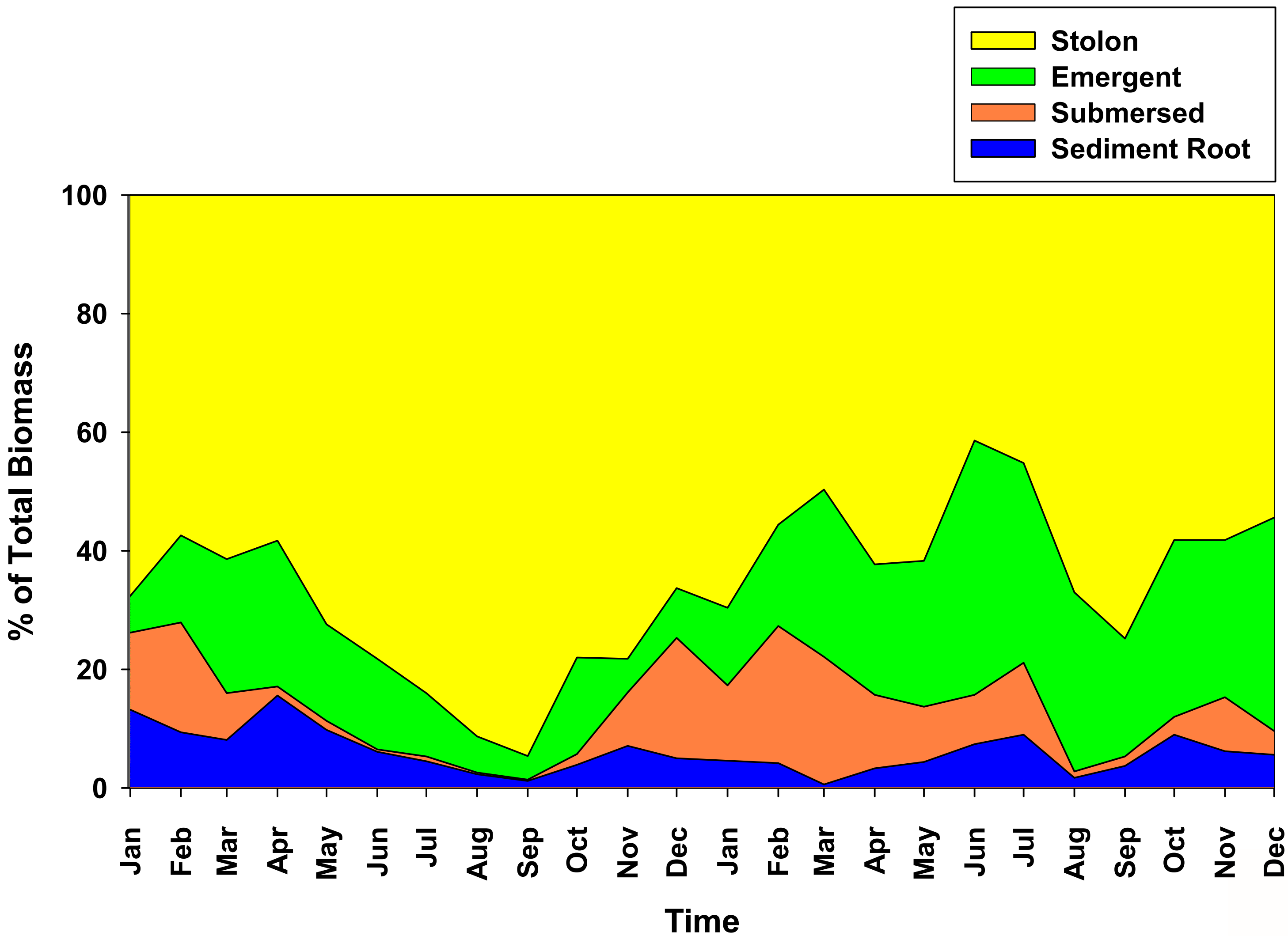
Madsen 1997

Eurasian watermilfoil



Madsen 1997

Parrotfeather



Wersal and Madsen 2011

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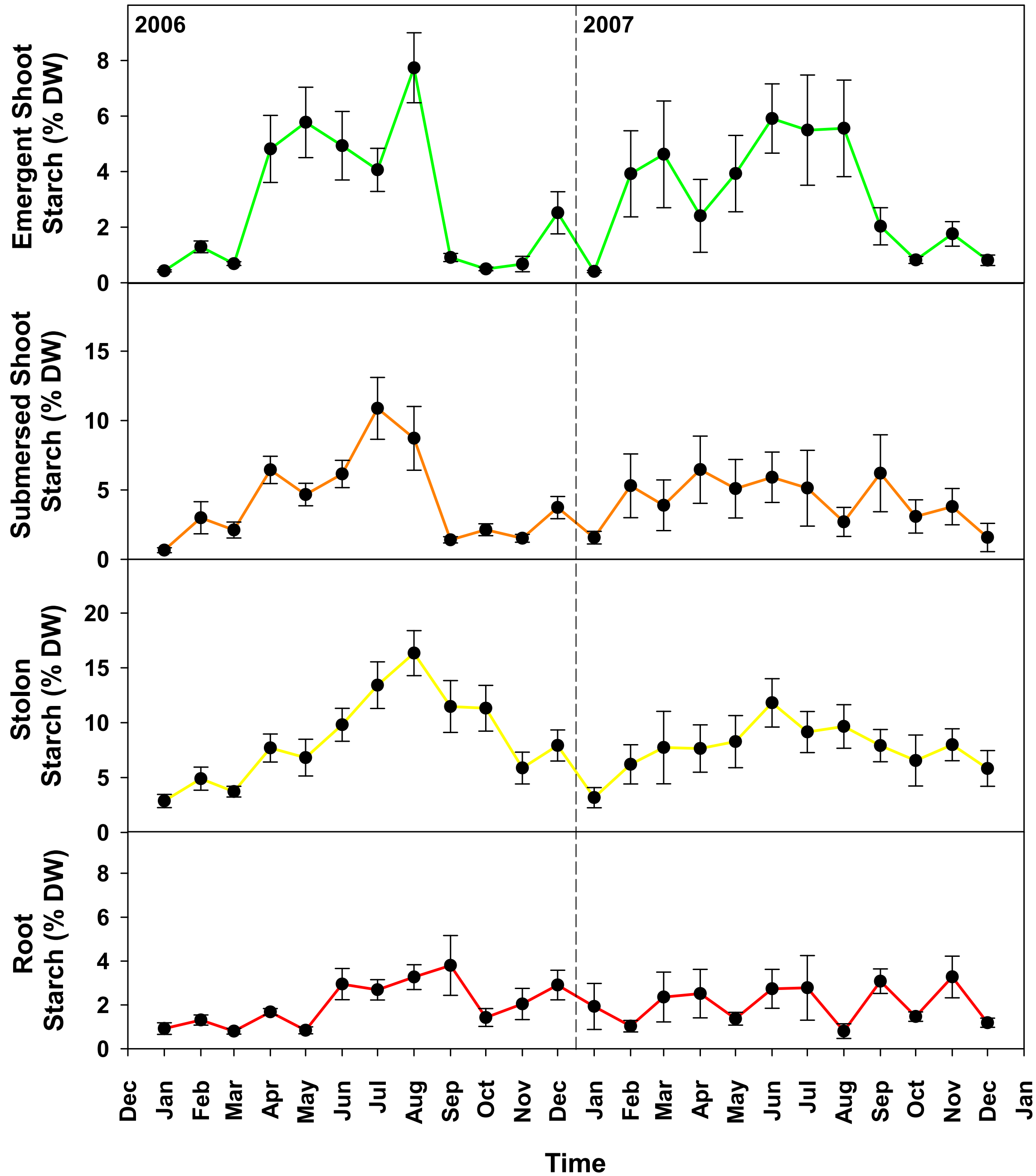
Parrotfeather



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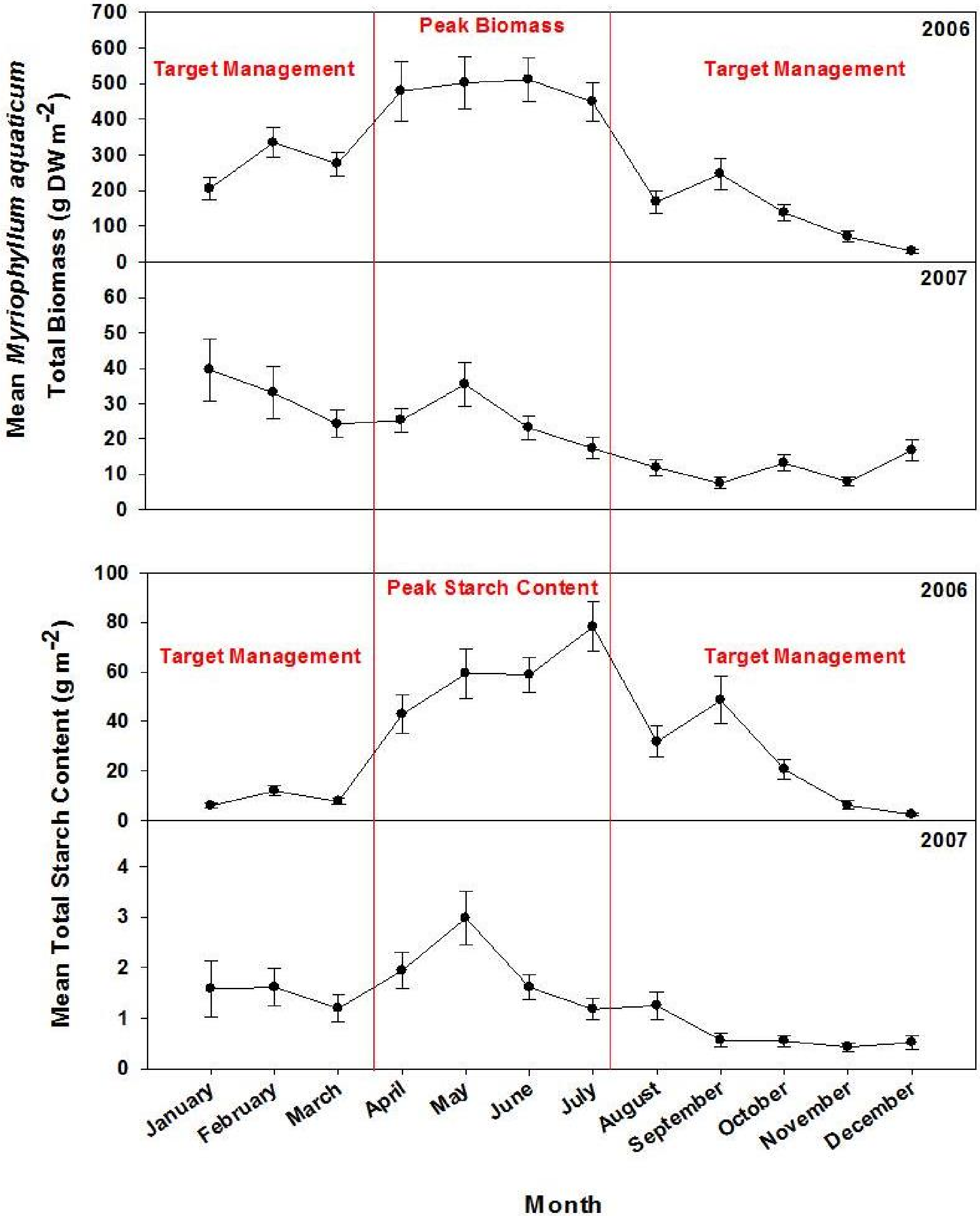
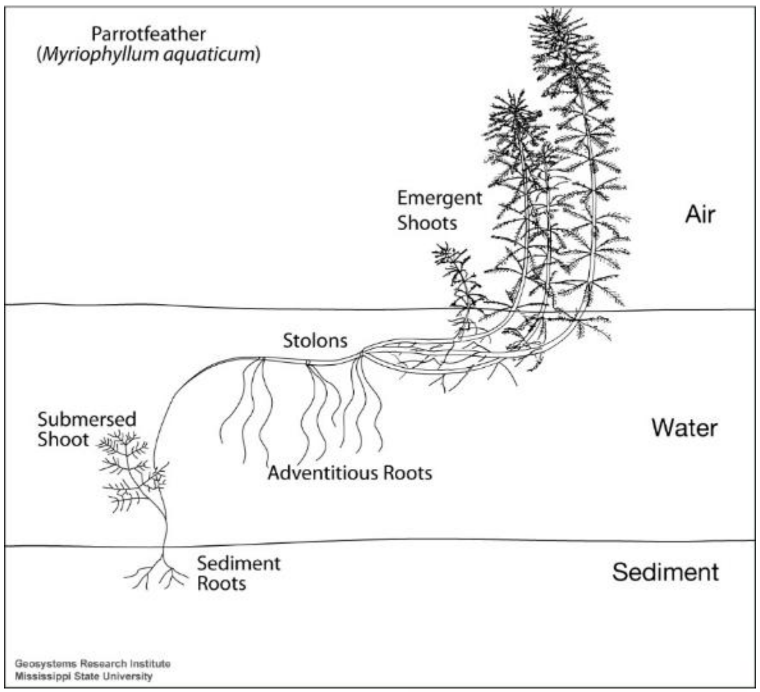
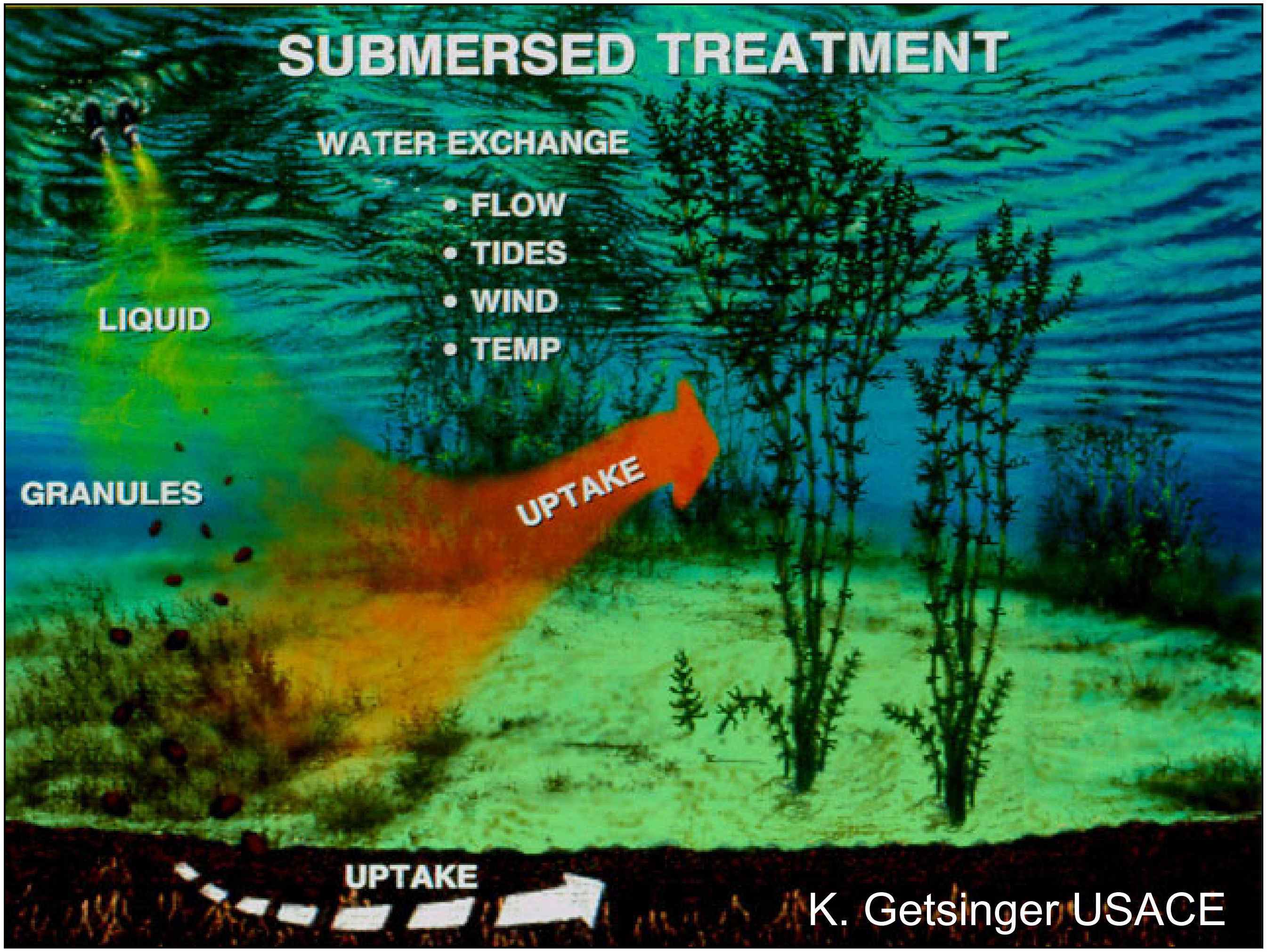




Management Examples



Eurasian Watermilfoil

Lake Pend Oreille, ID

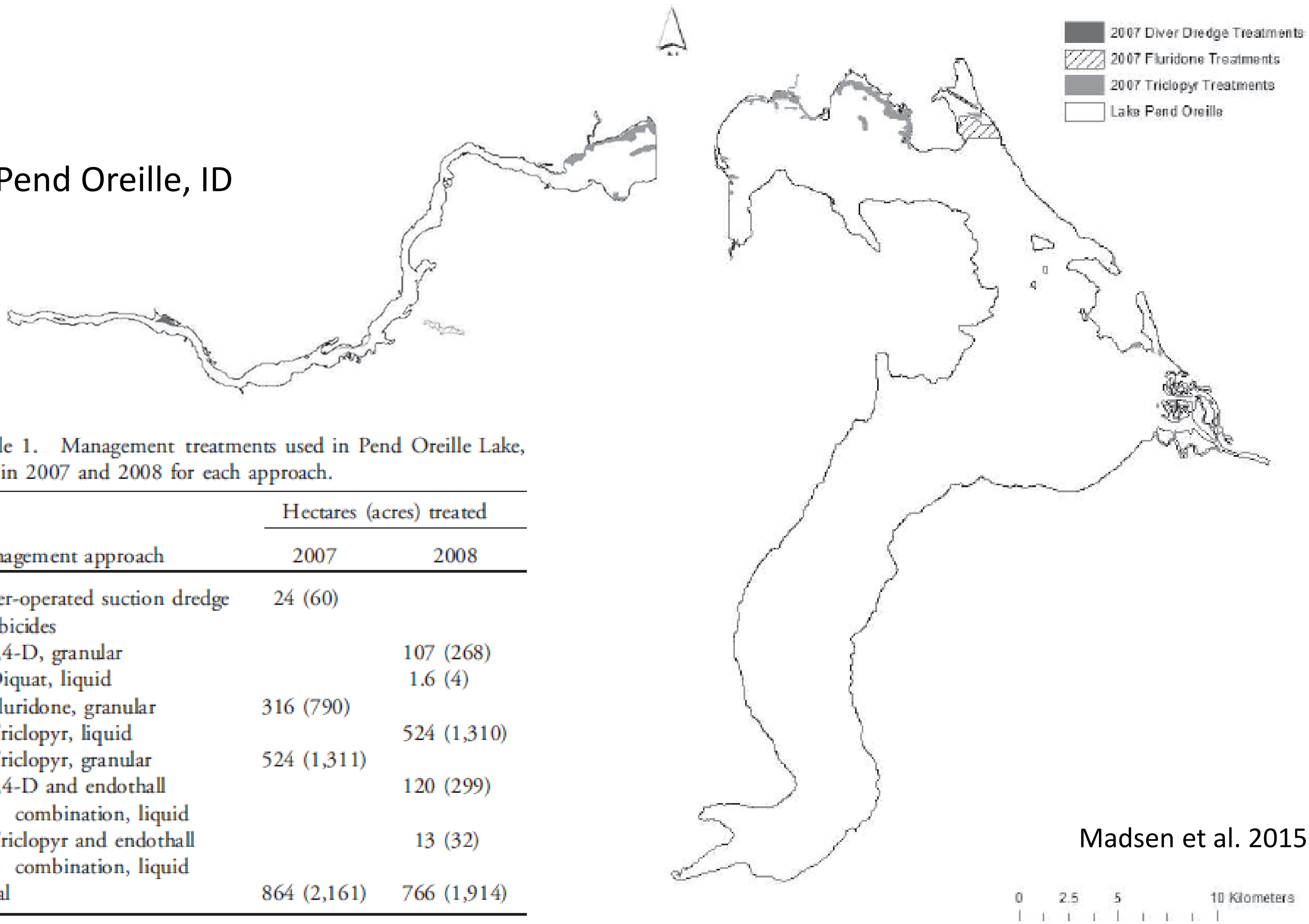


Table 1. Management treatments used in Pend Oreille Lake, ID, in 2007 and 2008 for each approach.

	Hectares (acres) treated	
Management approach	2007	2008
Diver-operated suction dredge	24 (60)	
Herbicides		
2,4-D, granular		107 (268)
Diquat, liquid		1.6 (4)
Fluridone, granular	316 (790)	
Triclopyr, liquid		524 (1,310)
Triclopyr, granular	524 (1,311)	
2,4-D and endothall combination, liquid		120 (299)
Triclopyr and endothall combination, liquid		13 (32)
Total	864 (2,161)	766 (1,914)

Madsen et al. 2015

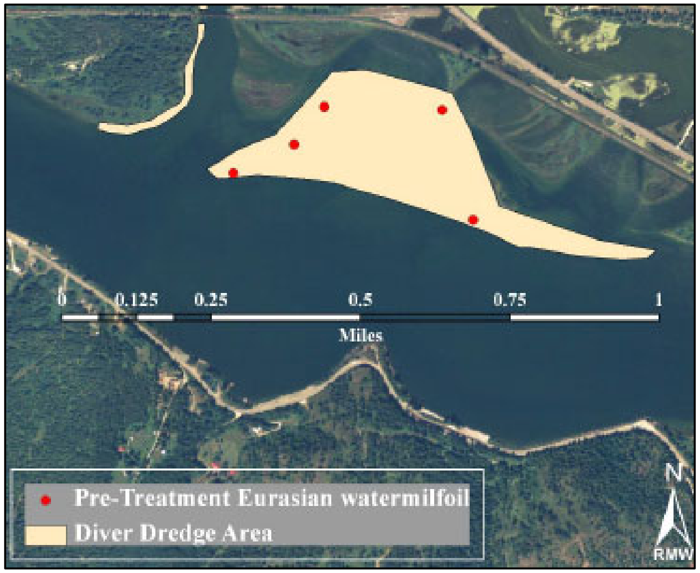
Eurasian Watermilfoil

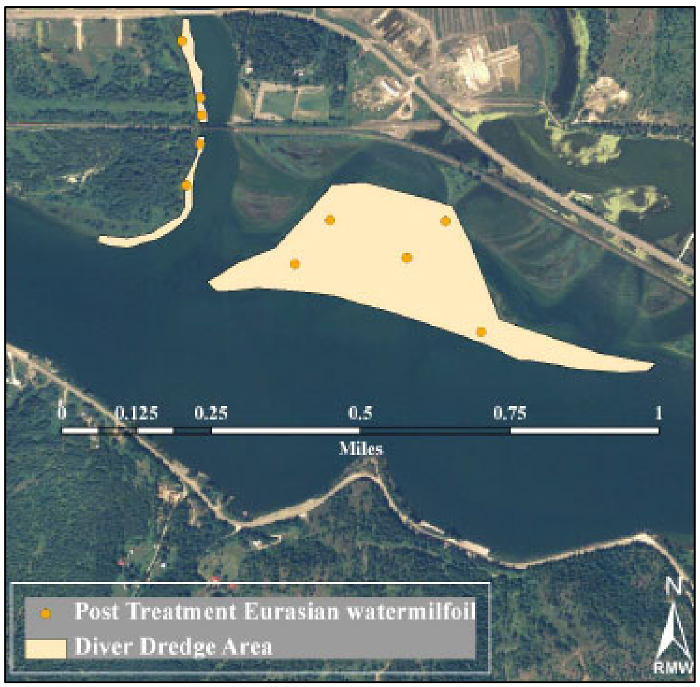
Species	Common name	% Occurrence 2007	% Occurrence 2008	P value ($n = 624$)
Ceratophyllum demersum L.	Coontail	14.8	11.3	0.18
Chara sp.	Muskgrass	20.2	18.3	0.55
Elodea canadensis Michx.	Elodea	31.4	33.0	0.68
Heteranthera dubia (Jacq.) Small	Water stargrass	0.41	0.52	0.84
Myriophyllum sibiricum Komarov	Northern watermilfoil	10.7	3.9	<0.01
Myriophyllum spicatum L.	Eurasian watermilfoil	64.5	23.6	<0.01
Myriophyllum verticillatum L.	Whorled watermilfoil	3.3	0.5	<0.01
Najas flexilis (Willd.) Rostk. and Schmidt	Slender naiad, bushy pondweed	4.5	4.1	0.83
Nitella sp.	Nitella	1.6	1.0	0.51
Phalaris arundinacea L.	Reed canary grass	0.0	0.4	0.24
Potamogeton crispus L.	Curly-leaf pondweed	6.2	20.1	<0.01
Potamogeton foliosus Raf.	Leafy pondweed	26.0	26.7	0.85
Potamogeton gramineus L.	Variable-leaf pondweed	5.3	5.5	0.94
Potamogeton natans L.	Floating-leaved pondweed	0.4	0.5	0.84
Potamogeton nodosus Poir.	American pondweed	1.6	0.0	0.01
Potamogeton praelongus Wulf.	White-stem pondweed	0.4	0.0	0.20
Potamogeton pusillus L.	Narrow-leaf pondweed	0.0	0.2	0.42
Potamogeton richardsonii (Ar. Benn.) Rydb.	Clasping-leaved pondweed	6.2	10.0	0.10
Potamogeton zosteriformis Fern.	Flat-stemmed pondweed	3.7	2.1	0.22
Ranunculus aquatilis L.	White water-buttercup	8.6	3.0	<0.01
Stuckenia pectinata (L.) Börner	Sago pondweed	14.8	9.4	0.03
Native species richness (per point)		1.5 ± 0.08	1.3 ± 0.04	<0.01
Mean species richness (per point)		2.2 ± 0.08	1.7 ± 0.05	<0.01

Eurasian Watermilfoil

		2008 % Occurrence		
Species	Common name	Treated	Untreated	P value
Ceratophyllum demersum L.	Coontail	10.5	18.3	0.04
Chara sp.	Muskgrass	19.4	15.8	0.42
Elatine minima (Nutt.) Fisch. and Mey.	Waterwort	0.0	0.8	0.21
Elodea canadensis Michx.	Elodea	34.0	35.0	0.86
Myriophyllum sibiricum Komarov	Northern watermilfoil	3.1	6.6	0.14
Myriophyllum spicatum L.	Eurasian watermilfoil	23.0	52.5	<0.01
Myriophyllum verticillatum L.	Whorled watermilfoil	0.5	0.8	0.73
Najas flexilis (Willd.) Rostk. and Schmidt	Slender naiad, bushy pondweed	4.2	1.6	0.21
Nitella sp.	Nitella	0.0	0.0	0.38
Potamogeton crispus L.	Curly-leaf pondweed	18.9	25.0	0.19
Potamogeton foliosus Raf.	Leafy pondweed	24.6	14.1	0.02
Potamogeton gramineus L.	Variable-leaf pondweed	6.8	6.6	0.96
Potamogeton natans L.	Floating-leaved pondweed	0.0	0.8	0.20
Potamogeton richardsonii (Ar. Benn.) Rydb.	Clasping-leaved pondweed	7.8	8.3	0.87
Potamogeton zosteriformis Fern.	Flat-stemmed pondweed	2.6	8.3	0.02
Ranunculus aquatilis L.	White water-buttercup	4.2	14.1	<0.01
Stuckenia pectinata (L.) Börner	Sago pondweed	10.9	5.0	0.06
Native species richness (per point)		1.3 ± 0.1	1.3 ± 0.1	0.65
Mean species richness (per point)		1.7 ± 0.1	2.0 ± 0.1	<0.01

Eurasian Watermilfoil





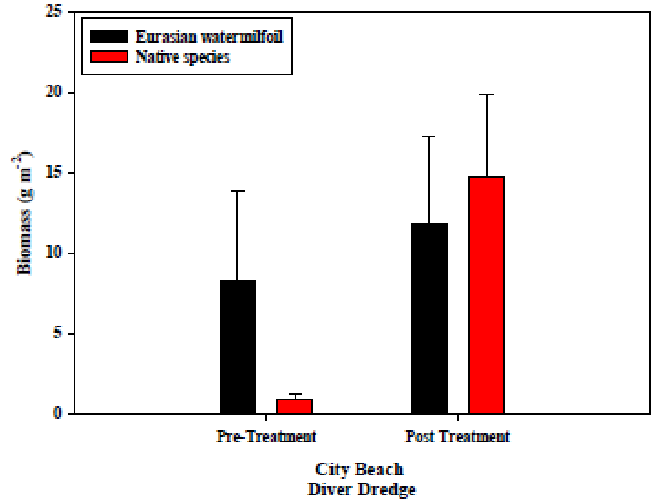


Figure 2-25. Mean pre-treatment and post treatment biomass of Eurasian watermilfoil and native plant species in the diver dredge area near City Beach in Sandpoint.

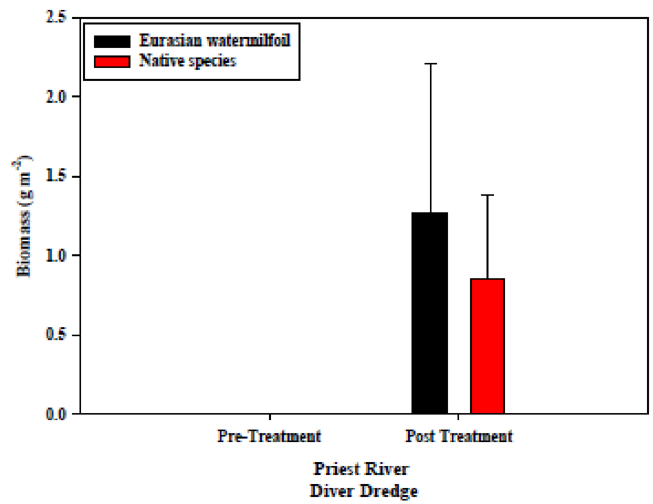


Figure 2-26. Mean pre-treatment and post treatment biomass of Eurasian watermilfoil and native plant species in the diver dredge areas near Priest River.

Madsen and Wersal 2008

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Eurasian Watermilfoil

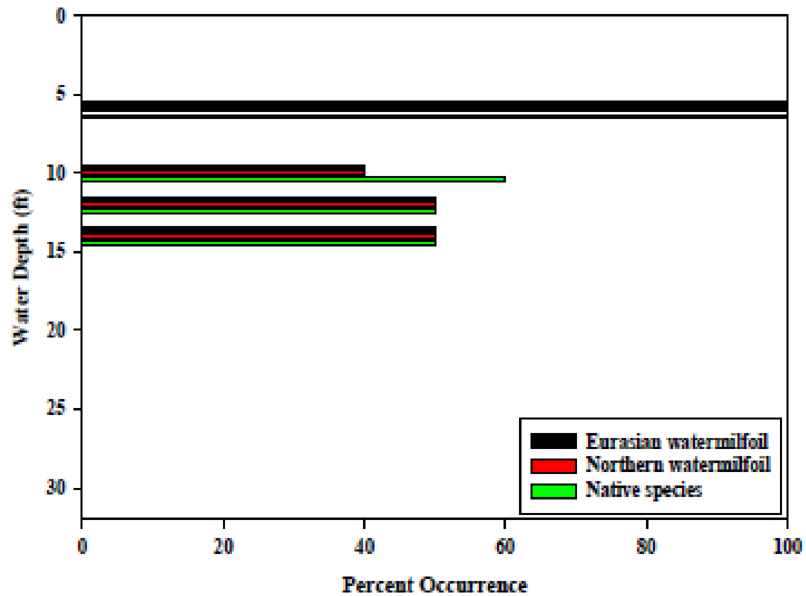

Figure 2.29. Depth distribution of the percent occurrence of Eurasian watermilfoil, northern watermilfoil, and native plant species during the pre-treatment assessment of diver dredge areas.

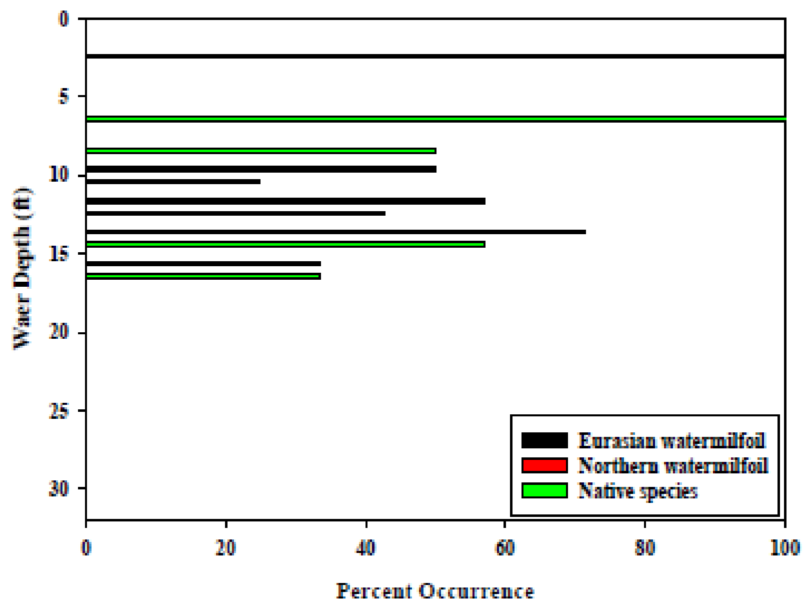

Figure 2.30. Depth distribution of the percent occurrence of Eurasian watermilfoil, northern watermilfoil, and native plant species during the post treatment assessment of diver dredge areas.

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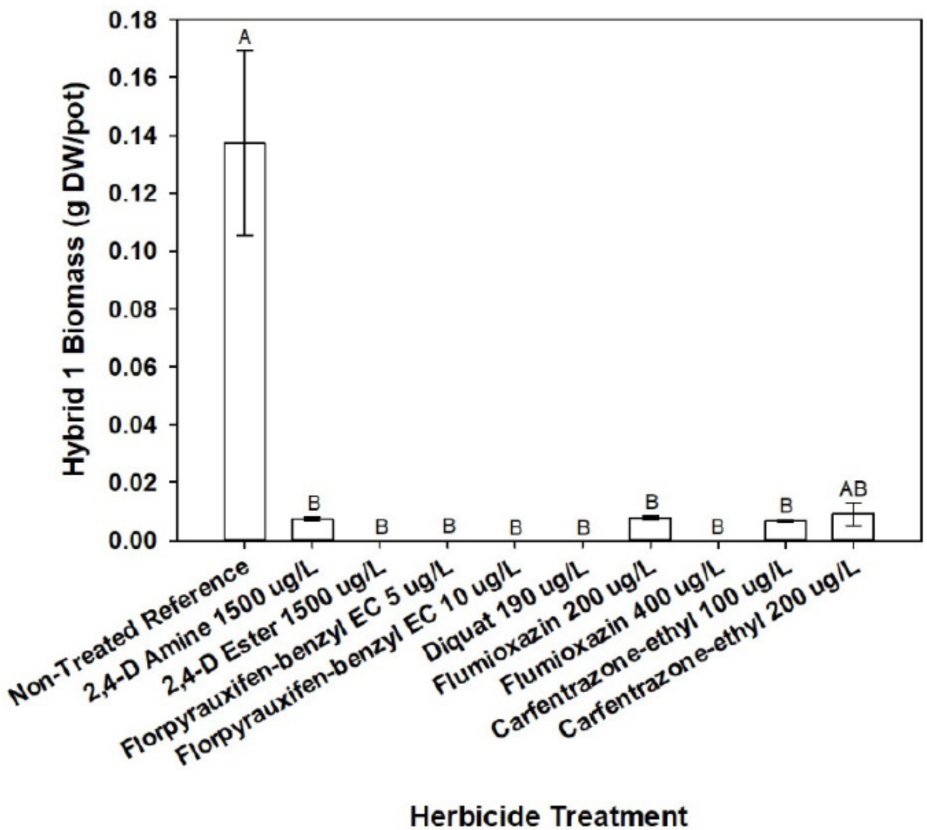
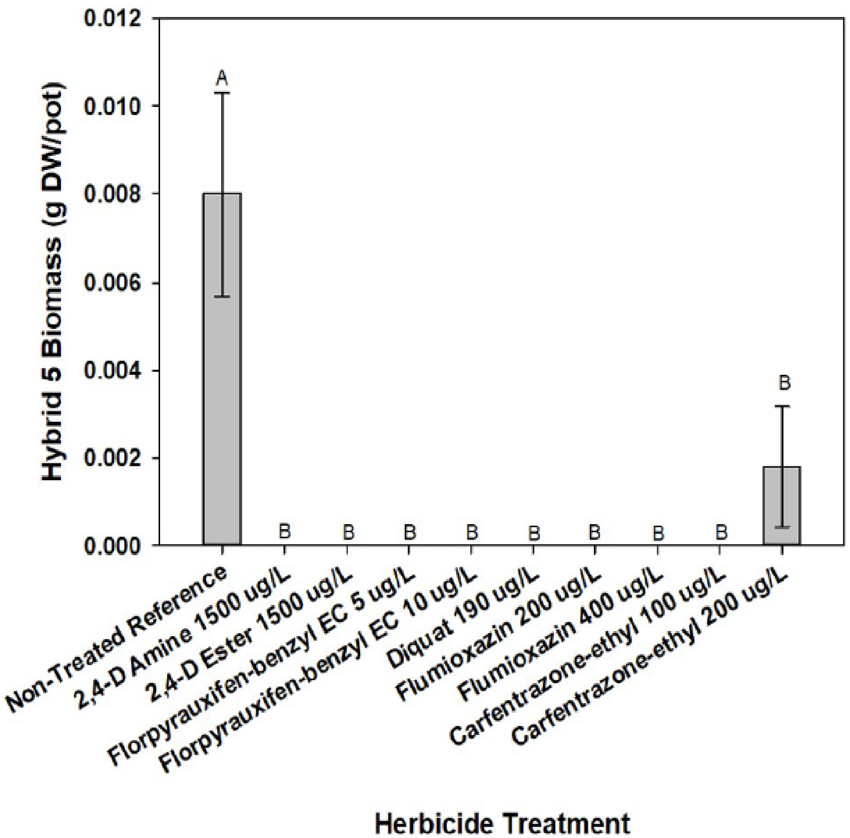
Hybrid Watermilfoil





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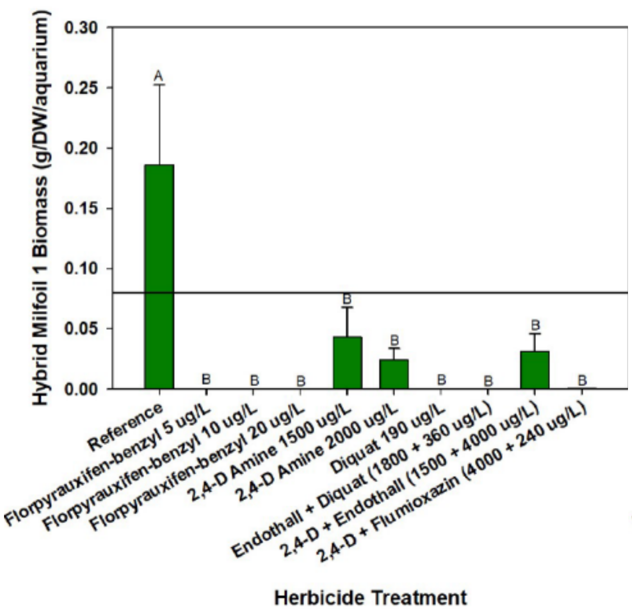
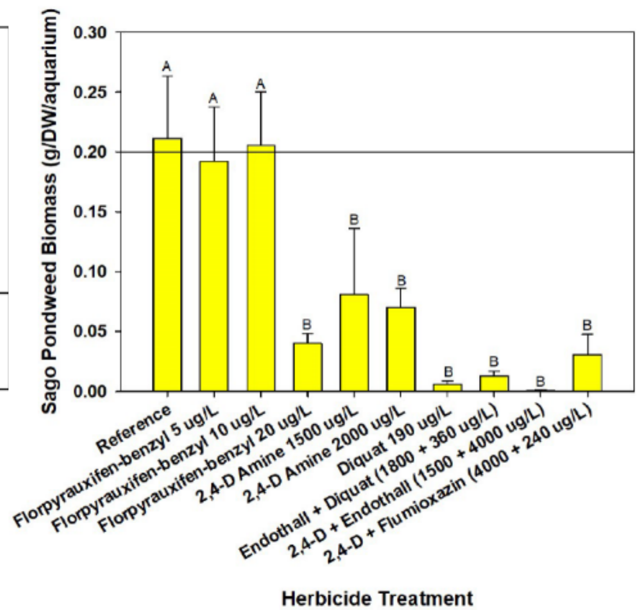
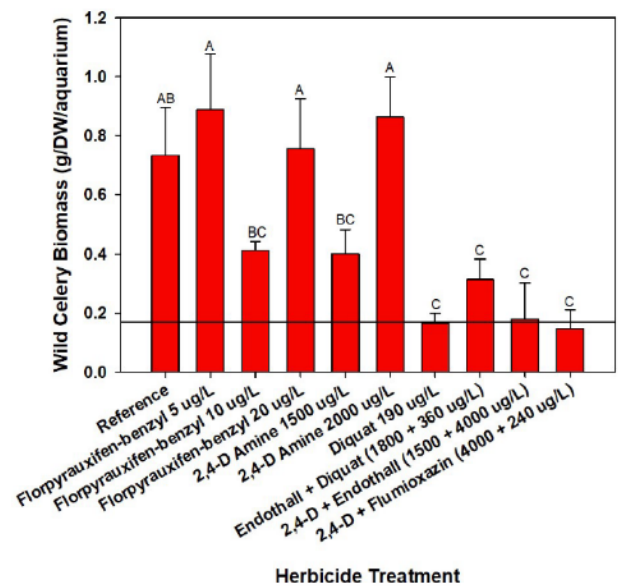
Hybrid Watermilfoil







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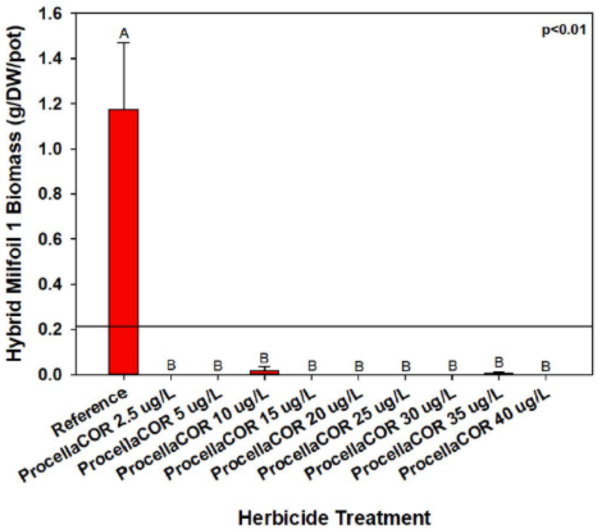
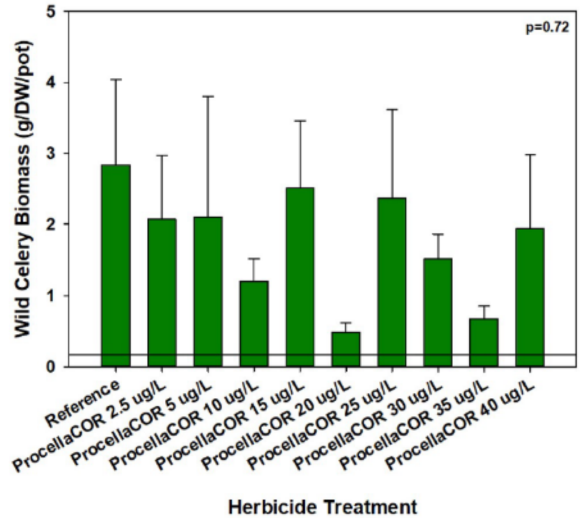
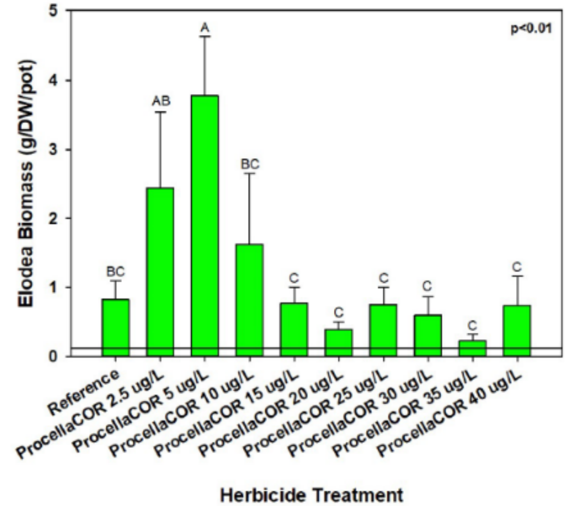
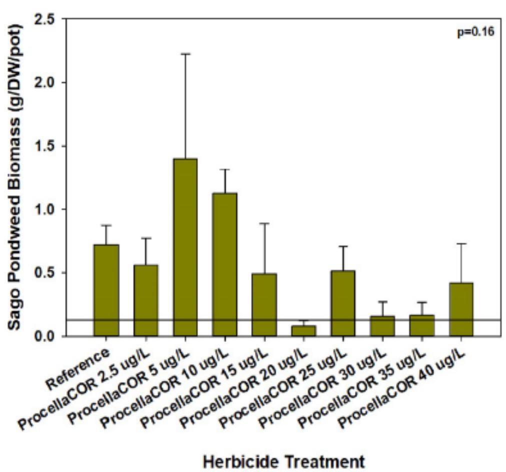
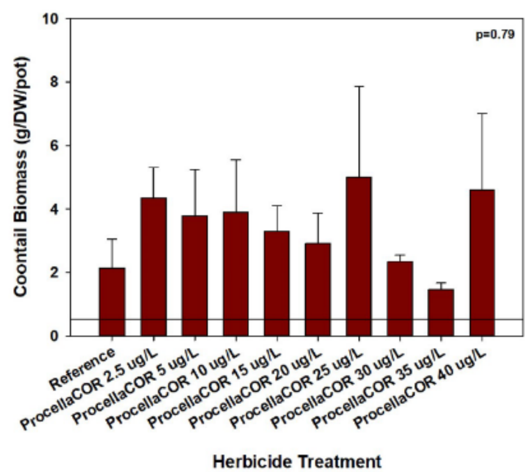
Hybrid Watermilfoil











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Hybrid Watermilfoil

Legend Lake, WI

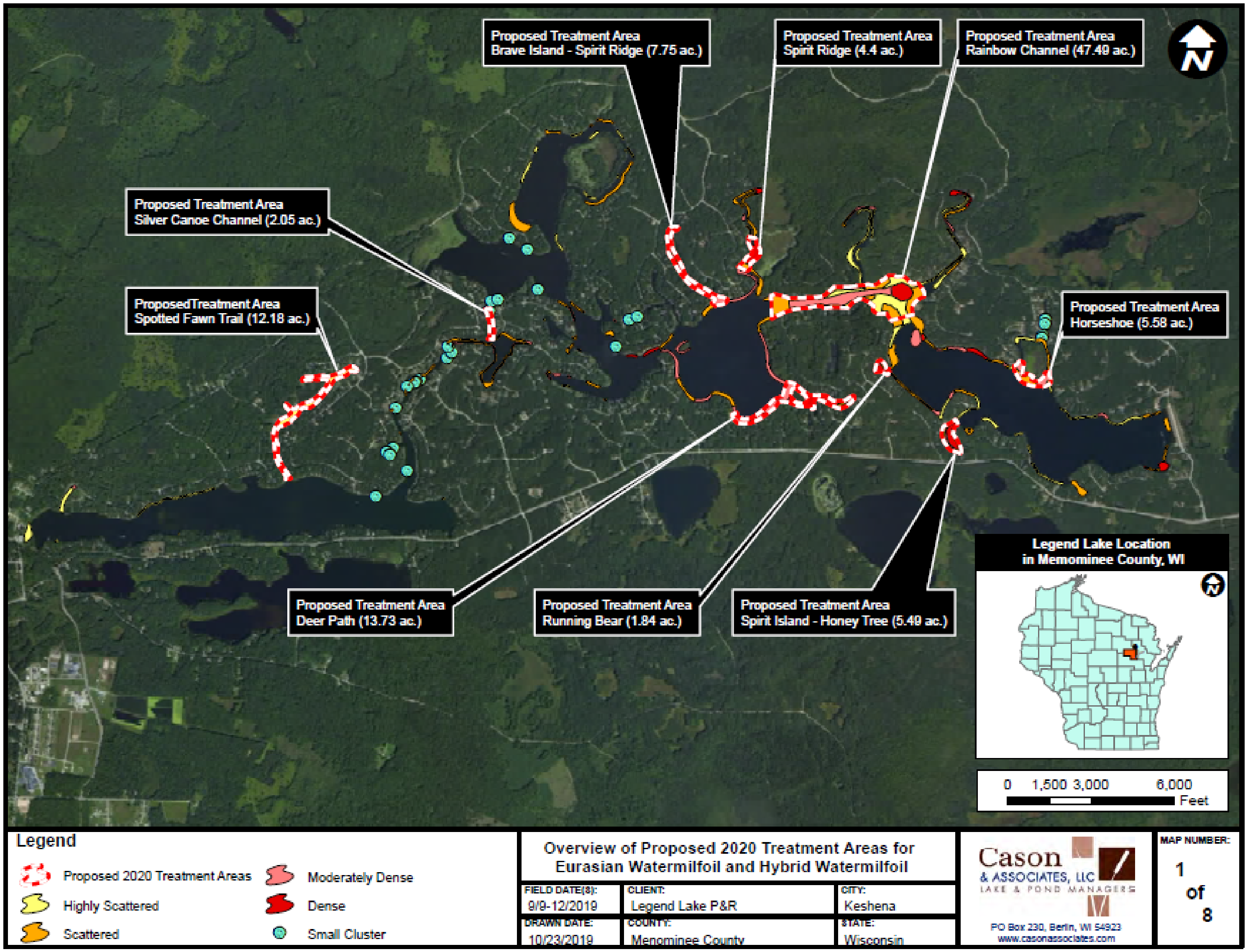

Table 8: Results of point-intercept surveys conducted in September 2019, and July 2020 for Brave Island in Spring of 2020. Plants are organized by 2020 percent frequency, with the highest frequency first. Green rows indicate significant increase and red rows indicate significant decrease in plant occurrence from 2019-2020. Invasive plants are indicated with red text.

Common Name	Scientific Name	September 2019 Percent Frequency	July 2020 Percent Frequency	Significant Change	Increase (I) or Decrease (D)
Filamentous Algae		5.7	24.6	***	I
Slender Naiad	*Najas flexis*	7	12.3	n.s.	I
Muskgrasses	*Chara spp.*	11.4	11.5	n.s.	D
Wild Celery	*Vallisneria americana*	17.1	10.8	**	D
Coontail	*Ceratophyllum demersum*	5.7	9.2	n.s.	I
Variable Leaf Pondweed	*Potamogeton gramineus*	6.3	9.2	n.s.	I
Flat Stem Pondweed	*Potamogeton zosteriformis*		6.2	**	I
Sago Pondweed	*Stuckenia pectinata*	1.3	6.2	*	I
White Water Lily	*Nymphaea odorata*	2.5	5.4	n.s.	I
Small Pondweed	*Potamogeton pusillus*		5.4	**	I
Spatterdock	*Nuphar variegata*	1.3	3.8	n.s.	I
Water Stargrass	*Heteranthera dubia*	3.2	3.1	n.s.	D
Common Waterweed	*Elodea canadensis*	1.3	2.3	n.s.	I
White Stem Pondweed	*Potamogeton praelongus*		2.3	n.s.	I
Common Bladderwort	*Utricularia vulgaris*	3.8	2.3	n.s.	D
Watershield	*Brasenia schreberi*	1.3	1.5	n.s.	I
Illinois Pondweed	*Potamogeton illinoensis*	10.1	1.5	***	D
Creeping Bladderwort	*Utricularia gibba*	0.6	1.5	n.s.	I
Eurasian Watermifoil	*Myriophyllum spicatum*	23.4	0.8	***	D
Nitella	*Nitella spp.*	0.6	0.8	n.s.	I
Pickerelweed	*Pontederia cordata*		0.8	n.s.	I
Large Leaf Pondweed	*Potamogeton amplifolius*	0.6	0.8	n.s.	I
Clasping Leaf Pondweed	*Potamogeton richardsonii*	1.3	0.8	n.s.	D
Large Duckweed	*Spirodela polyrhiza*		0.8	n.s.	I
Small Bladderwort	*Utricularia minor*		0.8	n.s.	I
White Water Crowfoot	*Ranunculus aquatilis*	1.3	Visual	n.s.	D
Aquatic Moss		1.3		n.s.	D
Hardstem Bulrush	*Schoenoplectus acutus*		Visual		

significant change (α = 0.05), ** more significant change (α = 0.01), * most significant change (α = 0.001)*

Data provided by Cason & Associates LLC

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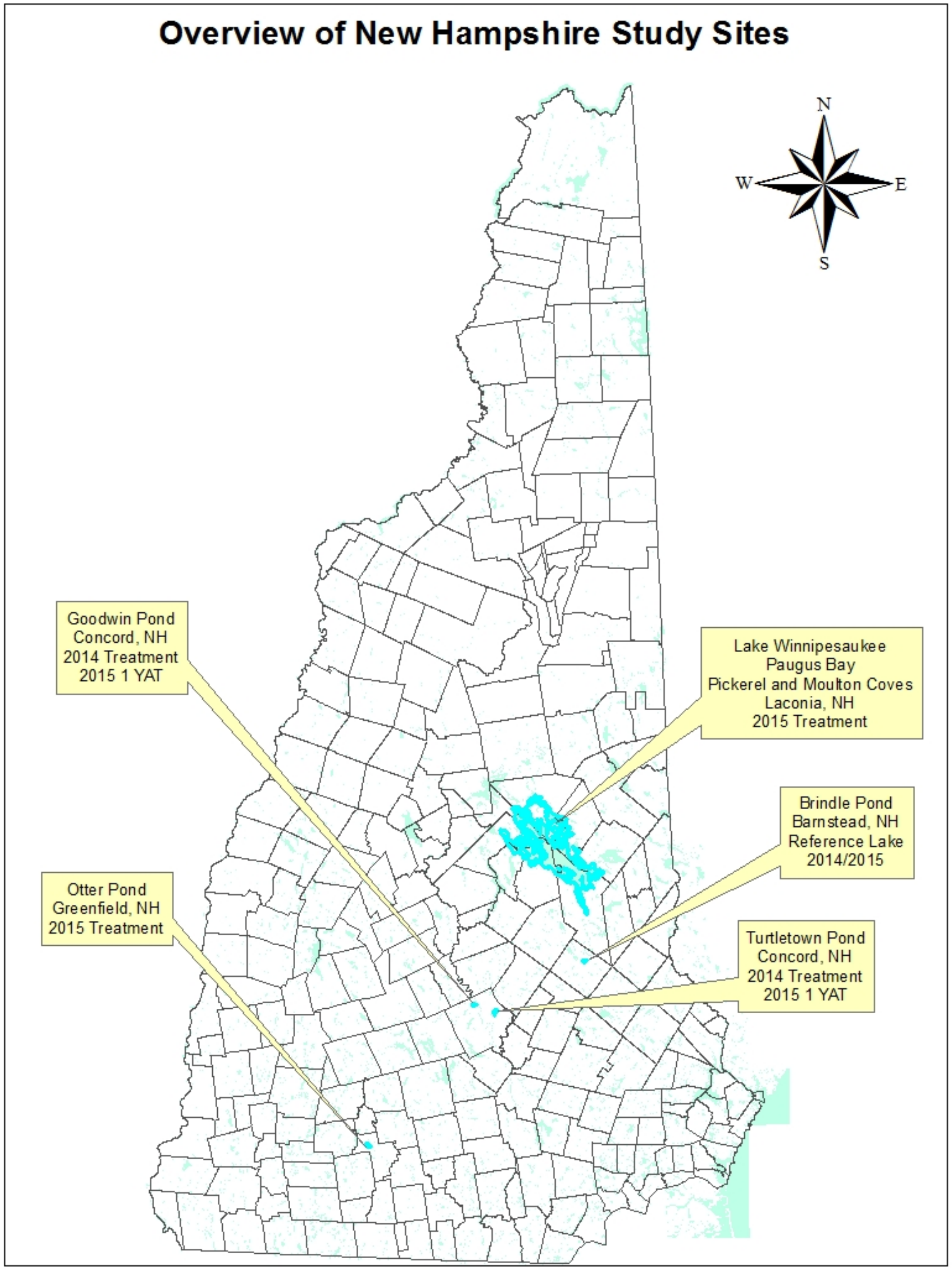
Variable-leaf Milfoil

- Previous studies indicated:
  - Triclopyr could be effective in a range of concentrations and exposure times (Getsinger et al. 2003)
  - Carfentrazone-ethyl and flumioxazin provided better control than endothall, diquat, copper, and diquat + copper combinations (Glomski and Netherland 2007; Glomski and Netherland 2008; Netherland and Glomski 2007)
  - The butoxyethyl ester (BEE) formulation of 2,4-D provided superior control of variable-leaf milfoil (Netherland and Glomski 2007)

Photo Credit: Amy Smagula



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Variable-leaf Milfoil

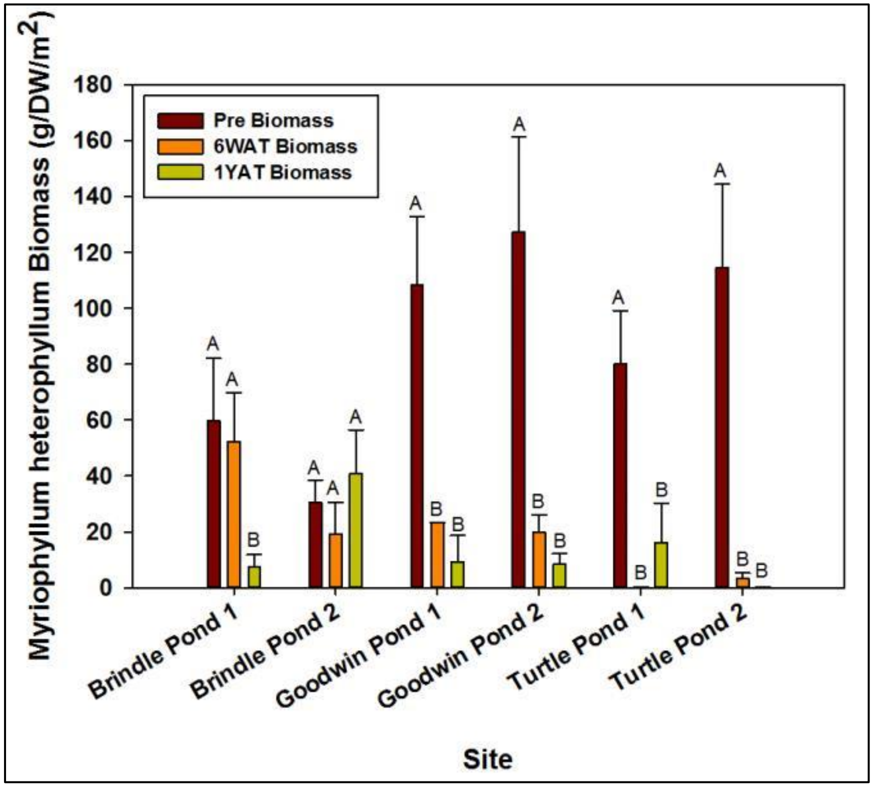
2014

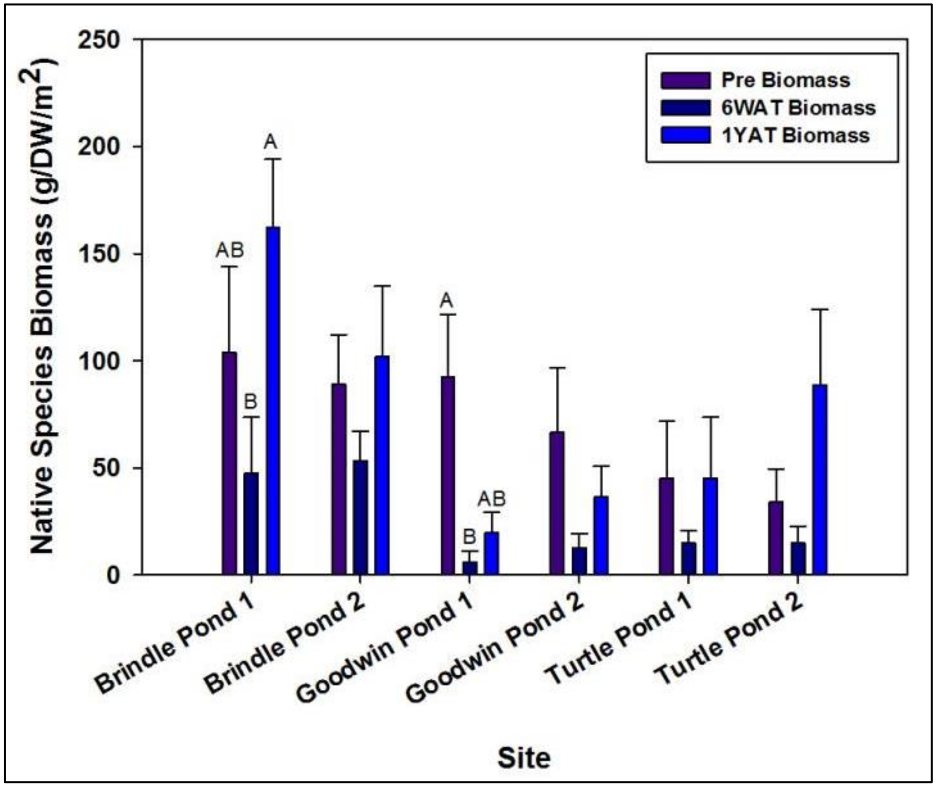
Table 1. Herbicide and treatment rates for variable-leaf milfoil control in small plots in New Hampshire ponds, 2014.					
Pond	**Plot**	**Copper EDA Granular (ug/L)**	**Copper EDA Liquid (ug/L)**	**2,4-D BEE (ug/L)**	**Triclopyr (ug/L)**
Brindle	BP-1	0	0	0	0
	BP-2	0	0	0	0
Turtle	TP-1	0	0	2000	0
	TP-2	750	0	1000	0
Goodwin	GP-1	0	0	0	2000
	GP-2	0	750	0	1000

2015

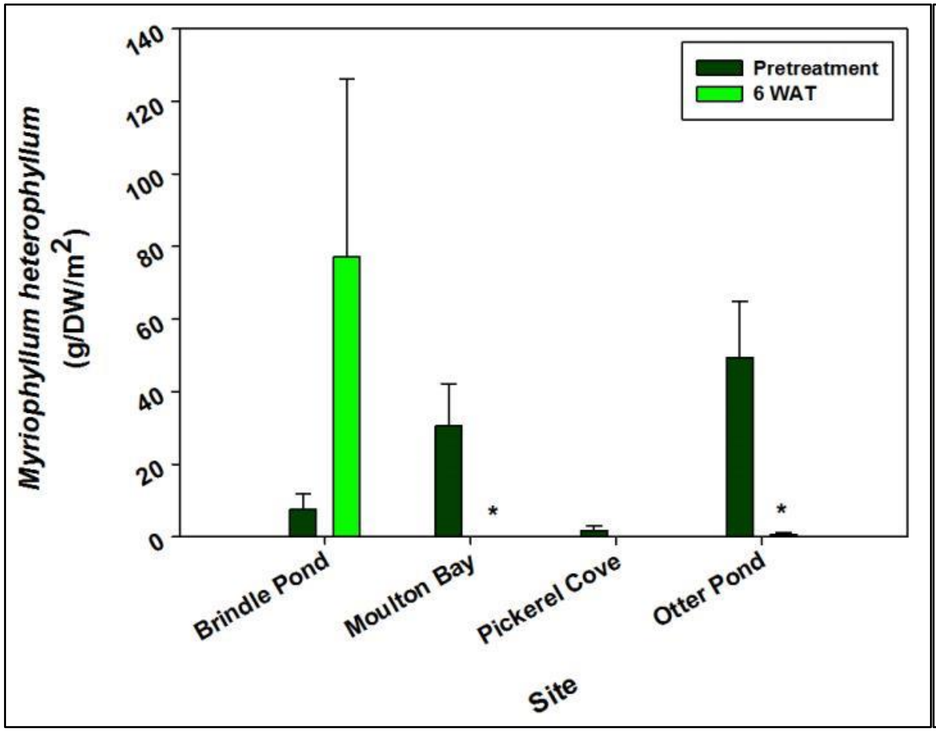
Table 2. Herbicide and treatment rates for variable-leaf milfoil control in small plots in New Hampshire ponds, 2015.				
Pond	**Plot**	**Copper EDA Granular (ug/L)**	**Surfactant (gal/acre ft.)**	**2,4-D BEE Granular (ug/L)**
Brindle	B-1	0	0	0
Otter	O-1	0	1	1500
Lake Winnipesaukee (Paugus Bay)				
Moulton Bay	M-1	750	0	1000
Pickerel Cove	P-1	0	0	2000

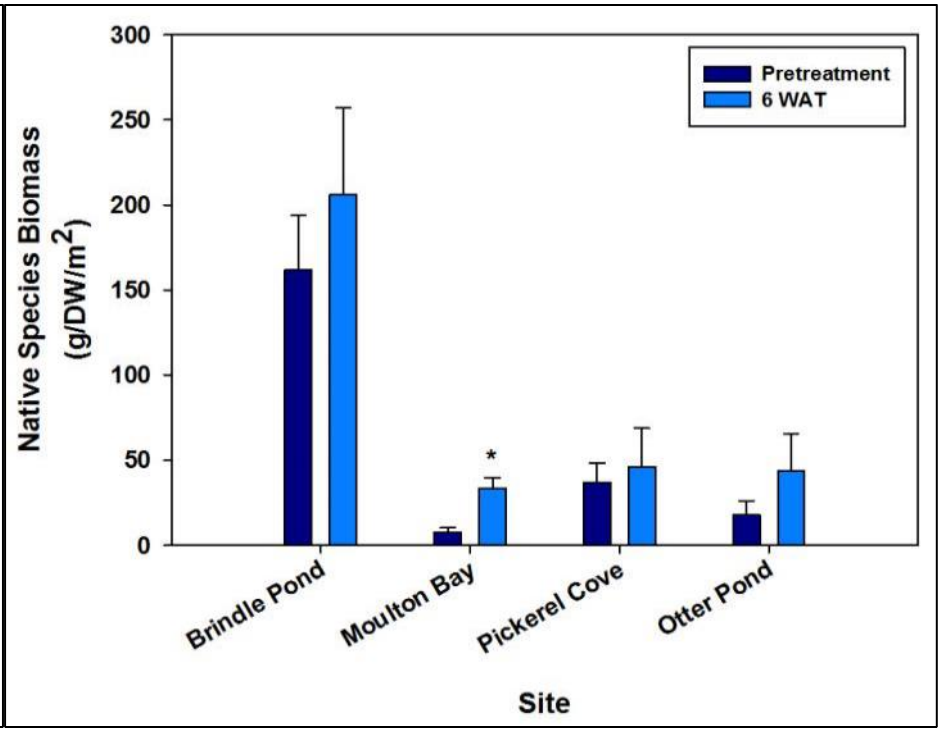
Results - 2014





Results - 2015





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Variable-leaf Milfoil

- 2,4-D BEE remains an effective treatment strategy for variable-leaf milfoil in New Hampshire at a target rate of 2 ppm
- All treatments conducted in 2014 provided at least one year of control at 75-99% biomass reduction
- Small plot studies conducted in 2014 and 2015 indicate that by combining copper EDA with 2,4-D BEE lower amounts of 2,4-D BEE can be used to achieve similar results
- These data also suggest that selectivity on most native species can be maintained with the combination treatment

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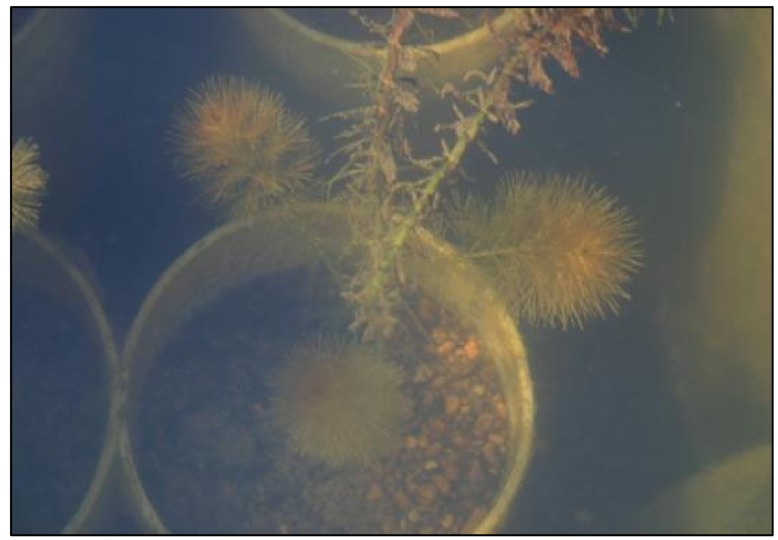
Parrotfeather

- Examine the efficacy of subsurface applications of seven herbicides labeled for aquatic use

- Compare those applications to herbicides that can also be applied to emergent foliage.



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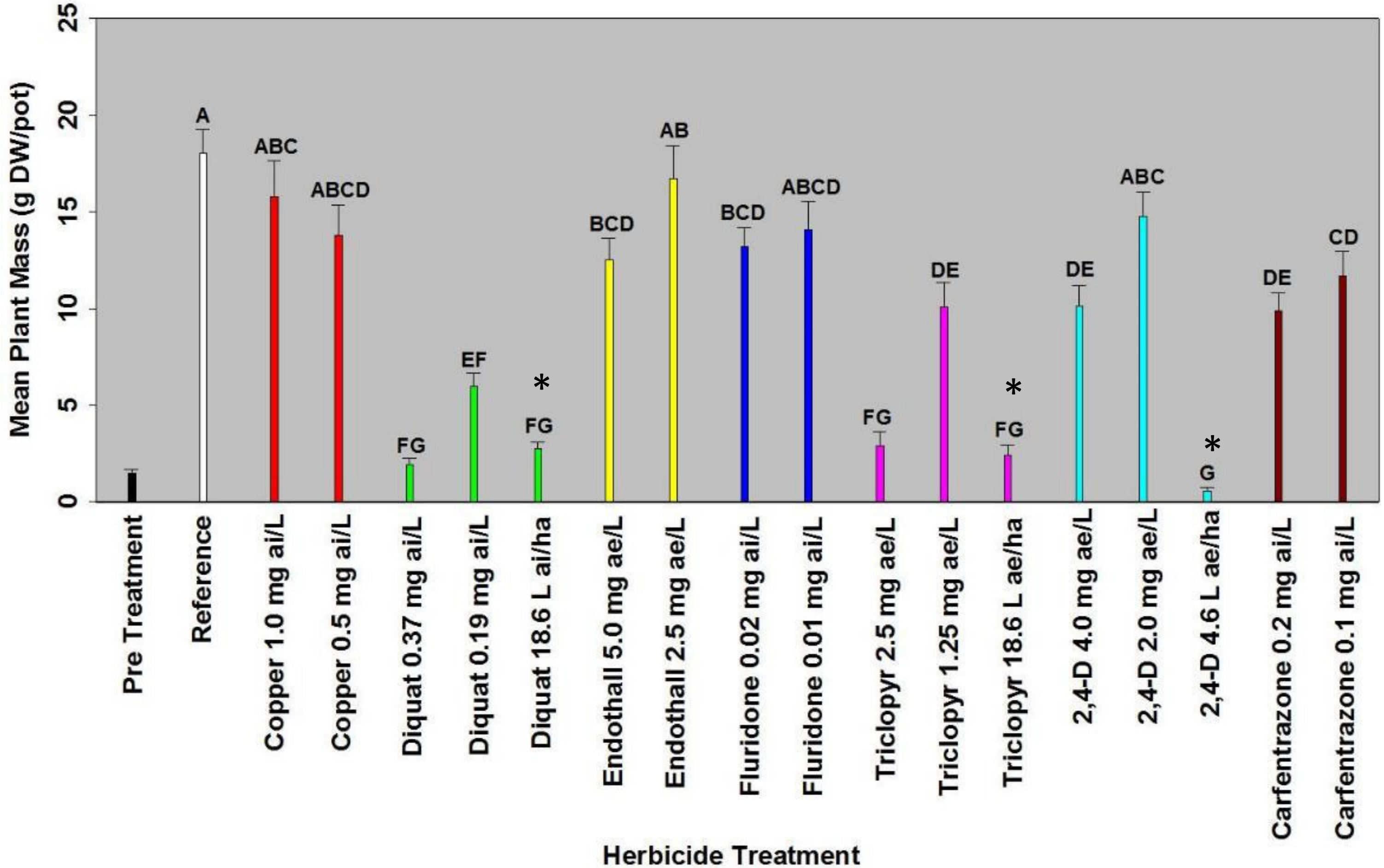
Parrotfeather



Wersal and Madsen 2010

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Parrotfeather

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Summary

- Eurasian watermilfoil and parrotfeather have low points in energy reserves
- Likely true for other milfoil species/genotypes, though more research is needed (hybrids)
- Age of infestation can offset low point vulnerability
- Application timing is important, but may shift due to geographic location, environmental factors, species, or genotype
- Management to reduce biomass is better than the "no management option"

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