

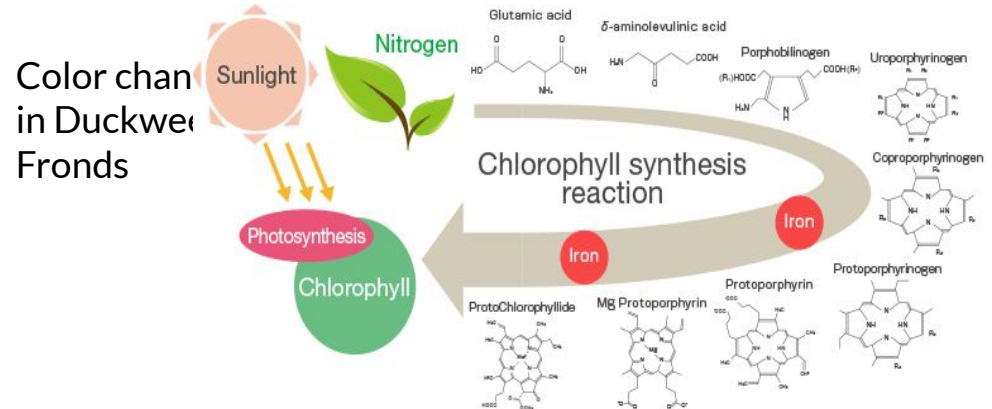
Duckweed was
used to study genetic and
morphological change

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Changes in the environment affects the biodiversity of populations.

Iron Chloride in ponds can impede morphological and physiological functions in Duckweed Species.

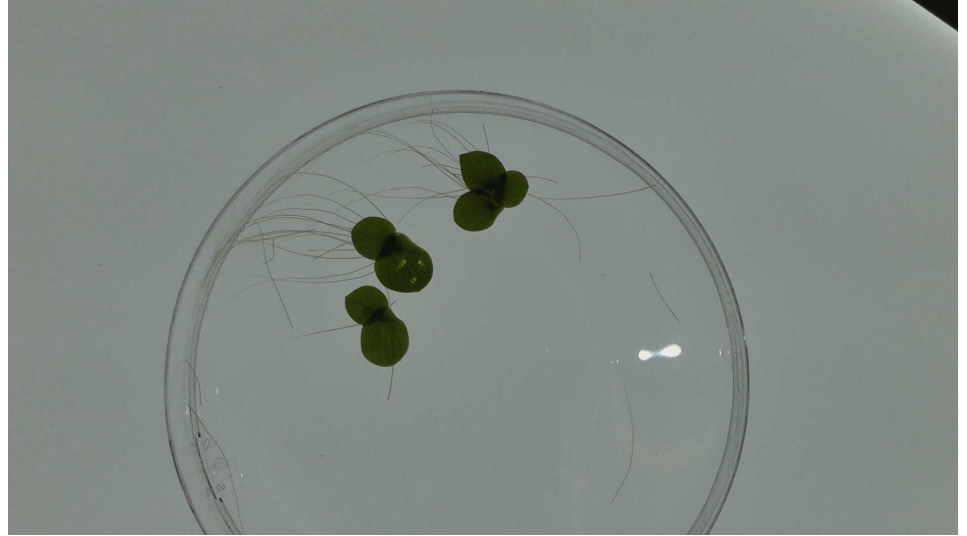


Inhibition of Metabolic processes

Morphological differences in *Lemna minor* and *Spirodela polyrhiza* are valuable for observing growth in polluted conditions.



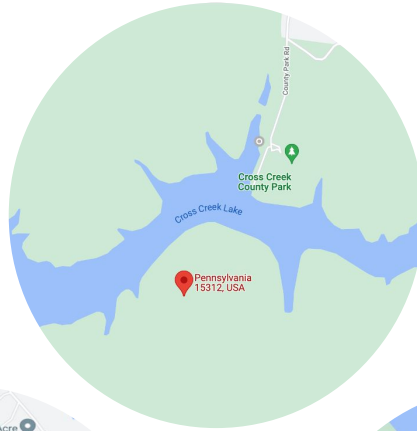
Lemna minor
1 root
Shape: Teardrop



Spirodela polyrhiza
Multiple Roots (Approx. 5 - 6)
Shape: Oval/Round

Duckweed clones were collected from pond sites in varied geographic regions.

S. polyrhiza
SP00003 Cross
Creek Lake, PA



L. minor
MI-MLSG5 LM3
Otter Lake, MI



S. polyrhiza
CT-CRLP SP56
Roseland Lake, CT

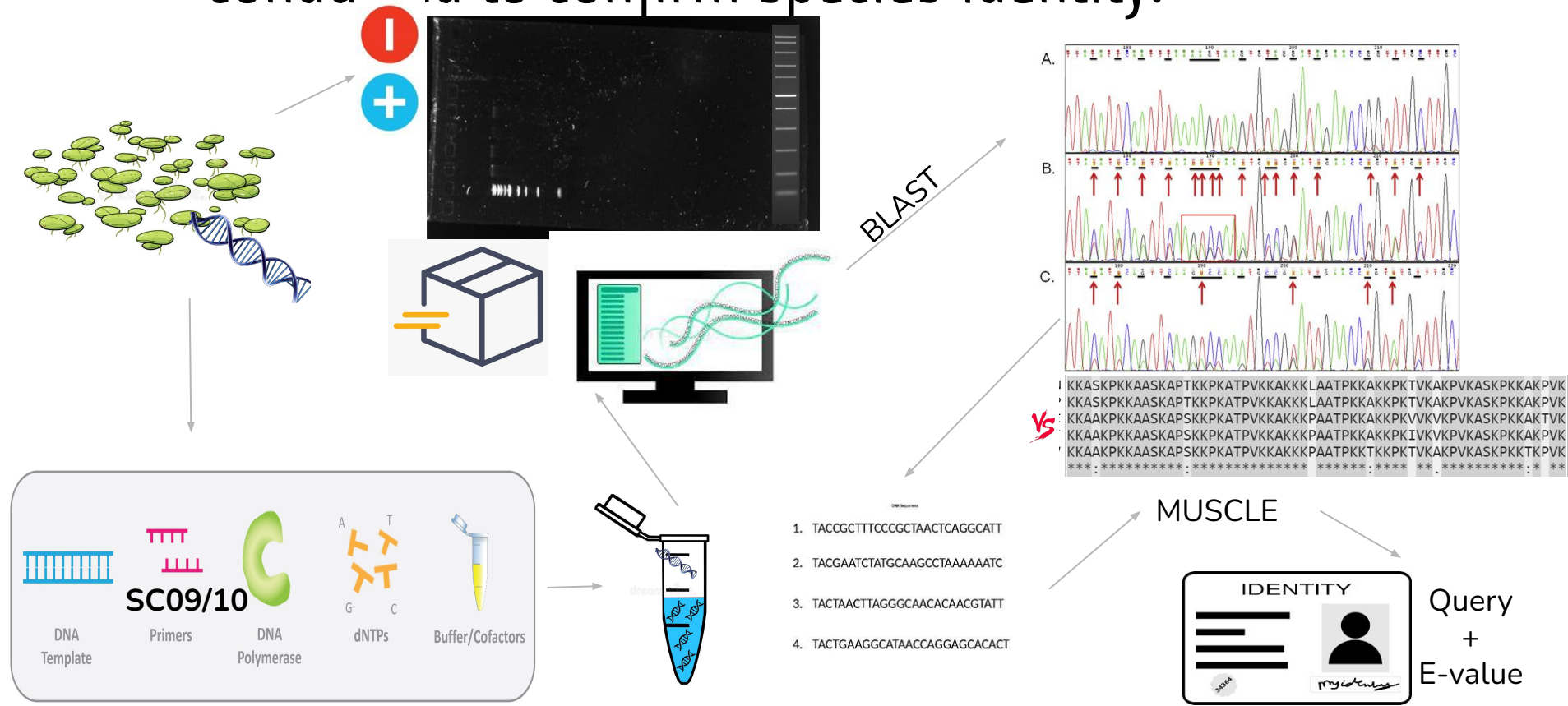


L. minor
WI-NIN LM66
Cumberland Lake, WI



Through development of a genetic barcode, an observational study was conducted to distinguish the identity of the duckweed.

Using various primers, PCR and DNA sequencing were conducted to confirm species identity.



The genetic material of 4 different clones of duckweed were amplified using PCR and the SC09/10 primer.



Each clone was positively identified using DNA sequencing of particular barcodes in their genomes.

Clone ID	SP3 (SP00003)	LM66 (WIWNINLM66)	LM30 (MIMLSGLM30)	SP56 (CTCRLPSP56)
Species ID	<i>Spirodela polyrhiza</i>	<i>Lemna minor</i>	<i>Lemna minor</i>	<i>Spirodela polyrhiza</i>
Useful primers for barcodes	matK rbcL	matK	SC09/10	matK rbcL
Expected product size	matK: 862bp rbcL: 580bp	862bp	170-256bp	matK: 862bp rbcL: 580bp

SC09/10 F primer revealed distinct SNPs between *L. minor* and *S. polyrhiza* barcode sequences.

CLUSTAL multiple sequence alignment by MUSCLE (3.8)

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CTCRLPSP56      TAAATAAAATAAAAAATATATATATATAGGATAATCCTGTAATCTTTCCGGTATTCT
CTCACRSP73      TAAATAAAATAAAAAATATATATATATAGGATAATCCTGTAATCTTTCCGGTATTCT
SP000003        TAAATAAAATAAAAAATATATATATATAGGATAATCCTGTAATCTTTCCGGTATTCT
WIWSXTSP71      TAAATAAAATAAAAAATATATATATATAGGATAATCCTGTAATCTTTCCGGTATTCT
MIMsDRSLM31     -----GAAAATATCTAGATGGGGTAATCTGTAATCTTTACGGTATTCT
WIWINLIM66      -----GAAAATATCTAGATGGGGTAATCTGTAATCTTTACGGTATTCT
MIMLSG5LM30     -----GAAAATATCTAGATGGGGTAATCTGTAATCTTTACGGTATTCT
MIMR13LM41      -----GAAAATATCTAGATGGGGTAATCTGTAATCTTTACGGTATTCT
                  ** **** ** ** ** **** ***** *****

CTCRLPSP56      AATATTGAATACGTGTAGATATAGAATAAAATTTAAATTCATTGATCATTACATATAATT
CTCACRSP73      AATATTGAATACGTGTAGATATAGAATAAAATTTAAATTCATTGATCATTACATATAATT
SP000003        AATATTGAATACGTGTAGATATAGAATAAAATTTAAATTCATTGATCATTACATATAATT
WIWSXTSP71      AATATTGAATACGTGTAGATATAGAATAAAATTTAAATTCATTGATCATTACATATAATT
MIMsDRSLM31     AGTATTCATATCTGTAGATCTAAAATAAAATGTAATTCATTGATCATTACATAGAATT
WIWINLIM66      AGTATTCATATCTGTAGATCTAAAATAAAATGTAATTCATTGATCATTACATAGAATT
MIMLSG5LM30     AGTATTCATATCTGTAGATCTAAAATAAAATGTAATTCATTGATCATTACATAGAATT
MIMR13LM41      AGTATTCATATCTGTAGATCTAAAATAAAATGTAATTCATTGATCATTACATAGAATT
                  * **** ** ** **** ***** *****

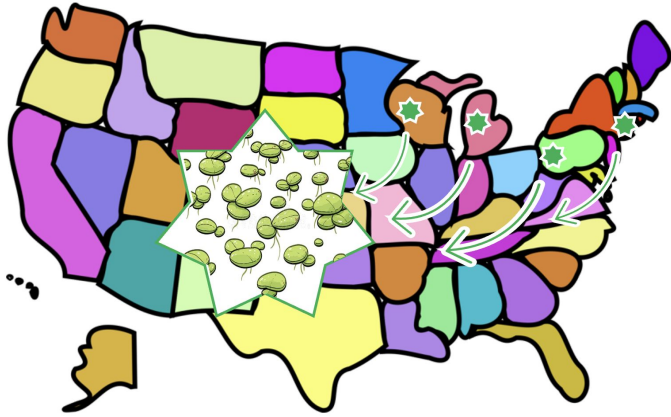
CTCRLPSP56      CAATTAAGATATTGTATGAAAGTATAATTCCTTGATTCTCATTGAAAATGTAAGGATT
CTCACRSP73      CAATTAAGATATTGTATGAAAGTATAATTCCTTGATTCTCATTGAAAATGTAAGGATT
SP000003        CAATTAAGATATTGTATGAAAGTATAATTCCTTGATTCTCATTGAAAATGTAAGGATT
WIWSXTSP71      CAATTAAGATATTGTATGAAAGTATAATTCCTTGATTCTCATTGAAAATGTAAGGATT
MIMsDRSLM31     CAATTAAGATATTGTATGAAAGTATAATTCCTTGATTCTCATTGAAAATGTAAGGATT
WIWINLIM66      CAATTAAGATATTGTATGAAAGTATAATTCCTTGATTCTCATTGAAAATGTAAGGATT
MIMLSG5LM30     CAATTAAGATATTGTATGAAAGTATAATTCCTTGATTCTCATTGAAAATGTAAGGATT
MIMR13LM41      CAATTAAGATATTGTATGAAAGTATAATTCCTTGATTCTCATTGAAAATGTAAGGATT
                  *****

CTCRLPSP56      TTTGATTGGATTTTTGGGGGAGTTCAAATCAAAGAAA-
CTCACRSP73      TTTGATTGGATTTTTGGGGGAGTTCAAATCAAAGAAA-
SP000003        TTTGATTGGATTTTTGGGGGAGTTCAAATCAAAGAAA-
WIWSXTSP71      TTTGATTGGATTTTTGGGGGAGTTCA-----
MIMsDRSLM31     TTTGATTGGATTTTTGGGGGAGTTCA-NTCAAAGA---
WIWINLIM66      TTTGATTGGATTTTTGGGGGAGTTCAAATCAAAGAAA-
MIMLSG5LM30     TTTGATTGGATTTTTGGGGGAGTTCAAATCAAAG---
MIMR13LM41      TTTGATTGGATTTTTGGGGGAGTTCAAATCAAAGAA--
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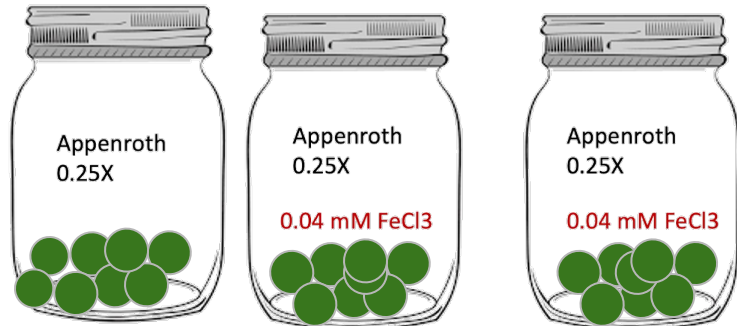
First 4 populations are *S. polyrhiza* and the following 4 are *L. minor*

What are the Physical Characteristics that we can Observe in the Jars of Duckweed with the Pollutants present?

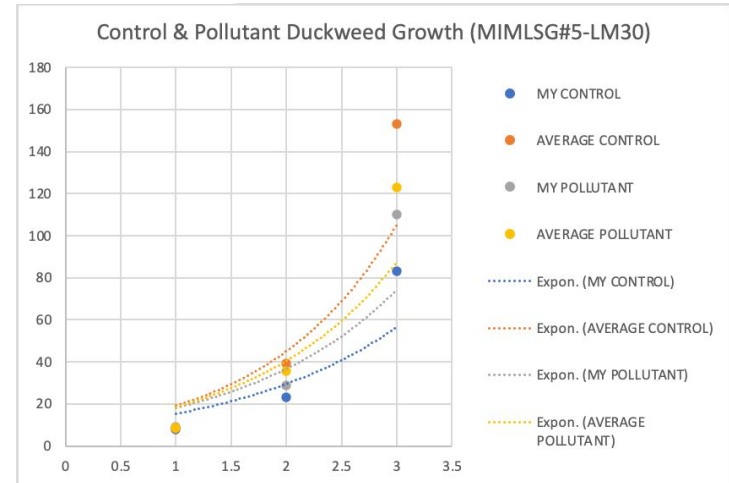
The collected duckweed was observed in an FeCl_3 solution.



1. Collect Duckweed from various regions



2. Add 8 fronds to a control jar and 2 pollutant jars



3. Using Fiji, count the number of fronds, and use that number to synthesize results.

The growth of *S. polyrhiza* and *L. minor* clones were compared under controlled conditions.

S.
Polyrhiza:
(SP00003)



D0:

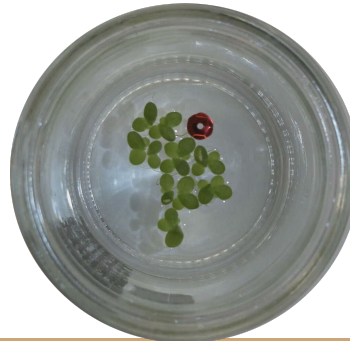


D7:

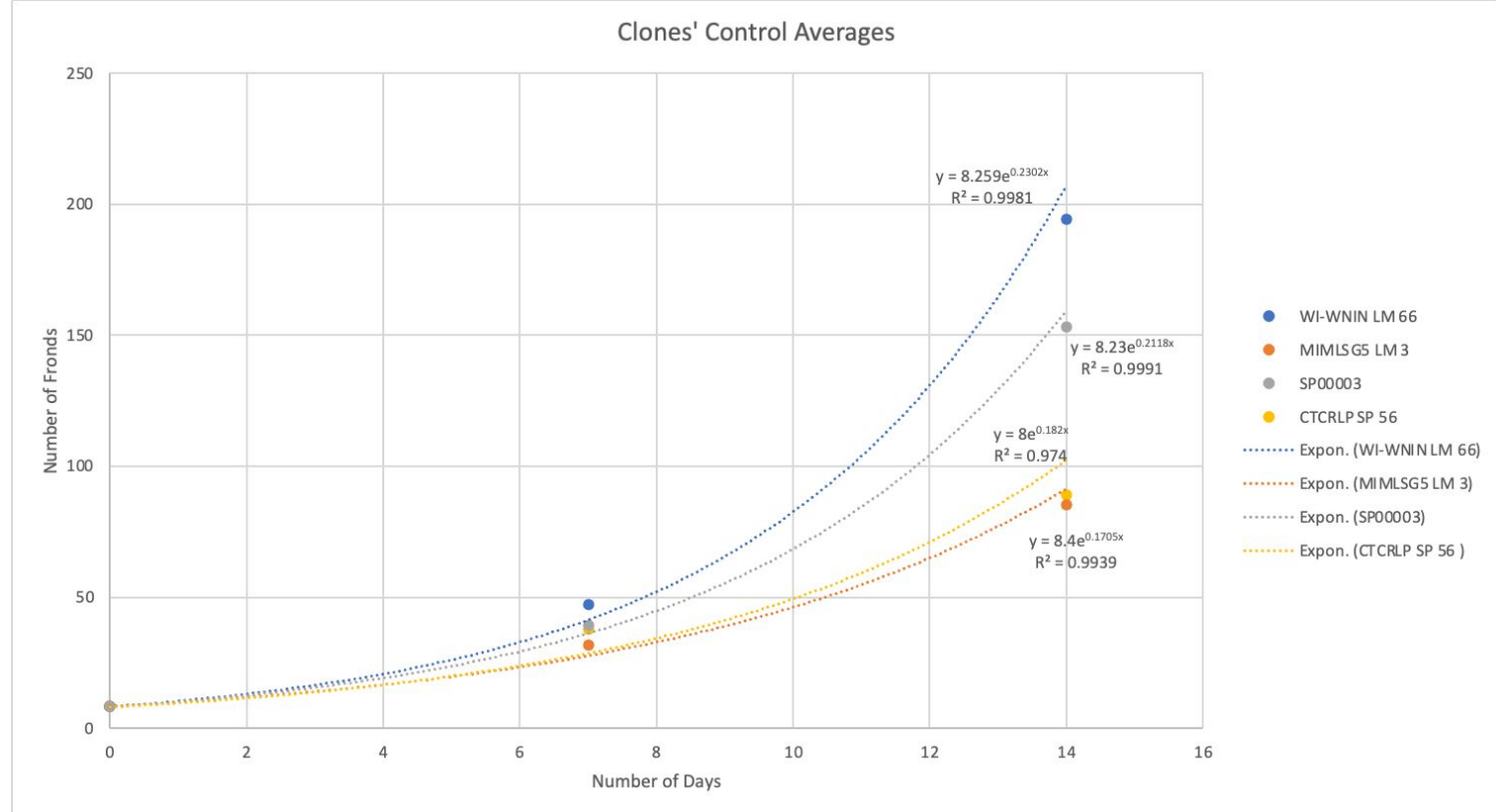


D14:

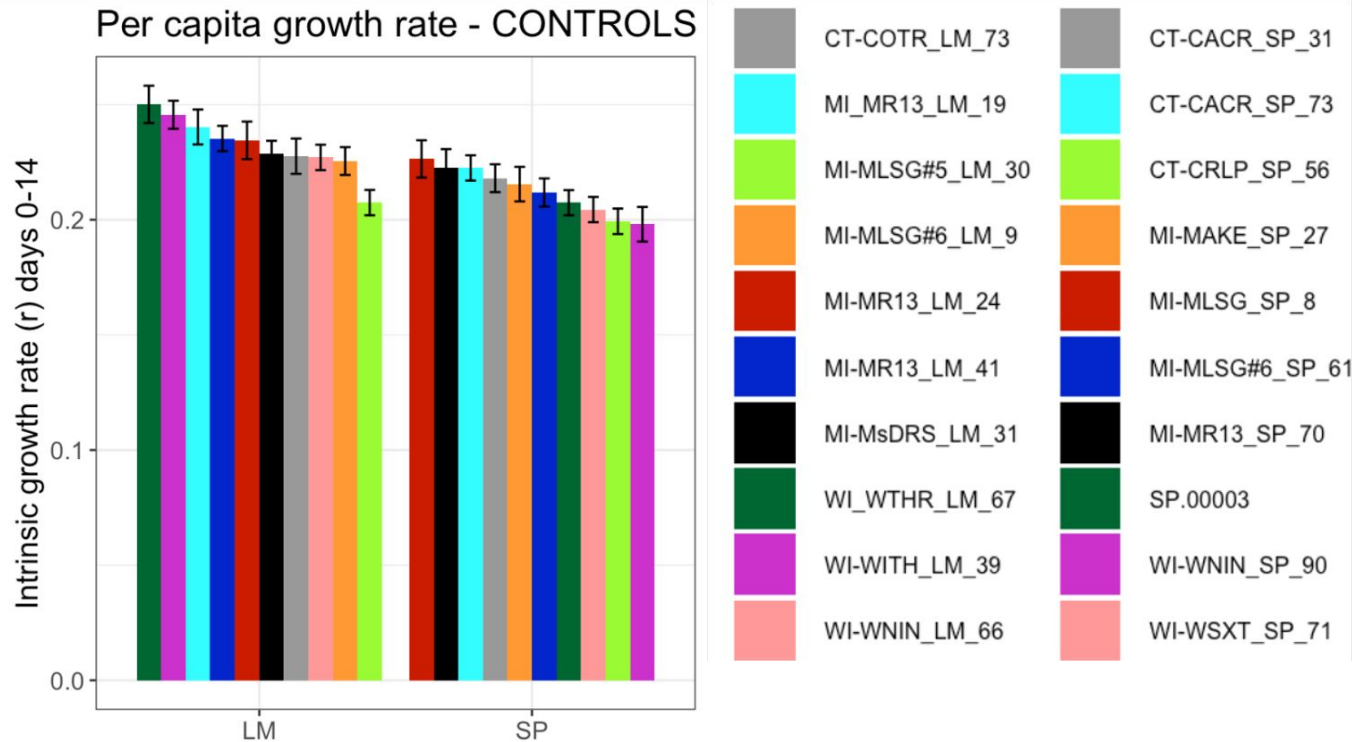
L. minor:
(WI-WNIN
LM 66)



Under controlled conditions, *S. polyrhiza* and *L. minor* clones followed an exponential growth trend.



Control Growth Rate Varied Between both Species and among Clones.



Bar Graph from Dr. Turcotte's Lab

14 days of exposure to the Iron contaminant negatively affected the growth of *Lemna minor* clones.



L. minor control
(WI-WNIN LM 66)



L. minor FeCl₃
pollutant
(MI-MLSG#5 LM 30)



L. minor FeCl₃
pollutant (WI-WNIN
LM 66)

14 days of exposure to the Iron contaminant negatively affected the growth of *S. polyrhiza* clones.



S. polyrhiza
Control
(SP.00003)

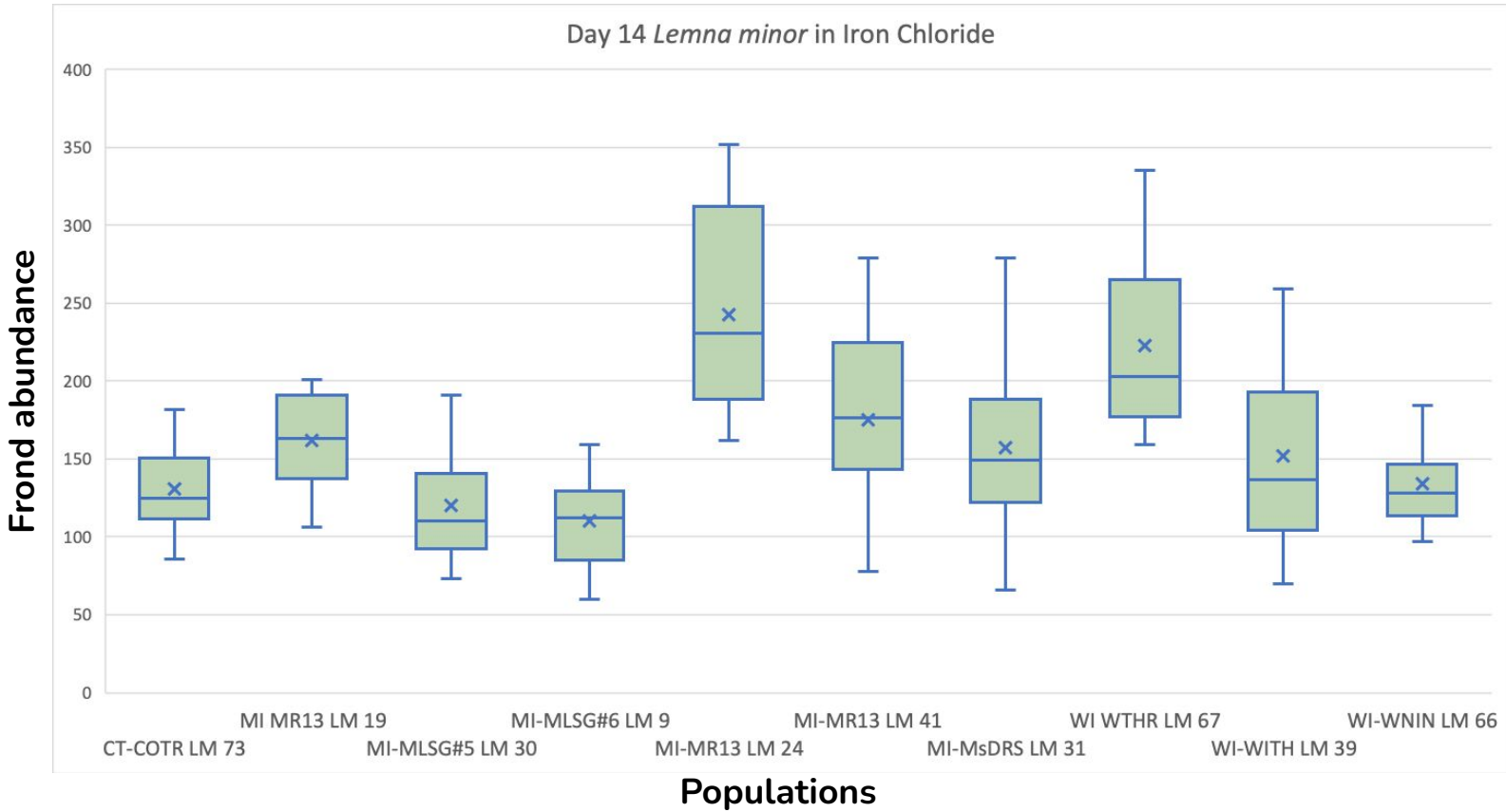


Spirodela FeCl₃
pollutant (SP.00003)



S. polyrhiza
FeCl₃ pollutant (CT-CRLP
SP 56)

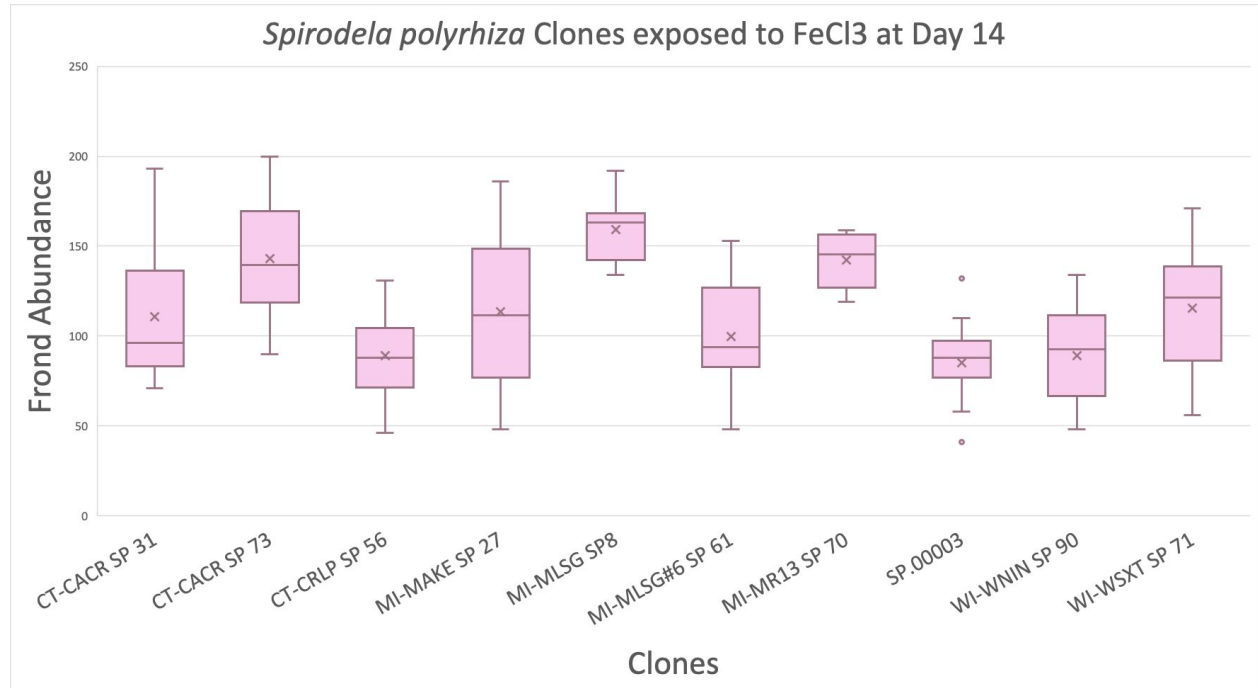
Lemna minor clones treated with FeCL3 caused variable frond abundance.



Average
abundance
of fronds in
L. minor
clones

Spirodela polyrhiza treated with FeCL3 caused variable frond abundance.

**Average
abundance
between 10
clones of
*Spirodela
polyrhiza***



Conclusions

- Molecular conclusions: Genetic differences and the effectiveness of the SC09/10 primer
- Pollutant conclusions: Effect of FeCl₃ pollutant and clone tolerances

Future Directions

- Repetition
- Effectiveness of SC 09/10 primer on more species
- Concentration variations to study FeCl₃ tolerance

Acknowledgements