Experimental transplant and ecology of the Michigan monkey-flower

Maple River, Emmet County

Prepared for the USFWS

by:

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Michigan Natural Features Inventory

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Cover: Michigan monkey-flower at the Maple River, Pellston, Michigan (June 19, 2018).

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Abstract

In 2018, the United States Fish and Wildlife Service (USFWS) requested Michigan Natural Features Inventory (MNFI) to re-assess and monitor the federally endangered Michigan monkey-flower (MMF) (*Mimulus michiganensis* (Pennell) Posto & Prather) along the Maple River in Emmet County, Michigan, because of a lake drawdown and dam removal project upstream of the population, and encroaching invasive forget-me-not (*Myosotis scorpioides* L.). This small semi-aquatic forb is endemic to Michigan and the Maple River population is the only known to set fertile seed, making it vital to conservation efforts. To migrate threats, MNFI monitored the site before, during, and after the dam project, transplanted stems from the Maple River to two other suitable sites, and developed strategies to manage water forget-me-not.

The water table at the Maple River dropped a mean of 2.5” (6.3 cm, n=3) from September 2018 to September 2019, while nearby monitoring sites increased a mean of 1” (2.5 cm, n=6), indicating the water table may be responding to the dam drawdown. Changes in groundwater from drawdown can take several years to be fully realized, and future monitoring is recommended at the Maple River site. During the same time period, the monkey-flower population varied in change in cover between sites. Decreases at two sites were attributed to competition with water forget-me-not interacting with decreases in the water table. Plants directly on springs faired the best. Transplanted colonies varied in growth between sites from a 500% increase in cover to 80% decrease in cover; most colonies established new roots and lateral shoots the first year (2018) then flowered and set fruit the second year (2019). Sites that performed poorly shared in low water flow rates (<0.1 m/sec) from the headwater seep or spring and low dissolved oxygen (<50%). Combining previous literature with this monitoring data, we propose new and expanded growth parameters for soil and water. We also propose best practices for managing water forget-me-not in sensitive areas.

Introduction

In 2018, the United States Fish and Wildlife Service (USFWS) requested Michigan Natural Features Inventory (MNFI) to re-assess the status of the federally endangered Michigan monkey-flower (*Mimulus michiganensis* (Pennell) Posto & Prather*)* along the Maple River in Emmet County, Michigan. This population is threatened by two factors: impact from significant invasion of non-native water forget-me-not (*Myosotis scorpioides* L.) and potential impacts of the Kathleen Lake Dam removal on Woodland Road. This project seeks to mitigate the risks and further the understanding of the ecology of the Michigan monkey-flower.

Michigan monkey-flower (MMF) is a Michigan endemic semi-aquatic perennial herb with lax stems that root at the nodes and short-tubular yellow flowers bore on upright stems. It occurs in marly springs, cold streams, and seepy calcareous lakeshores (Penskar 2012). There are twenty-three known locations in the Mackinac Straights region, and southward in Benzie and Leelanau Counties (MNFI 2018). Of these, the Maple River colony is the only population known to produce fertile pollen and set seed (Bliss 1986, Posto 2000, Posto & Prather 2003); all other colonies reproduce vegetatively. This makes the Maple River population critically important to the conservation of the species. The USFWS classifies Michigan monkey-flower as federally endangered with a recovery priority number of 8C, indicating a moderate degree of threat and high recovery potential (Payne et al 2010). The major threats across its range are destruction and modification of habitat for recreation or development and hydrological alterations (Payne et al 2010).

The Conservation Resource Alliance's (CRA) initiative *Free Span the Maple River* seeks to remove barriers to fish passage and improve water quality by updating infrastructure throughout the Maple River watershed. The largest project within this initiative is the removal of the Lake Kathleen dam, which is directly upstream of the Maple River monkey-flower population. This project included drawing down Lake Kathleen, which is a small impoundment lake on the river, removing the dam structures, and installing a free span bridge on Woodland Road. The biological assessment indicates dam removal is expected to affect water levels, flow, temperature, and sediment transport downstream and impacts "may affect, likely to adversely affect" the Michigan monkey-flower (Kowalski 2017). Additionally, the colony is threatened by competition from water forget-me-not. This project assesses the current status of the MMF population on the Maple River prior to dam removal, develops and implements a plan to monitor the population for impacts during and after the drawdown of Lake Kathleen, and assesses the future of the MMF at this site. A strategy to remove and monitor water forget-me-not is proposed. We also conducted experimental transplants of MMF from the Maple River population to two other sites as a precautionary measure and we summarize biotic and abiotic parameters preferred by MMF and associated growth rates.

Methods

Site Review

The project site is located at the Woodland Road crossing of the Maple River, 1.5 miles (2.4Km) south of Pellston in Emmet County, Michigan (T36N R4W Sec10/11, Fig. 1). Woodland Road crosses the Maple River at the confluence of the East and West Branches; historically, the East and West Branches joined south of Woodland Road, but with the installation of the original impoundment, the branches were dammed north of the road and Lake Kathleen was formed. The original dam structure let water through the East Branch but failed in the 1950s. The replacement dam and spillway was built on the former West Branch channel. The Conservation Resource Alliance began the dam removal project in August of 2018. Lake Kathleen was drawn down by siphon beginning August 23rd through the former East Branch of the river. The siphons were turned off in late October and removed in November 2018. The dam structures were removed, and bridge construction was completed in early 2019. The confluence of the East and West Branches remains north of Woodland Road and the river flows under the free span bridge through the West Brach channel.

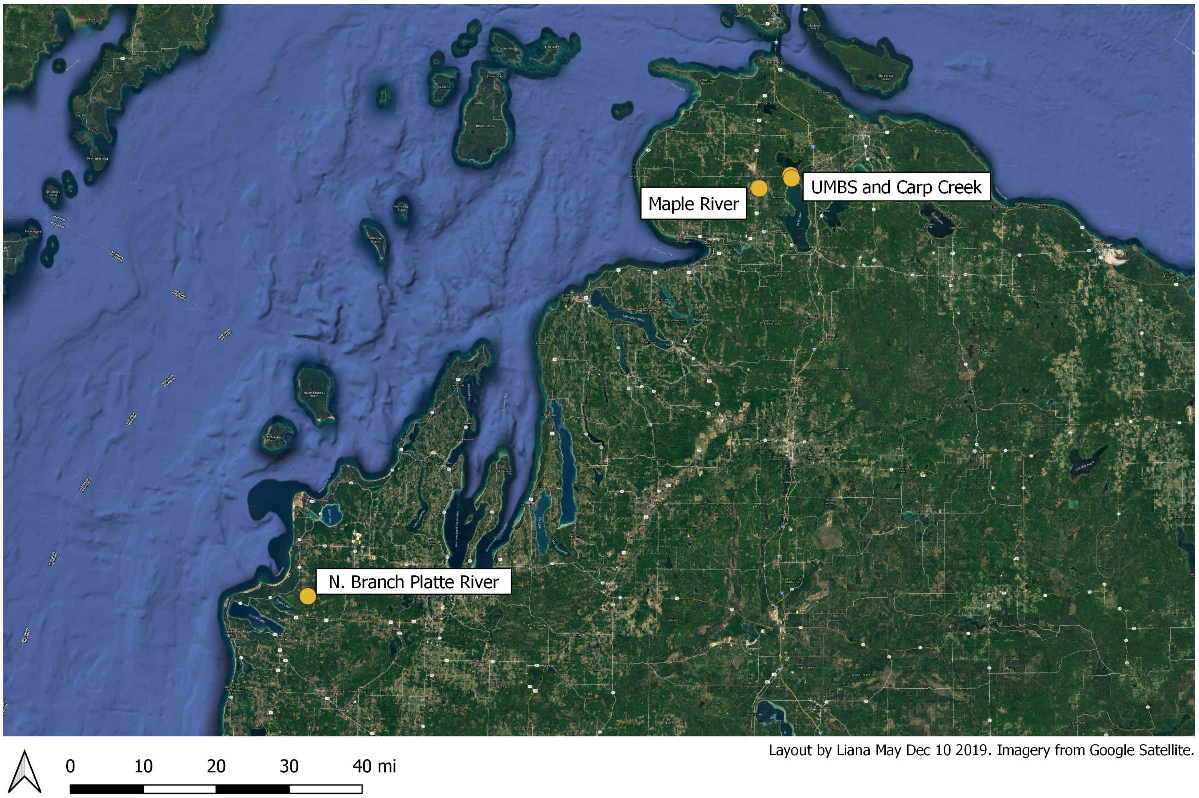


Figure 1. Map of the study site locations.

The primary Michigan monkey-flower colony is located in a small, spring-fed stream that runs parallel to the Woodland Road embankment and feeds into the Maple River just below the road crossing (Fig 2). The population consists of several thousand stems in an area of 0.25 acres (0.1Ha). In 2015, a second colony of several dozen stems was mapped downstream, in a small stream feeding the Maple River from the north (Slaughter 2015). Because both streams are spring-fed, concern was raised that drawing down Lake Kathleen will reduce groundwater discharge from these springheads. Additional concerns relate to the potential inundation of the streams and springheads with the release of water from Lake Kathleen, causing changes in temperature, sedimentation, and water chemistry.

In May and June of 2018, MNFI and USFWS staff conducted exploratory visits to the Michigan monkey-flower colony at the Maple River, colonies identified in the Natural Heritage Database within the vicinity of the Maple River, and potential MMF habitat outside of known populations. The purpose of the exploratory trip was to 1) observe the ecology of MMF in the area, 2) examine the locations of the springs, assess the direction of groundwater flow, evaluate risks, and select monitoring plots at the Maple River site, 3) locate a reference site that can be used to track changes in the MMF colony outside of impacts of dam removal and transplanting, and 4) explore the area for potential translocation sites. Additionally, discussions were held with local land managers and residents, ancillary geospatial data was gathered, and a literature review was conducted to further inform the site review (e.g. Bliss 1983 & 1986, Crispin 1981, Marquis 2011 & 2012, Posto 2001, Posto and Prather 2003). This information was used to design a monitoring protocol and select monitoring and translocation sites as described in the following sections.

Figure 2. Monitoring plots are located in the primary (MR1 and MR2) and secondary (MR3) Michigan monkey-flower colonies at the Maple River; note the spillway on the West Branch and remnants of the failed dam on the former East Branch of the Maple River, and Lake Kathleen, as of summer 2018.

Monitoring Plot Selection

To track changes during and after the river restoration project, three monitoring plots were established at the Maple River colonies. Field visits to the primary Maple River MMF colony showed that springs feeding the small stream are predominantly along the south bank, with additional springs within the stream bed and seeps on the north bank. Test wells dug along the south bank revealed strong groundwater flow from the south-southwest. This was supported by analysis of topographical maps and discussions with Chris Pierce, the hydrologist with CRA (pers. comm.). It was determined that the stream is not primarily fed by groundwater discharge from Lake Kathleen, though it likely supplements it. Based on this information, one monitoring well was located at the south bank of the stream (MR1), and a secondary monitoring plot was located on the north bank of the stream (MR2; Table 1, Fig 2).

The secondary colony of MMF is located on a small stream that appears to be fed mainly by groundwater discharge from the north. It was concluded that groundwater discharge at this site is likely to be reduced by the Lake Kathleen drawdown. A third monitoring plot was established here (MR3; Fig. 2). Because this area is imperiled by changes in hydrology, it was chosen to be the source for experimental translocation material. It is assumed that this colony is genetically similar to the primary colony and has fertile pollen.

An established, healthy MMF colony was used as a reference for comparison to all other sites. A search of the Natural Heritage Database identified three MMF populations near the Maple River: the North Braun Nature Preserve owned by the Little Traverse Conservancy (LTC); the Reese's Swamp population; and the Carp Creek population at Hogback Road. The latter two populations are on land owned by the University of Michigan Biological Station (UMBS). The Carp Creek site was chosen as a reference site because of its proximity to the Maple River population, university ownership, and road access. Three monitoring plots were established: Two in springy marsh along the west bank of Carp Creep, north of Hogback Road (CC1 and CC3), and one on the sandy creek bank south of Hogback Road (CC2, Fig. 3).

To select transplant sites, a literature review was conducted to establish the range of habitat preferences and ideal environmental parameters for growth (e.g. Bacon & Bozic 2012; Bliss 1986; Brushaber 2009; Marquis 2011, 2012; Penskar 2012). Potential translocation sites were identified by using geospatial data (satellite imagery, topographic maps, plat maps), consultation with land managers (LTC, UMBS, USFWS), and field visits. Public, university, or land trust ownership was preferred to ensure long-term access, and areas with existing MMF populations were ruled out to avoid outbreeding depression. Field investigations were conducted in June and July of 2018. During the field investigation, water pH and temperature were measured using a BlueLab pH pen, canopy cover was estimated visually, and site ecology, including plant community, groundwater discharge, and soil type, was also noted. Per the literature review, groundwater was required to have a pH between 6.8 and 8.2 and temperature between 8.9-18°C (Bliss 1986; Marquis 2012) and sunlight between partial ("dappled sunlight") to full sun (Marquis 2012; Penskar 2012).

Two transplant sites were selected. Nearest to the Maple River site, the Carp Creek headwaters at the Gorge Trail, owned by the University of Michigan Biological Station, has multiple springs discharging at the base of a large bluff ("the gorge") in northern white-cedar swamp (Fig 3). It is located approximately 0.5 miles (0.1Km) north of the reference population on Carp Creek at Hogback Road, but there is no MMF present in the gorge. Plots were located on a seepy spring (UMBS1), on a sandy springhead (UMBS2), and along the edge of a small sandy stream (UMBS3).

The second transplant site is located along the North Branch of the Platte River south of the corner of Gudemoos and Hooker Roads in Benzie County (Fig. 4). This site is characterized by northern white-cedar swamp along the base of a north-facing moraine where multiple springs discharge to feed the North Branch of the Platte River. MMF is also known from this river; surveys in 2018 located a few dozen stems 1.5 miles (2.4Km) upstream of the transplant site (May & Higman 2018). Three plots were established, one in a small stream below springs (PR1), a second on a sandy springhead (PR2), and a third on a seepy bank of a stream (PR3).

Table 1. Monitoring site names, locations and plot labels.

Site	Plot Name	Description	Latitude	Longitude
Maple River Plot 1	MR1	primary colony, south side of stream	45.528756°	-84.776589°
Maple River Plot 2	MR2	primary colony, north side of stream	45.528771°	-84.776003°
Maple River Plot 3	MR3	secondary colony, transplant source population	45.528189°	-84.773341°
Carp Creek Plot 1	CC1	reference site	45.548691°	-84.682624°
Carp Creek Plot 2	CC2	reference site	45.548514°	-84.682529°
Carp Creek Plot 3	CC3	reference site	45.548865°	-84.682694°
The Gorge at UMBS Plot 1	UMBS1	experimental transplant colony	45.553589°	-84.683944°
The Gorge at UMBS Plot 2	UMBS2	experimental transplant colony	45.553503°	-84.684140°
The Gorge at UMBS Plot 3	UMBS3	experimental transplant colony	45.554424°	-84.685563°
North Branch of Platte River Plot 1	PR1	experimental transplant colony	44.707643°	-86.024337°
North Branch of Platte River Plot 2	PR2	experimental transplant colony	44.706991°	-86.024340°
North Branch of Platte River Plot 3	PR3	experimental transplant colony	44.707216°	-86.024632°

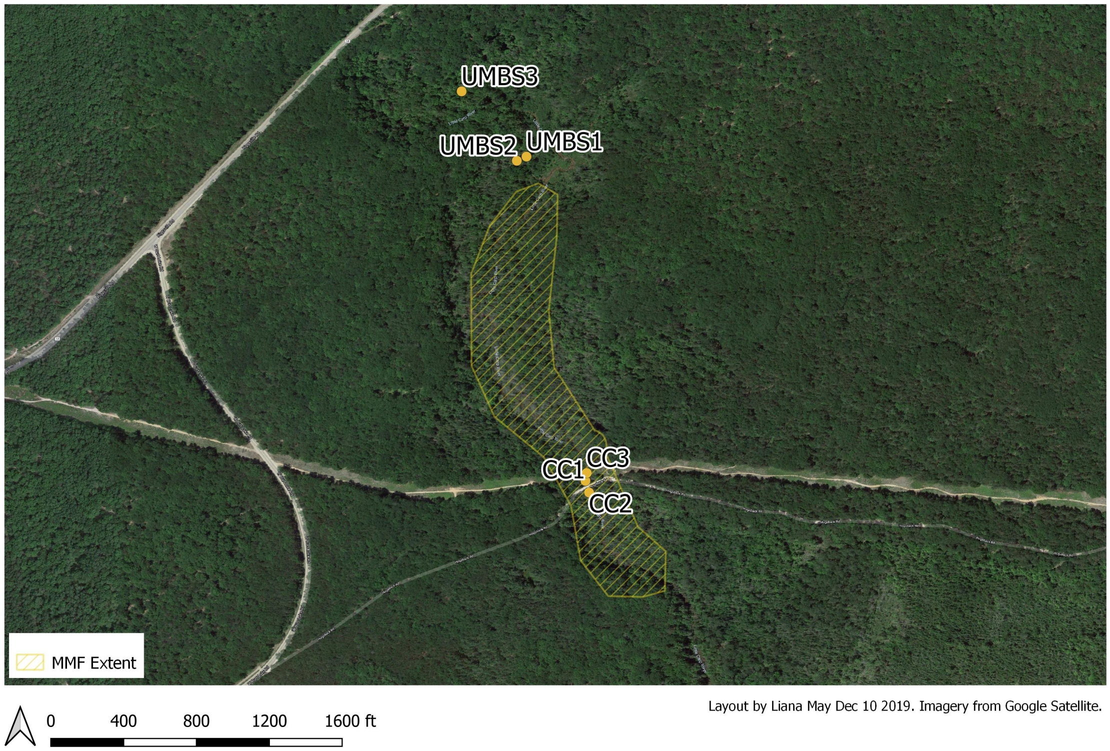


Figure 3. Aerial of the reference (CC) and translocation (UMBS) sites in Cheboygan County.

Figure 4. Aerial of the translocation sites (PR) on the North Branch of the Platte River in Benzie County.

Baseline Parameters

At each of the twelve plots, baseline environmental parameters were collected in the early summer of 2018, and peizometers were installed. Soil samples were taken from each plot following the Michigan State University Soil and Plant Nutrient Laboratory (SPNL) methodology. Samples were analyzed by the SPNL for pH, organic matter content, nitrate, ammonium, calcium, phosphorous, potassium, magnesium, percent exchangeable bases, and total cation exchange capacity. Percent canopy cover was estimated visually within 10% increments. Species composition and percent cover was recorded for the area immediately adjacent to each plot. Water flow rate was measured at each site by recording the number of seconds it takes a standardized unit to travel one meter; three separate measurements were averaged to give the final estimate.

To measure changes in the water table, peizometers were installed at each plot using a basic PVC pipe design. Boreholes were dug using a hand auger and a 5-foot (1.5m) piece of 1.5-inch (3.8cm) PVC pipe, with hand-slotting along the bottom 2 feet (0.6m), was installed at a depth of 3 feet (0.9m). Two feet (0.6m) of pipe was left exerted above ground, with a 1/8th inch (0.3cm) pressure equalizer hole and a PVC end cap. One gallon (3.8l) of silica sand was used to backfill the borehole along the slotted section, and a half-gallon (1.9l) of bentonite clay was used to fill the hole to ground level. After the clay was allowed to swell for several days, a cement collar was poured at the ground surface. The distance from the ground to the top of the pipe was measured ("stick up").

Translocation Process

At each of the six transplant sites, plots were prepped to receive MMF by removing existing vegetation. The source population of MMF (MR3) was surveyed to identify clumps that were large enough to provide adequate material for transplanting. Because of the creeping, clonal growth habit of MMF, counting stems was not a practical method to standardize transplants; instead, clumps of approximately 12" x 6" (30.5 x 15.2cm) were selected. Plants were carefully removed from the soil using a spade and by hand-digging, following procedures laid out by Mama Bear Restorations, Inc (Marquis 2011, 2012). All roots were less than 6" (15.2cm) deep, and often less than 3" (7.6cm), making it easy to remove plants without damage. Allospecific plants were hand-pulled from the clumps and the roots were gently rinsed in stream water to remove excess soil that may hold invasive forget-me-not seeds. Clumps were placed in a standard cooler with a small amount of stream water and immediately driven to the receiving site.

Transplanting to the UMBS site occurred on July 20th, 2018, and to the Platte River site on July 27th 2018 (Table 2). The UMBS clumps were replanted within an hour, while the Platte River clumps were replanted within 4 hours. Once on site, plants were immediately taken to each plot and gently worked into the prepared beds. Rinsing soil from the stems resulted in the stems becoming more lax allowing the clumps to be spread over a larger area upon planting. Each end of the planting was flagged. The initial amount of material received by each plot varied and was measured in square inches at the time of planting.

Monitoring

To track changes in the population, the transplant sites, reference sites, and source population at the Maple River were monitored monthly (as possible) during the growing season of 2018 and 2019 (see table in the Appendix for monitoring dates and activities). The water table was monitored at each plot by dropping an electronic tape measure down the peizometer and recording the distance from top of pipe to water level. Groundwater height was calculated by subtracting the distance to water table from the stick-up. The Maple River site was primarily monitored for impact of the Lake Kathleen drawdown, while other sites were measured to understand seasonal water table changes, and for comparison to the Maple River site. The first two measurements (July and August 2018) were taken pre-drawdown of the lake, the third (September) taken during the drawdown, and subsequent measurements (October 2018 and onward) after drawdown was complete.

The pH, temperature, conductivity, and dissolved oxygen of the surface water were measured at the point of interaction with the MMF using a Hydrolab Quanta Multi-probe meter. Data were collected for the Carp Creek, Maple River, and UMBS sites from July-October 2018, and April 2019. Because of an equipment malfunction, there were no measurements for June-September 2019. Data were taken at the Platte River site July-October 2018, and May-September 2019.

MMF population size was measured in square inches of area covered during the monitoring trips. At each plot, a standard tape measure was placed between the flagged areas and photographs were shot top-down. MMF cover in square inches was calculated for each of the transplant sites by laying a 1-inch (2.5cm) grid over the reference photographs and counting each grid square that is covered at least 50% by MMF material. For transplant plots (UMBS and PR sites) the entire colony was measured. Existing populations at Carp Creek and Maple River were measured within a 36” by 12” (91.4 x 30.5cm) area marked by flags. Notes were taken on presence of browse or insect herbivory, number of flowers, number of capsules, and qualitative descriptions of the health of the population and other disturbances.

Maple River Vegetation Transects and Forget-me-not Management

At the primary Maple River population, two point-intercept vegetation transects were established to monitor the main population of MMF and associated invasive species during and after the dam removal. In October 2018, a 40-foot (12.2m) long permanent baseline was laid out along the north side of the stream and marked with pink flags. Five 20-foot (6.1m) long transects were extended south from a random point on the baseline. The transect point on the baseline was determined using a random number generator with whole numbers between 0 and 40 that corresponded to feet along the baseline. Along each transect, a rod was dropped at 2-foot (0.6m) intervals and all of the plant species that intersected with the rod were recorded. This provided an estimate of MMF and other species abundance in 50 sample points across an 800 square foot (74.3 m^2) area. This process was repeated on the south side of the stream in May of 2019 to gain adequate coverage of the population. The data is expressed as percent cover per species by dividing the number of sample points in which a species is present by the total number of sample points (50).

During the growing season in 2019, two efforts were made to hand pull forget-me-not within the vegetation transect areas by MNFI and USFWS staff. Hand-pulling was chosen as the most appropriate management strategy because of the interspersed nature of the MMF and the sensitive wetland habitat; this strategy is also used to control forget-me-not in similar projects (Marquis 2012). Transects will be repeated in future years after the management of the forget-me-not by the USFWS, using the same baseline and new randomly spaced transects, to monitor long-term growth or decline.

Results

Baseline Parameters

Soil characteristics were similar within sites but varied between sites. Calcium and magnesium were the most abundant soil nutrients at all sites, with ranges two magnitudes larger than nitrogen, phosphorus, and potassium (Table 2). These nutrients were particularly high at the Carp Creek and the Platte River sites, with parts per million (ppm) ranging from 3000 to over 5000. Soil nitrogen varies from around 1 ppm up to 22 ppm, again with highest concentrations at the Carp Creek and Platte River sites. Phosphorus and potassium range from around 5 ppm to 26 ppm across sites with most values between 10-15 ppm. Calcium made up the preponderance of exchangeable bases, therefore cation exchange capacity was highest in plots with high calcium concentrations.

Organic matter makes up less than 8% of soil by mass at all sites except PR1, where levels are about twice that of others. Soil pH was within the growing range of MMF (6.8-8.2) in all plots except for UMBS1, where it falls to 6.7.

Table 2. Soil characteristics were measured at each of the plots before transplanting; note the CC and MR sites are of existing MMF populations.

SITE	Soil pH	Organic Matter (%)	Nitrate (ppm)	Ammonium -N (ppm)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	K CEC (%)	Mg CEC (%)	Ca CEC (%)	CEC (meq/ 100g)
CC1	7.3	5.3%	6.1	4.4	11	17	3100	414	0.2	18.2	81.6	19
CC2	7.7	2.3%	22.3	3.7	15	15	1509	191	0.4	17.3	82.3	9.2
CC3	7.5	3.7%	10	4.8	12	13	2567	424	0.2	21.5	78.3	16.4
MR1	7.3	7.0%	0.6	0.9	12	26	731	60	1.6	11.8	86.6	4.2
MR2	8	4.0%	0.7	0.9	6	24	891	43	1.3	7.4	91.4	4.9
MR3	7.5	2.2%	0.2	1.4	7	22	897	112	1	17	81.9	5.5
UMBS1	6.7	2.2%	0.7	3.8	10	9	1553	189	0.2	16.8	82.9	9.4
UMBS2	7.4	2.3%	1.6	2.1	14	13	1205	169	0.4	18.9	80.7	7.5
UMBS3	7.9	7.0%	1.5	1.8	21	14	653	65	0.9	14.1	85	3.8
PR1	7.4	15.3%	6.8	3.7	12	22	4725	677	0.2	19.2	80.6	29.3
PR2	8.2	8.4%	0.9	0.5	10	10	620	60	0.7	13.8	85.5	3.6
PR3	7.5	8.1%	4.2	0.5	10	15	4020	514	0.2	17.5	82.3	24.4

Surface water flow rates are between 0.10 and 0.25 m/sec in all plots except for two; UMBS1 has a flow rate of 0.05 m/sec, and the seeps at PR3 did not have enough concentrated flow in one area to form a channel, therefore it was not possible to measure flow.

Canopy cover at most sites is dominated by northern white-cedar *(Thuja occidentalis,* 9 plots*)* and tag alder *(Alnus incana*, 10 plots*)*. Associated species occurring within the surrounding area include Eastern hemlock *(Tsuga canadensis)*, bigtooth popular *(Populus grandidentata),* with red maple *(Acer rubrum)* and yellow birch *(Betula alleghaniensis)* at the Platte River site. Cover ranges from nearly full sun at the Carp Creek site to about 50% canopy cover at the shadiest of the Platte River sites.

Understory community across all sites is composed of common northern Michigan wetland species. The most common understory species (found at 50% of plots or more) are marsh marigold *(Caltha palustris),* field horsetail *(Equisetum arvense),* fowl manna-grass *(Glyceria striata),* jewelweed *(Impatiens capensis),* and watercress *(Nasturtium officinale).* Water forget-me-not is the most common invasive species at the Maple River plots. Cover is as high as 95% in MR1, lesser in the other two plots. Other invasive species found are Canada thistle *(Cirsium arvense)* at Maple River and Platte River sites, willow-herb *(Epilobium parviflorum)* at the Carp Creek site, curly dock *(Rumex crispus)* at the Maple River site, and bitter dock *(Rumex obtusifolius)* at the Platte River site. Invasive species cover is minimal except for water forget-me-not at the Maple River.

Water Chemistry

The results of monthly monitoring during the growing seasons give us the range of abiotic conditions that the MMF experience across 12 sites and help identify conditions associated with growth, and, changes at the Maple River site after the drawdown of Lake Kathleen. Additional line graphs of temporal changes in these parameters are included in the Appendix.

The average water temperature in the root zone during the growing season was 12 ±2.2°C (Fig. 5). Temporal trends in water temperatures at the Carp Creek, Maple River, UMBS, and Platte River sites were similar, with temperatures of approximately 12-16°C in July, holding fairly steady through August and September, then dropping off in October to 8-11°C. Plots MR1 and MR3 showed a slightly different trend, increasing in temperature from July (~8.5°C) through September (~11°C), with October temperatures remaining slightly above July temperatures (~10°C). MR1 saw the most increase in temperature from July to October, from 8.5 to 11.1°C.

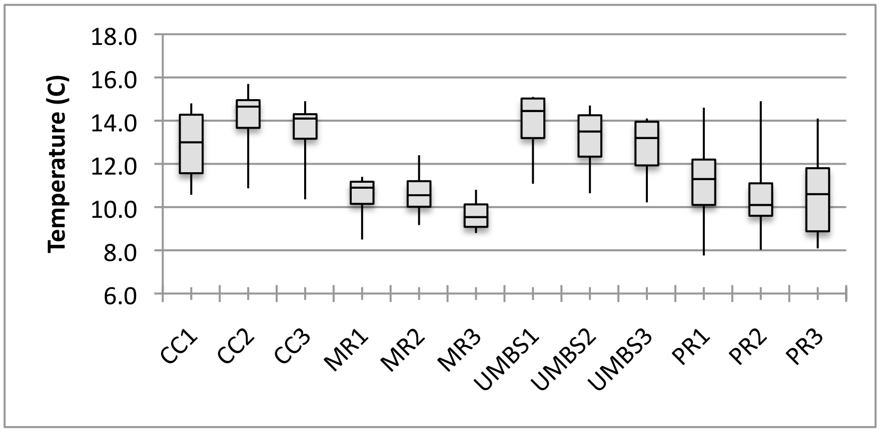


Figure 5. Water temperatures ranged from 8°C to 16°C during the growing season.

Dissolved oxygen in the water at the root zone ranged between 33% and 98%, with a mean value of 70.9 ±15.9% (Fig. 6). There a similar pattern throughout the growing season across the plots, with oxygen staying steady or increasing through September then dropping off in October by as much as 30%. The Platte River plots were unique in that they dropped in dissolved oxygen steadily from July to October in 2018, but rose in PR2 and PR3 from September to October slightly in 2019.

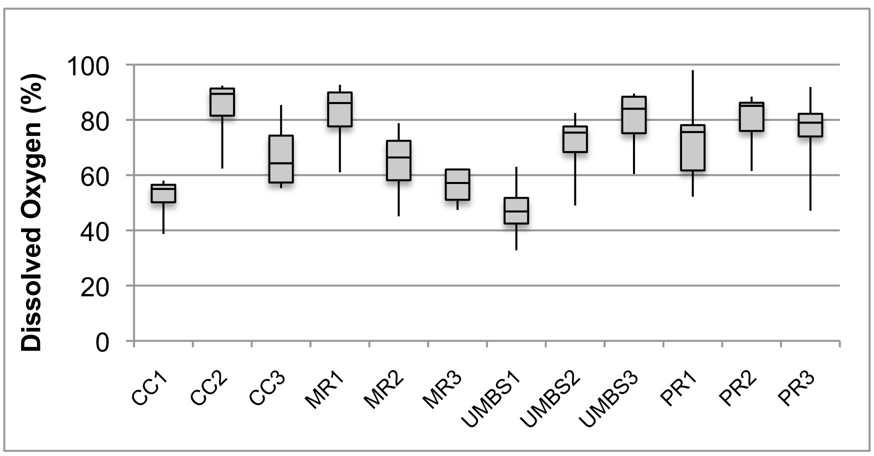


Figure 6. Dissolved oxygen ranged from 33% to 98%, with a mean of 70% across all plots.

Water pH across all sites had an average of 7.9 ±0.4, staying fairly even across all plots (Fig 7). Temporal trends show water pH increased between July and September from ~7.7-8.1 to ~8-8.3, then dropped back down roughly to July levels in October. The minimum pH levels (6.5) were made at the three Platte River sites in September 2019. The maximum pH levels (up to 8.45) were recorded in April of 2019 at the Carp Creek and UMBS sites.

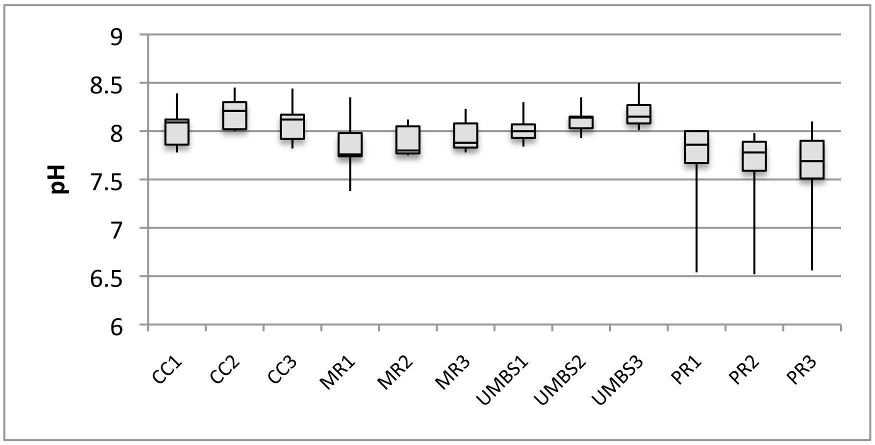


Figure 7. The pH across all plots had a mean value of 7.9 with a narrow standard deviation of 0.4.

The average electrical conductivity of the water was 352 ±76 µS across all sites during the growing season (Fig. 8). Generally, the EC slowly rose throughout the growing season, with some sites showing that it lowered slightly in the fall.

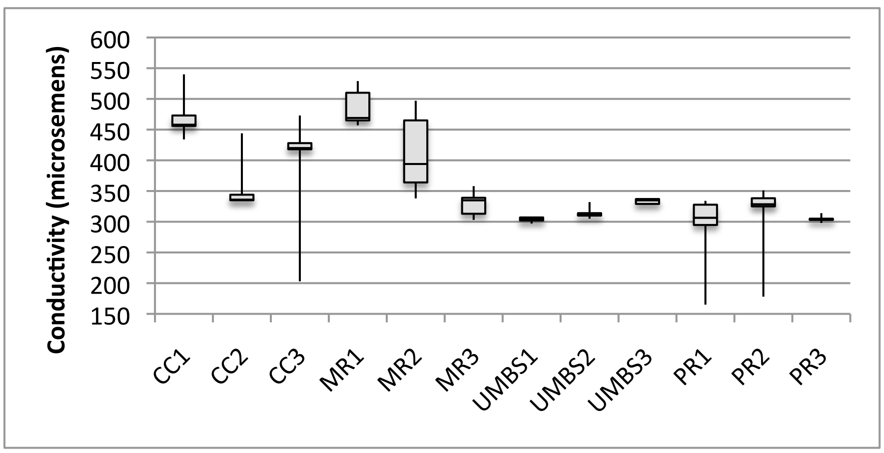


Figure 8. Boxplots of water electrical conductivity at MMF sites.

Water Table Depths

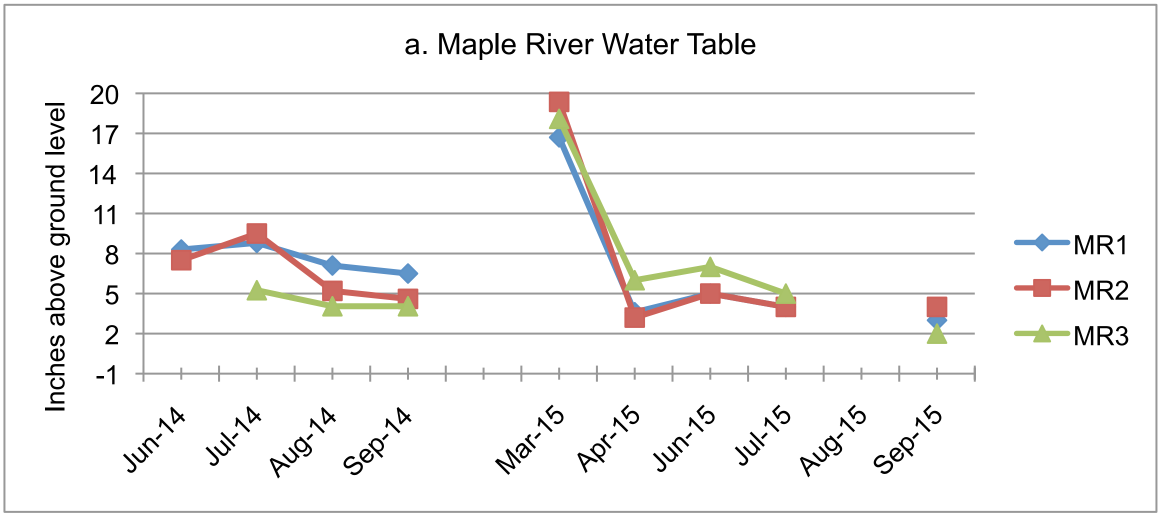
Groundwater depth was monitored by measuring the water depth in the peizometers; because of the positive pressure of the groundwater at the monitoring sites, the water table was above ground surface within the peizometers, thus the water table readings appear as inches above ground.

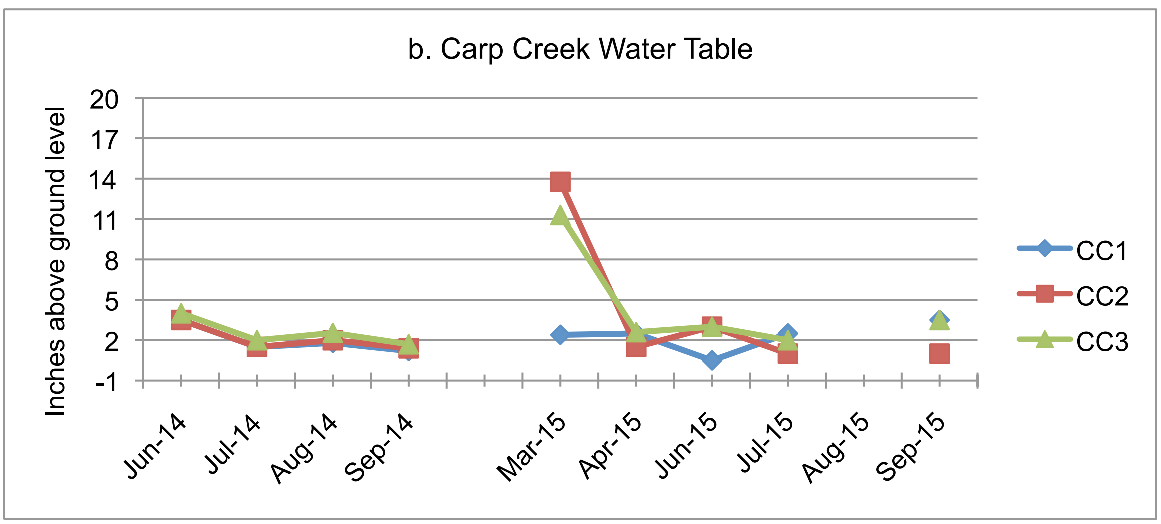
At the Maple River monitoring site, the water table decreased a mean of 2.8” (7.1cm, n=3) during the Lake Kathleen drawdown between August and October (Fig. 9a). In April of 2019, when monitoring commenced, the water table was approximately 10-15” (25.4-38.1cm) higher than October measurements the year before, but dropped rapidly to 2018 levels by May. In July 2019, the water table was a mean of 3.9” (9.8cm) below the 2018 levels, and in September, was a mean of 2.4” (6cm) below 2018 levels.

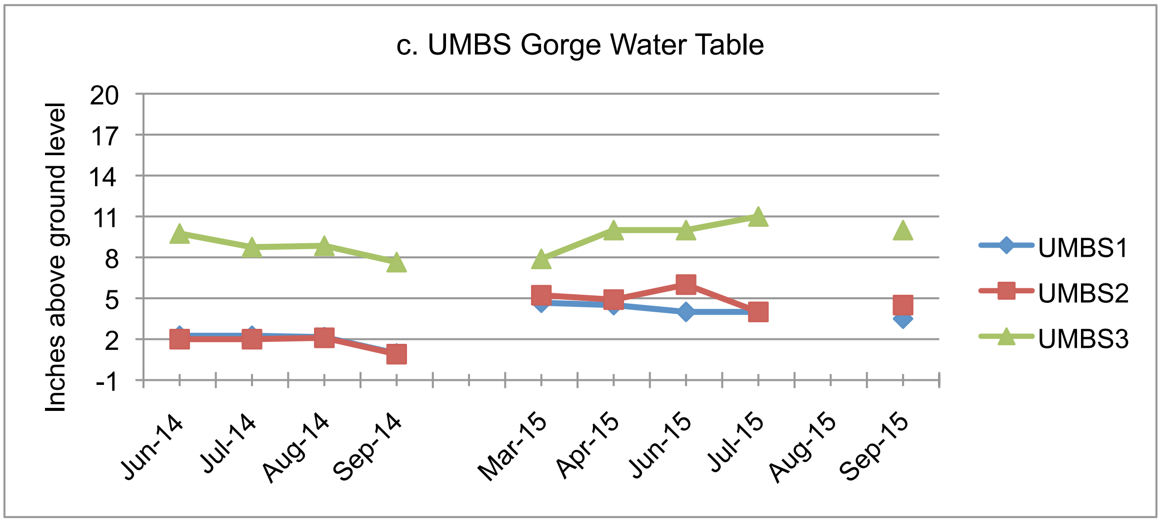
The water table at the monitoring sites nearby the Maple River, Carp Creek and UMBS, showed similar patterns throughout 2018 (Fig. 9b and c). Carp Creek also had very high water in April 2019 (10-12” (25.4-30.5cm) above October levels) which then returned to normal by May. Year to year comparisons show in July 2019 groundwater was 1-2.5” (2.5-6.4cm) below 2018 levels at Carp Creek, and 1.25-2”

(3.2-5.1cm) above 2018 levels at UMBS. By September of 2019, the water table at both Carp Creek and UMBS had a mean increase of 1.1” (2.8cm, n=6) above 2018 levels.

The Platte River sites displayed less homogenous behavior in groundwater, with some sites declining during summer months of both years, and others increasing. Year to year comparisons show a range of changes from -4.8” (-12.2cm) lower to 8” (20.3cm) higher than 2018 September levels (Fig. 9d).







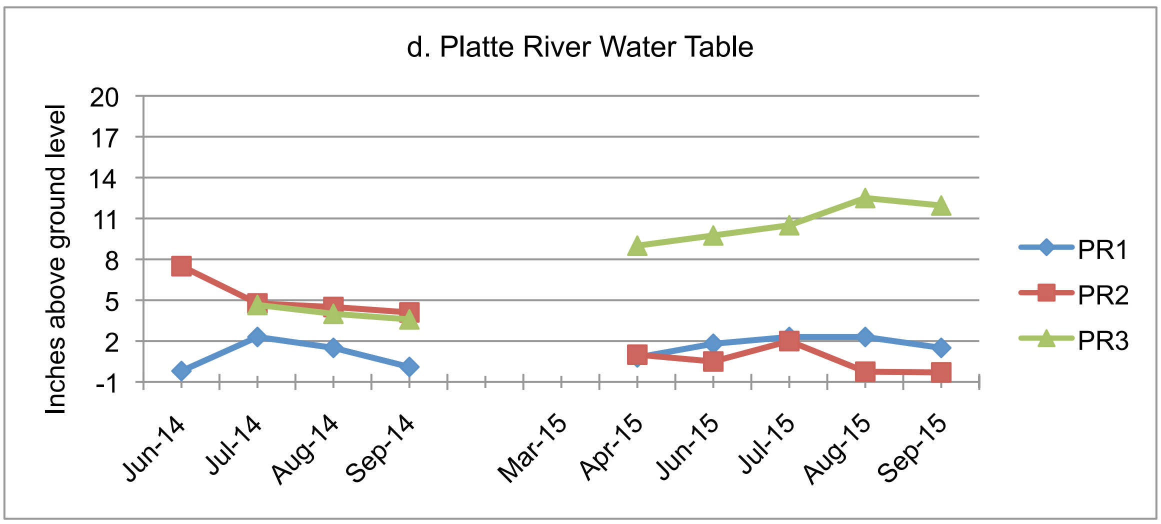


Figure 9 (a-d). Water table as measured in peizometers at the four sites over the course of the monitoring period.

Trends in MMF Growth at Monitoring Plots

Transplant populations were monitored throughout the growing season. After transplanting, initial monitoring visits noted anthocyanin pigments in larger stems and leaves and some desiccation of larger leaves in all plots except PR2, and minimal amounts at UMBS2. September 2018 visits confirmed the leaves that had anthocyanin pigmentation had proceeded to desiccate, with dieback at UMBS1, UMBS3, PR1, and PR3; UMBS2 and PR2 had minimal dieback. While main leaves desiccated, stems began to produce new growth at nodes and by October 2018, most plots had replaced large leaves with new lateral shoots, new root growth, and many smaller leaves.

The growth of new lateral and terminal shoots increased the area covered by the colonies across most experimental transplant plots throughout the 2019 growing season (Fig. 10). The best growth was measured at PR1 with a ~500% increase in cover, followed by PR2 and UMBS2 with a ~250% increase in cover. UMBS3 had a slightly lower increase in cover (150%) but showed steady increase throughout the season. Two plots saw no growth or decreased in cover, PR3 and UMBS1. These plots also had the most anthocyanin pigments and desiccation immediately after planting.

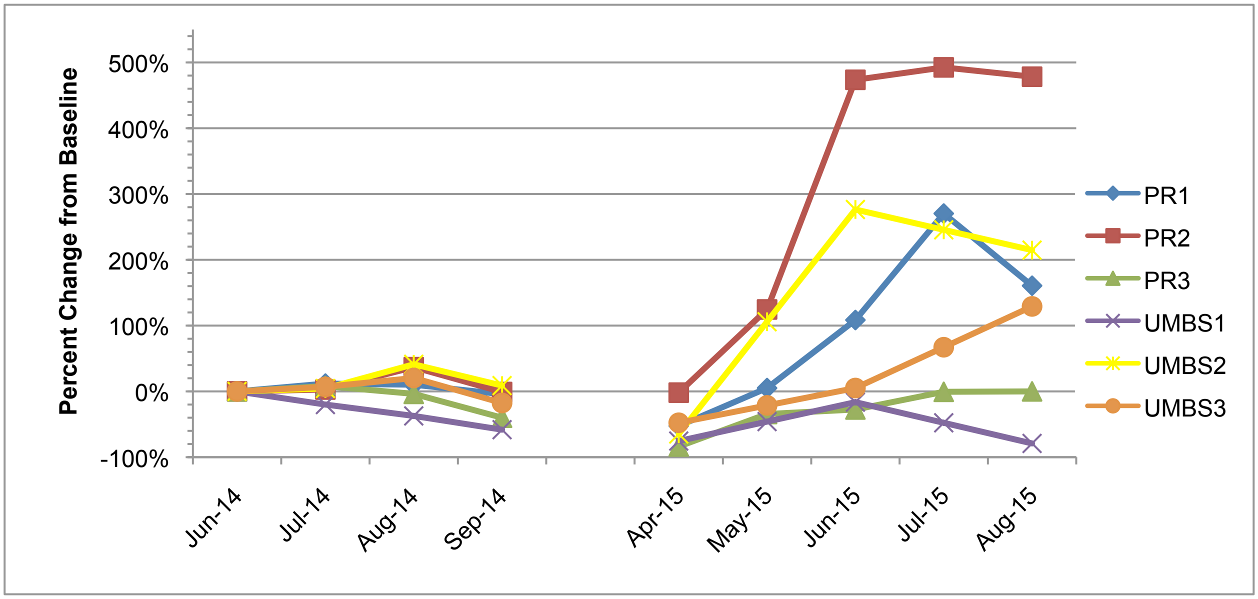


Figure 10. Transplanted populations at PR and UMBS were measured in the percent change in areal cover from the original planted colony.

Michigan monkey-flower cover at the Maple River site saw a decrease in cover within all plots between 2018 and 2019, ranging from small decreases at MR2 (8%), to large decreases in MR1 (92%) and MR3 (87%) late summer of 2019. The reference plots at CC1 and CC2 increased (73%) and decreased (24%) from the baseline measurements, respectively. While all other populations measured (including the transplant populations) showed a seasonal increase from May through August, then decline in and after September, the Maple River populations held steady cover, or decreased, in the 2019 growing season. The CC3 reference site data was not usable because one of the marker flags was missing in the spring of 2019.

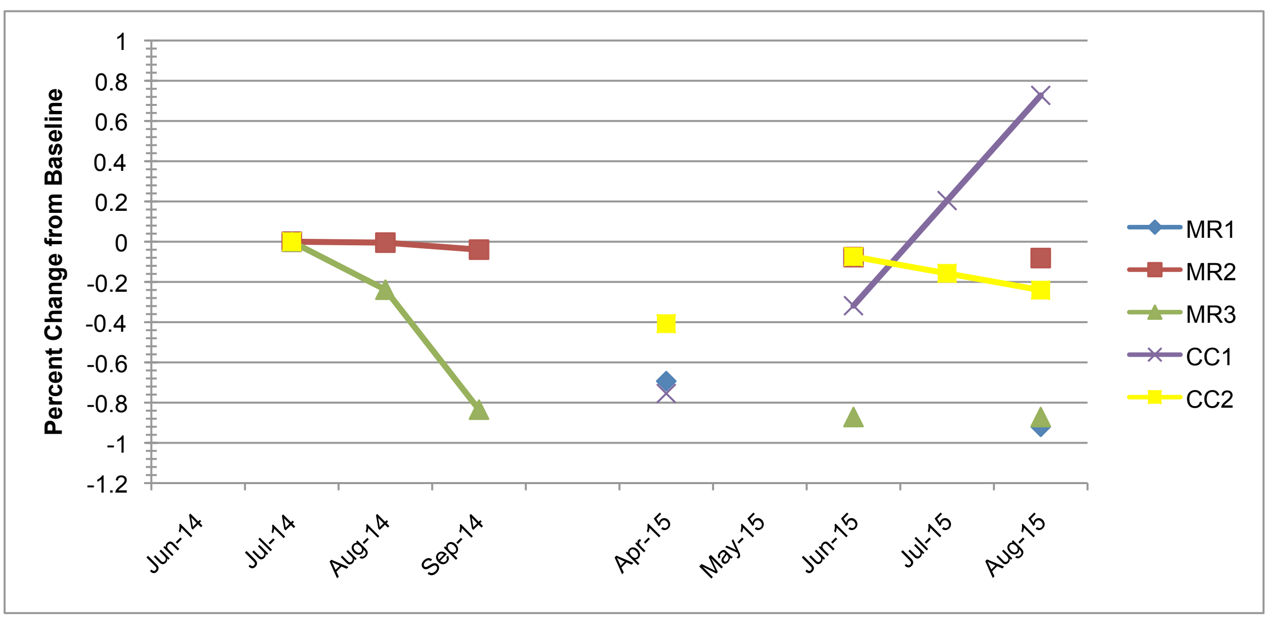


Figure 11. Percent changes in established colonies were calculated from a baseline of cover within each plot before Lake Kathleen drawdown (August 2018).

Vegetation Transects and Forget-me-not Cover

The point-line transects at Maple River show a 64% cover of water forget-me-not, 22% cover of Michigan monkey-flower, 18% cover of American brooklime *(Veronica beccabunga var. americana)* and watercress *(Nasturtium officinale)*, 16% cover of duckweed *(Lemna turionifera),* and 12% cover of invasive Canada thistle *(Cirsium arvense)*, with 10% or less cover of other species. In the areas where forget-me-not was removed, there was an immediate decrease in cover but the return rate of forget-me-not (or other species) in these areas will require further study.

Discussion

The Maple River Population

The water parameters measured at the Maple River site stayed within the known range tolerated by MMF throughout the monitoring period, and generally followed the magnitude and direction of changes seen at other plots in the area. One exception was water temperature at the Maple River site; July water temperature at two of the Maple River sites, MR1 and MR3, were slightly below the known low temperature (8.9°C) that MMF occurs, 8.5°C and 8.8°C respectively. This contributed to the relatively high fall temperatures at these sites, with October water temperatures remaining above July temperatures. The other ten plots saw a net decrease in temperature between July and October. Since this is an established colony and has been growing in these conditions historically, the tolerance range for temperature should be expanded (it should also be assumed that water temperature drops below 8.5°C during winter months).

The initial concern of the Lake Kathleen dam removal was that drawdown would inundate the small streams where MMF occurs and significantly change water quality. This was mitigated by the slow release of water and the location of the siphons, which were discharging Lake Kathleen into the historic East Brach channel downstream, so the primary MMF colony was not inundated. The water table decreased at the Maple River sites during the drawdown (Aug-Oct 2018), but this change was mirrored at the Carp Creek and UMBS sites and was likely a seasonal fluctuation. Reduction in groundwater discharge from the drawdown of Lake Kathleen could take several years to be fully realized, and it is not likely to be reflected immediately in the monitoring wells. However, in 2019, the water table was lower than 2018 levels by several inches at the Maple River, while the Carp Creek and UMBS sites mostly saw an increase in the water table. These sites are both in the Cheboygan River watershed (different sub-watersheds) and experience similar rainfall, so the lower water table could be attributed to reduced groundwater flowing from the north (Lake Kathleen).

Between 2018 and 2019, the MMF population at the Maple River saw slight to sharp declines, while the reference site populations at Carp Creek held steady or increased. The most drastic declines were at MR1 and MR3. MR1 is located in the area with dense water forget-me-not growth, and the decline over the season (May-June 2019) was, in part, because the forget-me-not overtopped the MMF later in the summer, covering it from view of the monitor (Fig 12). The monitoring only counted vegetative that was visible from a top-down view, and it did not account for the lower strata of vegetation. Even though there is MMF growing under some of the forget-me-not, these plants are vigorous, as competition for resources and reductions in flowering stems will lead to declines. Depending on the needs of future monitoring projects, this could be designed to include lower strata of vegetation. The declines at MR3 were nearly as sharp as those at MR1, but forget-me-not, though present, was not abundant in this plot. The major declines, from August to October of 2018, seemed to be related to desiccation of the plants (though water levels at MR3 did not differ from patterns at MR1 and MR2), or perhaps insect herbivory. Monitoring notes and photos indicate the plants that were there at an early monitoring period were not there on follow-up visits.

Figure 12. Forget-me-not growth (blue flowers) crowds and overtops MMF (yellow flower, center) at monitoring plot MR1 (June 20, 2019)

The reduction in MMF cover at MR2 was only slight from the year before (8%) which is probably within the normal variation of a plant population. This plot was located in an area of dense MMF growth on top of a cold spring with little forget-me-not present. Browse was a factor in this plot, and many of the stems were nipped off after they sent up their flowering shoots, from about 8-12” (20-30cm) height with flowers to 1-2” (2.5-5cm), between June and August (Fig. 13). This suggests the MMF is quite palatable (likely to white-tailed deer) and browse has a significant impact on reproduction.

Figure 13. Heavy browse was noted on most of the upright flowering stems, which emerge in late June and flower through August (MR2, June 20, 2018).

Experimental Translocation

All of the experimental transplant colonies survived the transplanting process and developed new roots and shoots within one month, and all plots grew in areal cover during the first month except one. The plots that were the most successful (PR1, PR2, UMBS2, and UMBS3) were planted on fine silt sand overlaying a mixture of muck and sand sediments with the highest water flow rates of all the experimental plots (0.2-0.3 m/sec) and consistently high dissolved oxygen. These populations all sent up flowering shoots over 13” that produced multiple flowers and capsules within one year of transplant (Fig. 14). The rate of growth is promising and reflects the plant’s vigor as a colonizer.

Figures 14 & 15. Flowers (left, July 1, 2019) and capsules (right, Sept. 3, 2019) at Platte River.

The poorest performing colonies (UMBS1 and PR3) saw no growth or a reduction in colony size over the monitoring period. These sites have the lowest water flow rates of all the plots (0-0.05m/sec). This supports the findings of Marquis (2012) on the importance of springhead discharge at the planting site, and rates over 0.1m/sec seem to be our threshold for good growth in this study. Since it is important that the substrate that MMF is rooted in does not incur a hard freeze while overwintering, good groundwater flow is also important to keep temperatures closer to the average Michigan groundwater temperature, 5.5°C. Additionally, the surface water at UMBS1 had a large amount of iron-oxidizing bacteria (visual assessment) and the lowest dissolved oxygen of all the plots with a mean of 47%. Iron-oxidizing bacteria colonize areas where anaerobic groundwater flows into an aerobic environment, indicating that the water at the rooting zone of the MMF at UMBS1 is anoxic. None of the other plots in this study have iron-oxidizing bacteria in the area where MMF is rooting, though some had similar DO measurements. Plots CC1 and MR3 had relatively low DO, with a mean of 51% and 56% respectively, and MMF cover declined in both plots in the 2019 growing season. The percentage of DO measured at the surface water may not be the threshold for survival, rather the discharge of anoxic groundwater in the root zone may be impacting root development. MMF may not have physiological structures for transporting oxygen to roots.

Poor performing plots did show some promising signs too. Remaining healthy plants grew long, thin lateral branches that spread quickly across the substrate, rooting and forming new upright growth in nearby habitat. In the cases of UMBS1 and PR3, these areas are slightly above the muckiest portion of the substrate on moss-covered organic material such as rotting wood. This concurs with the findings of Mama Bear Restorations at the Big Glen population, who noted that they often see MMF moving along the shoreline, and fragments broken off by water and ice root readily in new suitable locations (pers. comm.).

Other environmental parameters (including soil characteristics) did not seem to have a strong correlation to transplant success, though they were mostly within preferred ranges. The water pH of the experimental plots varied between plots and over time, but generally stayed within the parameters observed at the established colonies. Bliss (1983) suggested water pH needs to be between 6.8 and 8.2, however, we observed healthy populations with water pH ranges from 6.5 (PR, October 2019) to 8.5 (UMBS and Carp Creek, April 2019). The low water pH of 6.5 may have been recorded after a rain event, thus a temporary flux. Additionally, the poor performing site UMBS1 had a soil pH of 6.7. MMF may not be tolerate extended exposure to pH <6.8, but a pH up to 8.5 appears acceptable.

Anthocyanins were noted in the leaves at several MMF transplant plots in August 2018. The development of anthocyanins in plants is a response to stress and may be related to the transplant process, or the change in soil and water chemistry from the source site (Chalker-Scott 1999). Anthocyanins were also noted in MMF in a reciprocal transplant experiment with James' monkey-flower (*Mimulus glabratus* (Bentham) Grant) and were associated with smaller leaves and stunted growth (Bliss 1983). In this experiment, the transplant colonies with the most anthocyanin pigmentation were those with greatest leaf desiccation in September 2018, and a net decrease in cover. The initial development of anthocyanins may be able to be used as an indicator of future growth.

Leaf loss and stem desiccation was noted at the Platte River site on otherwise healthy plants and it seemed to be associated with a large population of slugs on the plants. There are tentatively identified as the non-native dusky slug *(Arion subfuscus* (Draparnaud)) (Fig. 16). During the September surveys, as much as a third of the flowering stems had been eaten and the tops had died (Fig 17). The slug damage was unexpected because it had not been mentioned elsewhere in the literature, and may be unique to this site, i.e., a large population occurring on a less palatable host plant such as watercress *(Nasturtium spp.)*. The slug damage reduced the number of capsules set.

Figures 16 & 17. Slugs were noted consuming the MMF at the Platte River plots on the Aug. 2nd (left) and Sept. 3rd (right) 2019 monitoring visits.

The best possible measures were taken to prevent transplant colonies from being sited near existing colonies to isolate them from gene flow between populations and reduce chances of out-breeding depression. Out-breeding depression occurs when genetically distant individuals produce progeny that have lower overall fitness in their environment than either of the parents. However, sites that met our criteria for establishing new colonies were limited and both PR and UMBS sites are along streams that contain historical MMF populations. The plots on the North Branch of the Platte River are located two miles downstream from the historical site, so fragments, seeds, or pollen are not likely to intermix. The

UMBS plots are at the headwaters of Carp Creek population, and it may be possible for insects to pollinate Carp Creek population with the fertile Maple River pollen. Posto (2001) showed that plants from the Maple River population can cross with the Carp Creek population and set seed, but they did not test if this produces fertile offspring. The Maple River population can also self-pollinate (Posto 2001), so seed produced could be transported downstream to the Carp Creek population.

Expanding the Understanding of MMF Ecology

Taking baseline and seasonal environmental measurements added to the general knowledge of the growth requirements of MMF. Previous studies have focused on water parameters and have not quantified soil characteristics, therefore the range of nutrients, CEC, pH, and organic matter presented here gives us a baseline range for parameters to work with in the future. Additionally, water flow rate has been noted as important in other literature, but measurements were not provided (Marquis 2012; Penskar and Higman 2001). Two sites, Carp Creek and the Maple River, are historical MMF populations so the soil and water parameters should be within the "safe" range for MMF long term survival and growth.

Combining the measurements taken at CC and MR sites (established populations) with those of previous publications (Bliss 1983, Penskar and Higman 2001), MMF sites across the state have water parameters with the following ranges: pH between 7.2-8.5, electrical conductivity between 203-540, DO between 39%-93% (though note earlier discussion on best growth with >60%), water temperatures 8.5°C-18°C, and water flow rates of 0.12-0.25 m/sec. It should also be noted that in seed germination experiments, Posto (2001) found that seeds had 67% germination at 23°C and 0% germination at 8°C, demonstrating the importance of warm sites for germination.

Soil parameters taken at the CC and MR sites are within the following ranges: pH between 7.3-8, 2.2-7% soil organic matter, 0.2-22.3 ppm nitrate, 0.9-4.8 ppm ammonium-N, 6-15 ppm phosphorus, 13-26 ppm potassium, 731-3100 ppm calcium, and 43-424 ppm magnesium. The cation exchange capacity is 4.2-19 meq/100g CEC, with exchangeable bases of 0.2-1.6% potassium, 7.4-21.5% magnesium, and 78.3-91.4% calcium. Sunlight ranges from full sun at Carp Creek and at the Glen Lake populations to dappled sunlight at other sites around the state; shady sites often do not produce flowers (Penskar and Higman 2001).

Conclusion

The Maple River population will require continued monitoring to understand the long-term changes in groundwater discharge and to see if the population shows continuing declines. We recommend monitoring in the growing season of 2020, particularly of the water table and population size at the Maple River and the reference site, Carp Creek, and to keep collaborators informed on the status of the population. Additionally, substantial effort is needed to protect this population from continuing threats from invasive species and browse. The experimental hand-pulling of forget-me-not conducted during this study showed initial success and a large-scale effort over an extended period of time, followed by another point-line intercept, is recommended. Currently, the level of infestation is none to low at other sites and prompt removal will minimize the chances of infestations overtaking them. We also recommend the installation of a deer fence around the larger intact portions of the Maple River population (e.g. south side of stream at the primary colony). If resources are available, deer fences could also be installed at the experimental transplant sites. A more detailed study would be needed to parse the relative impact of each factor (water table, browse, invasive species) at the Maple River site.

Transplanting was generally successful, and MMF is easy to remove from its substrate and takes readily to new sites as long as the basic environmental parameters are within the ranges discussed above. This is encouraging from a conservation perspective and reflects the USFWS recovery classification 8c, having a high potential for recovery. With the correct conditions, plants quickly form new lateral shoots, rooting at nodes, and flowered within one year of the transplant date at the healthier populations. Within the transplant sites, the most important limiting factors to transplant success was water flow above 0.1

m/sec and high (>60%) dissolved oxygen in the root zone (all other parameters were within the known suitable ranges). All other parameters were pre-determined to be within the known growth parameters. The two new populations of Maple River genetic material appear to be self-supporting and should continue to grow and reproduce at their new sites. This will serve as a reserve for the genetic material in the case that Maple River population continues to decline. This material may also be moved back to the Maple River in the future after the site stabilizes, if needed.

Stakeholders have discussed the possibility of introducing Maple River plants, with fertile pollen, to non-reproducing populations such as the one at Big Glen Lake. Benefits may include the formation of a new seed-producing population, perhaps with seed-producing off-spring, that could help in the dispersal and long-term survival of MMF. Drawbacks include the potential for out-breeding depression of the population by producing offspring that are not suited for the new conditions. Non-reproductive populations have been isolated a long time and may have un-expressed genes that could reduce or improve fitness. If this is pursued at some point in the future, it may be preferential to mix Maple River genetics with a non-reproducing population in isolation, such as in a laboratory, greenhouse, or a tertiary site where the two can be transplanted to, without changing the genetic expression at an existing site.

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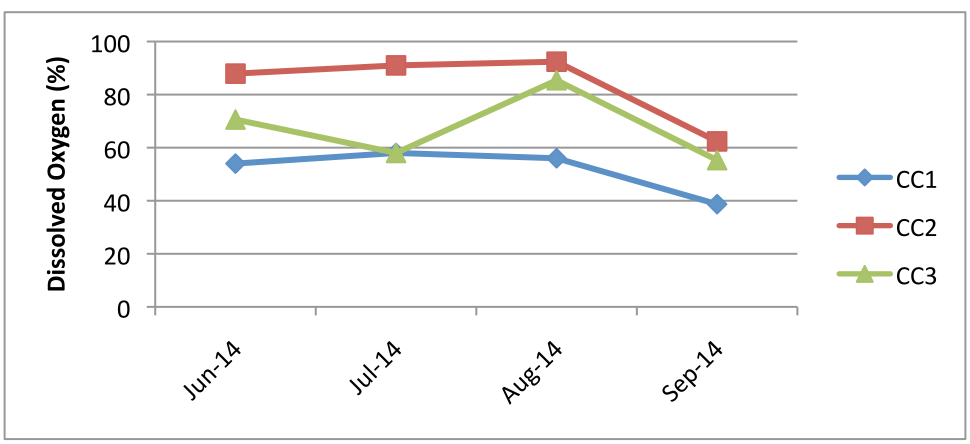
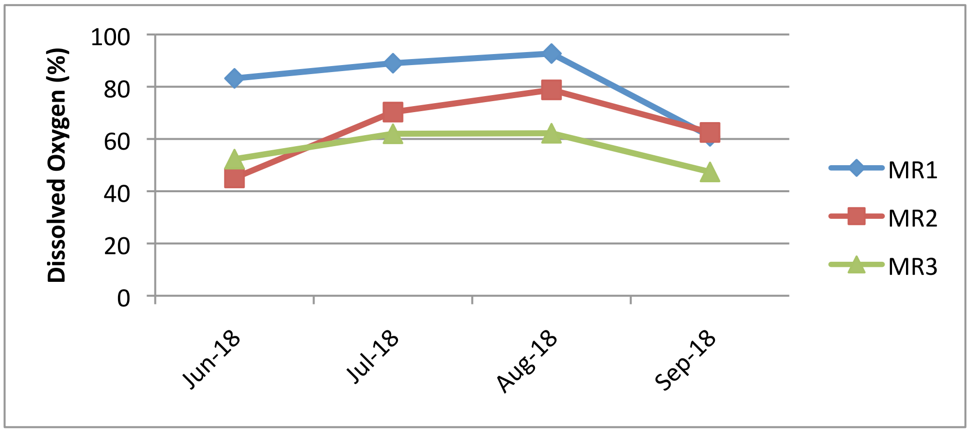
Appendix

1. Monitoring dates and activities.

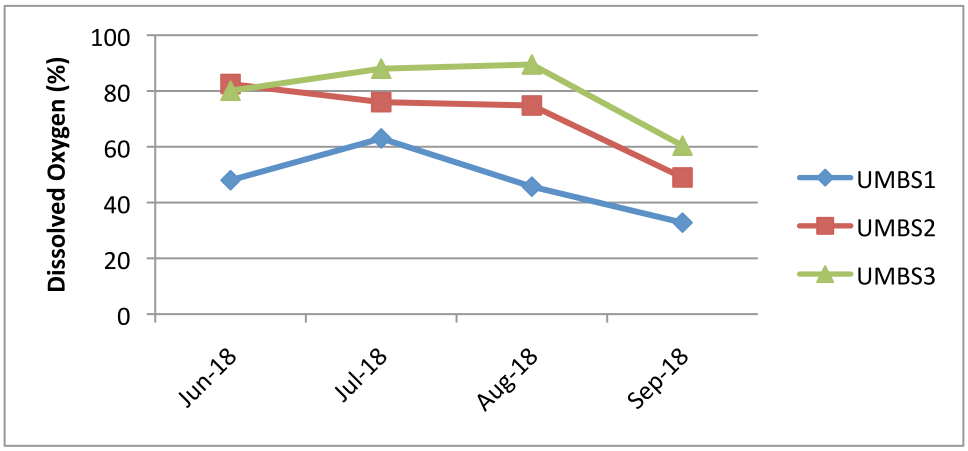
Date	Activity
06/19/2018	Monitoring wells installed and baseline parameters taken at MR1, UMBS, CC
07/20/2018	Translocate MMF from MR3 to UMBS; Monitoring wells installed and baseline parameters taken at MR2, MR3
07/25/2018	Monitoring wells installed and baseline parameters taken at PR1, PR2
07/27/2018	Translocate MMF from MR3 to PR; monitor MR and PR plots
08/03/2018	Monitoring wells installed and baseline parameters taken at PR3; monitor PR plots
08/18/2018	Monitor UMBS, MR, and CC plots
09/07/2018	Monitor UMBS, MR, and CC plots
10/11/2018	Monitor UMBS, MR, CC plots; conduct MR point-line transect
10/18/2018	Monitor PR plots
05/03/2019	Monitor PR plots
05/20/2019	Monitor UMBS, MR, CC plots
05/22/2019	Conduct MR point-line transect
05/31/2019	Monitor PR plots
06/19/2019	Monitor UMBS, MR, CC plots
07/01/2019	Monitor PR plots
07/24/2019	Monitor UMBS, MR, CC plots
08/02/2019	Monitor PR plots
09/03/2019	Monitor PR plots
09/12/2019	Monitor UMBS, MR, CC plots

2. Temporal graphs of dissolved oxygen

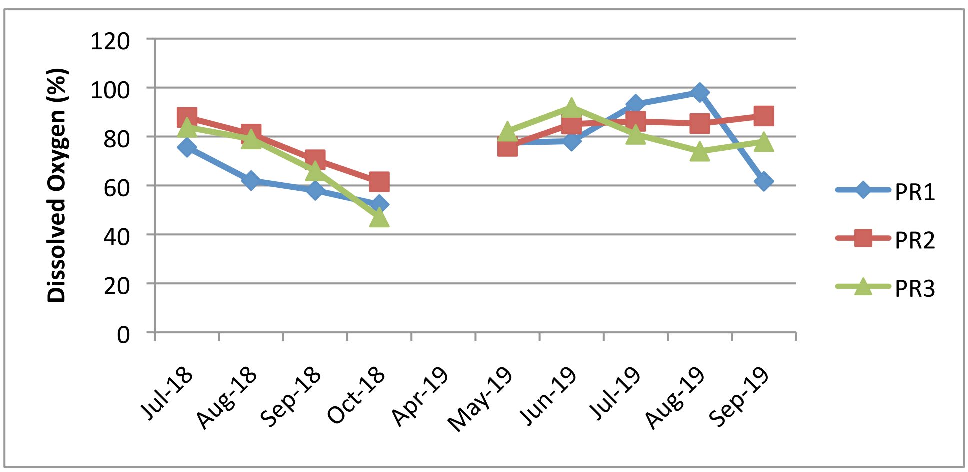




Dissolved Oxygen (%)
100
80
60
40
20
0
Jun-18
Jul-18
Aug-18
Sep-18
MR1
MR2
MR3

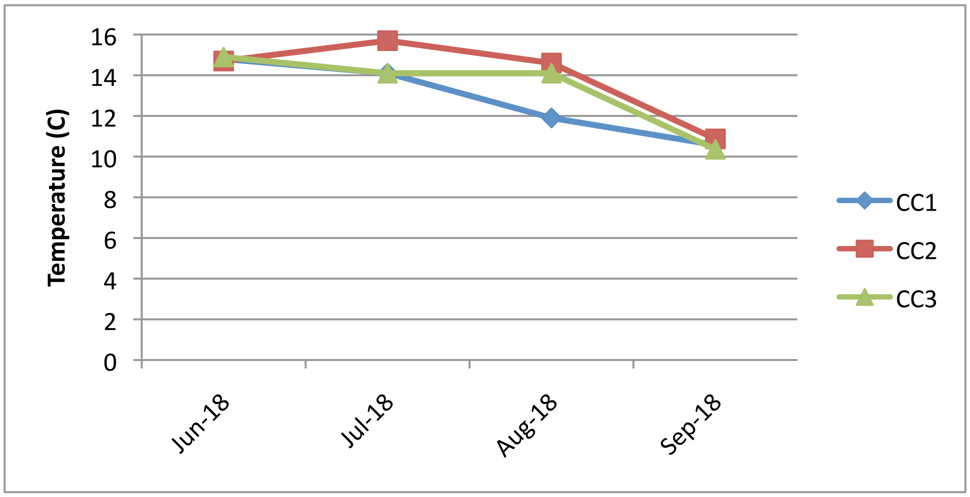


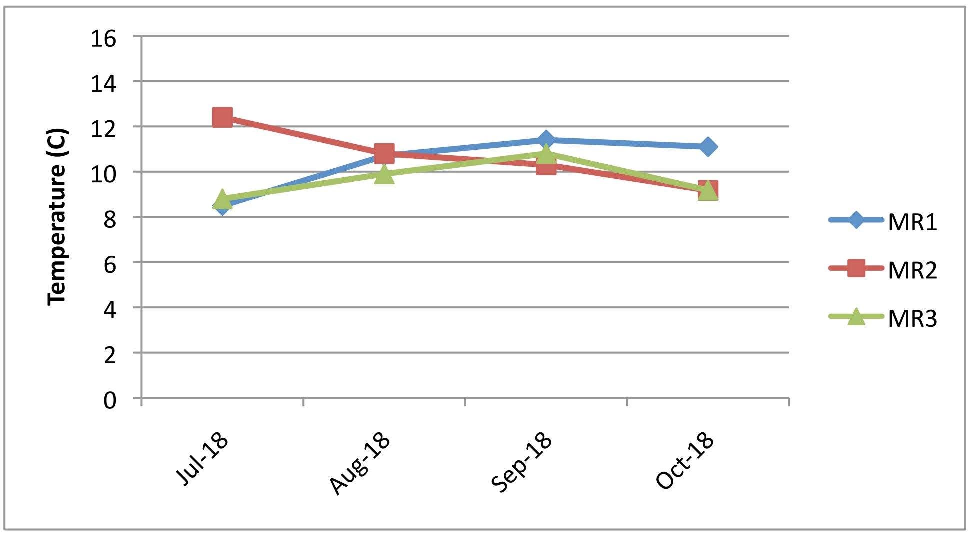
Dissolved Oxygen (%)
100
80
60
40
20
0
Jun-18
Jul-18
Aug-18
Sep-18
UMBS1
UMBS2
UMBS3

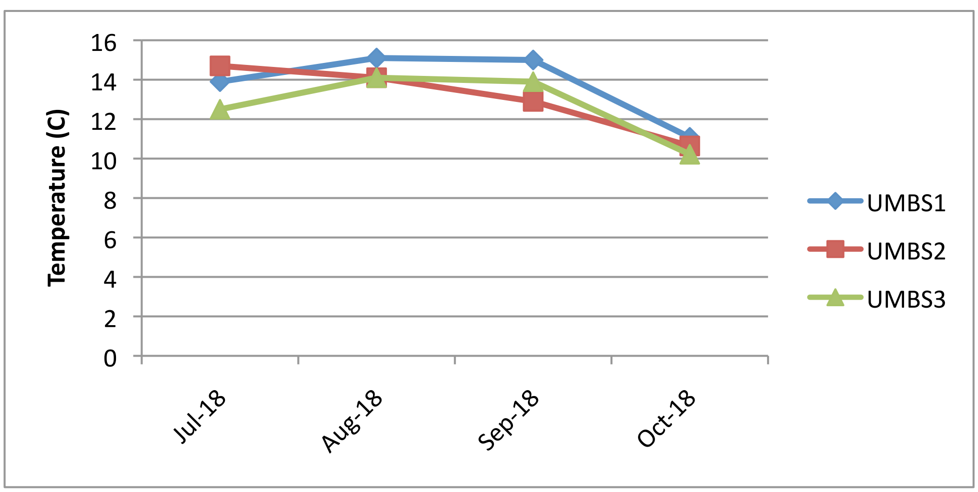


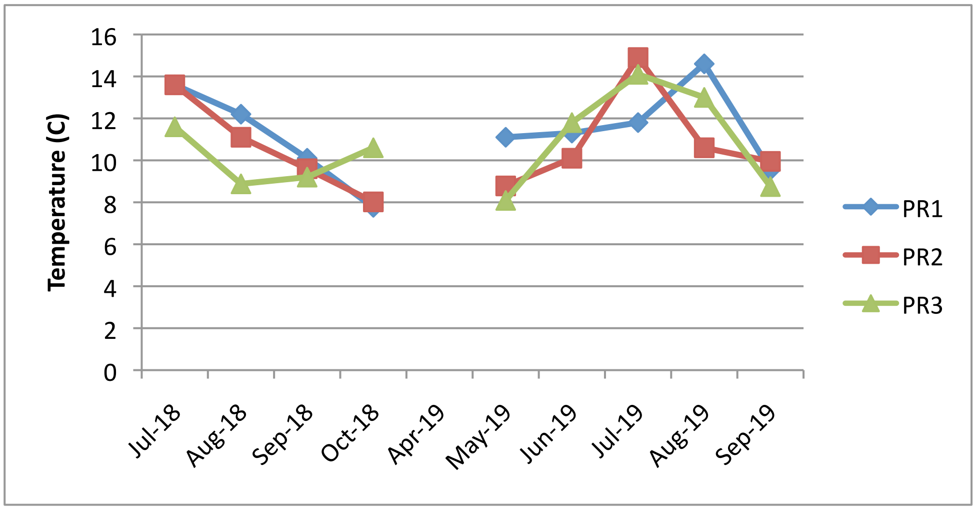
Dissolved Oxygen (%)
120
100
80
60
40
20
0
Jul-18
Aug-18
Sep-18
Oct-18
Apr-19
May-19
Jun-19
Jul-19
Aug-19
Sep-19
PR1
PR2
PR3

3. Temporal graphs of temperature

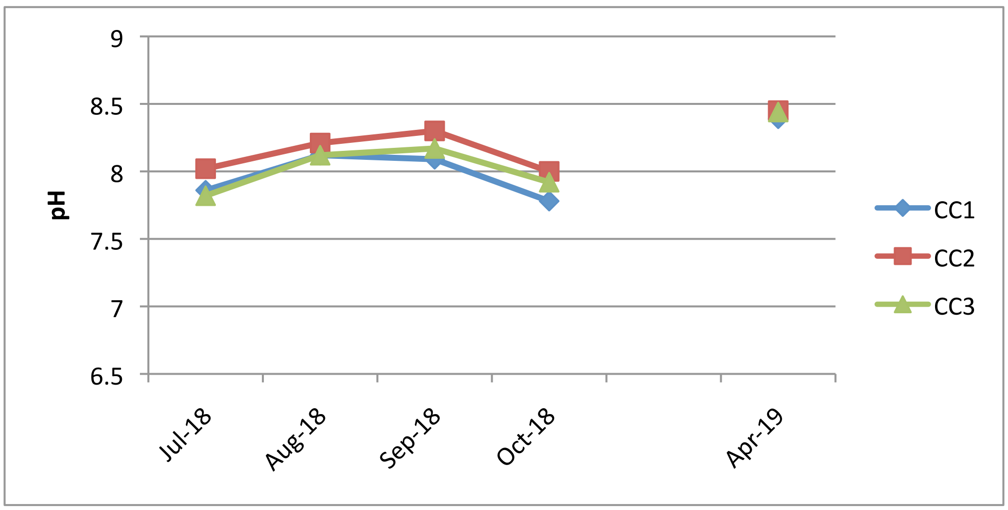


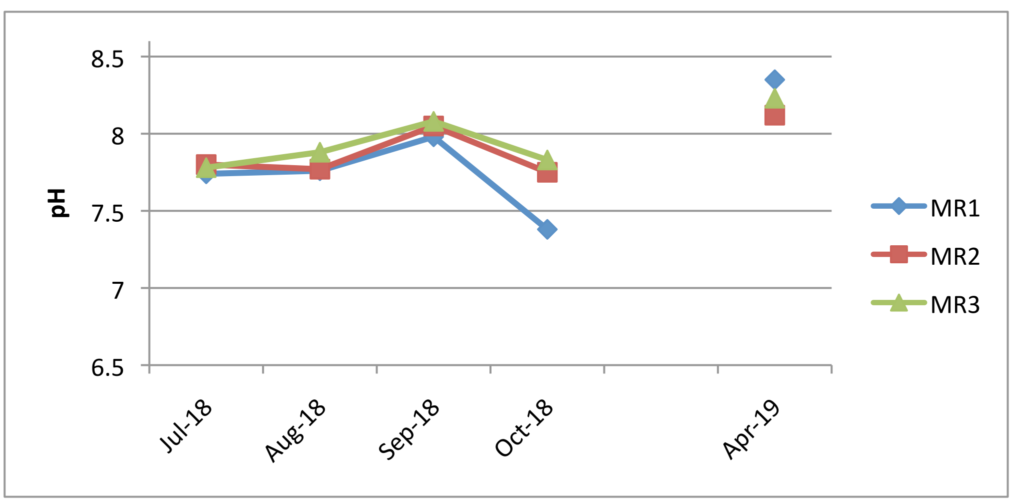


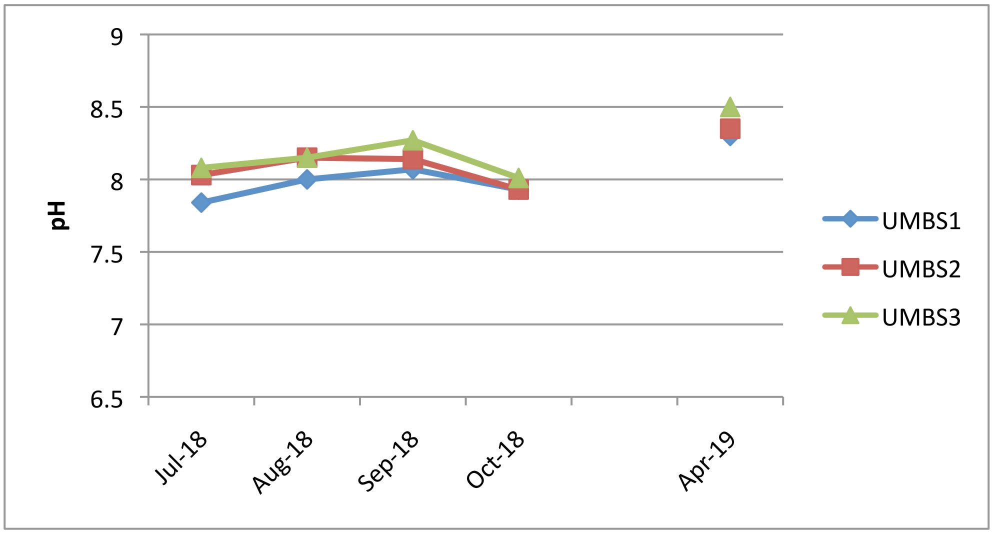


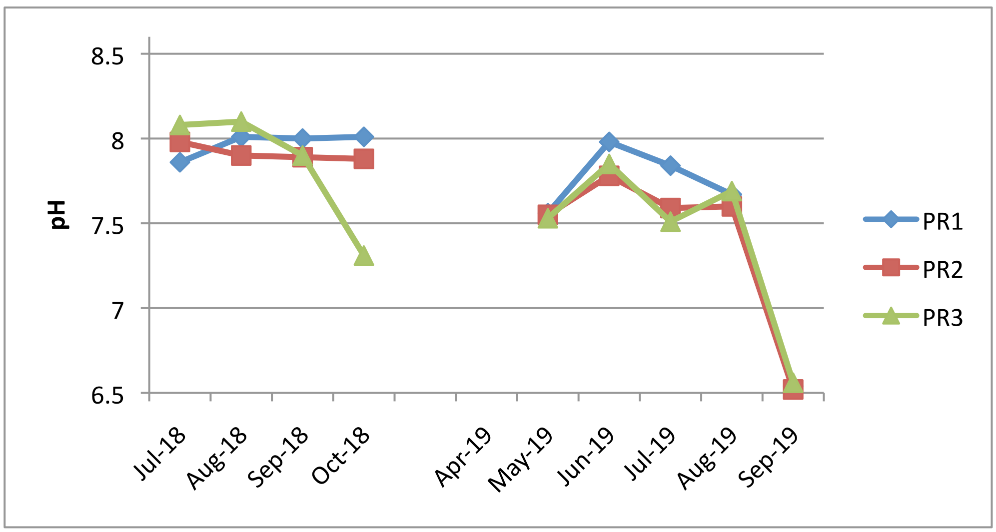


4. Temporal graphs of pH

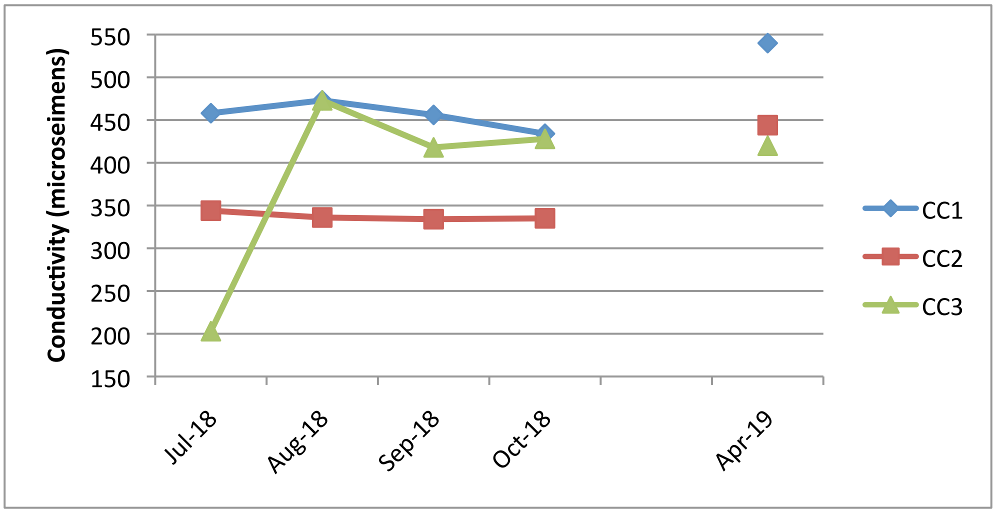


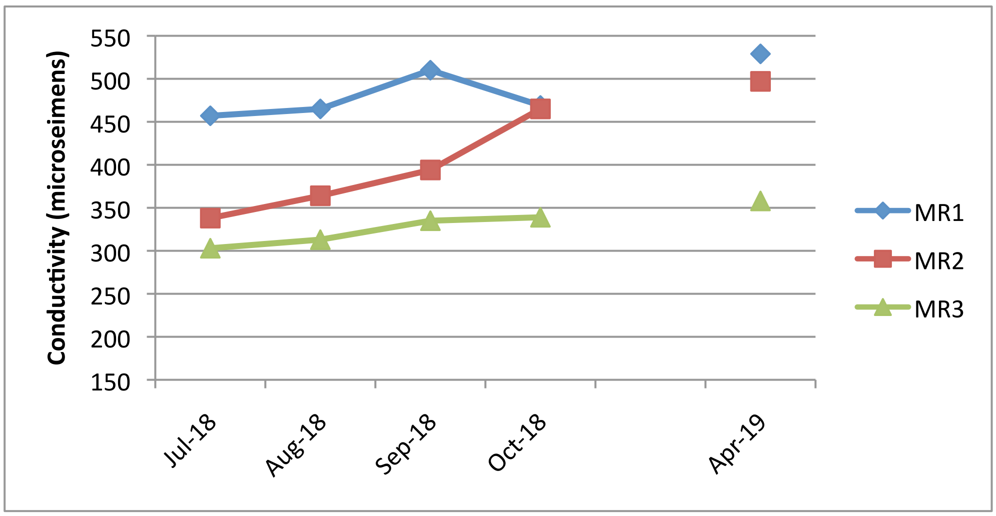


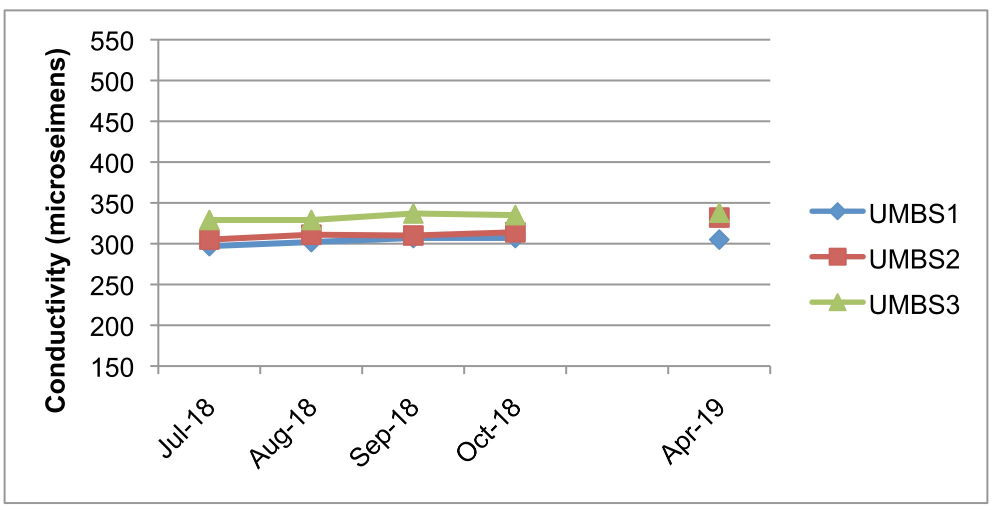

9
8.5
8
7.5
7
6.5
pH
Jul-18
Aug-18
Sep-18
Oct-18
Apr-19
UMBS1
UMBS2
UMBS3

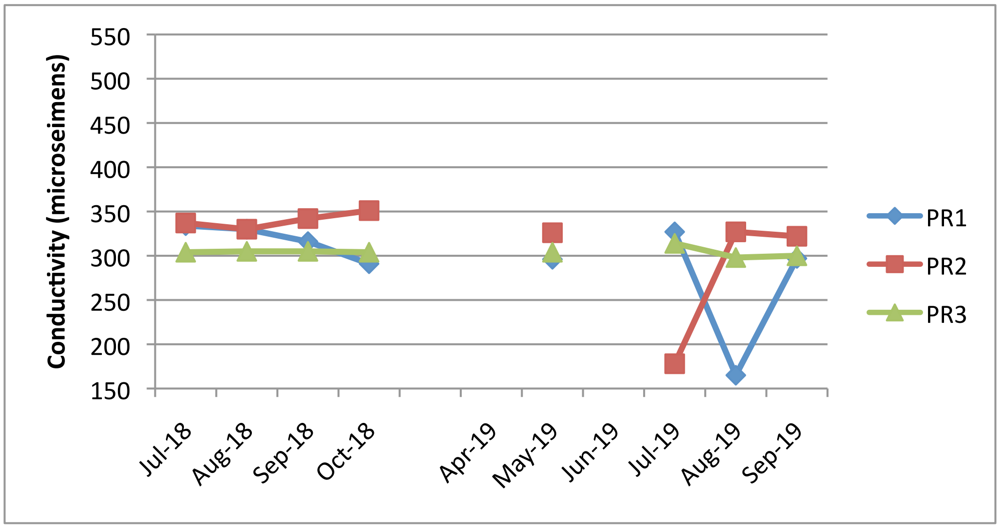

8.5
8
7.5
7
6.5
pH
Jul-18
Aug-18
Sep-18
Oct-18
Apr-19
May-19
Jun-19
Jul-19
Aug-19
Sep-19
PR1
PR2
PR3

5. Temporal graphs of electrical conductivity









Conductivity (microseimens)
550
500
450
400
350
300
250
200
150
Jul-18
Aug-18
Sep-18
Oct-18
Apr-19
May-19
Jun-19
Jul-19
Aug-19
Sep-19
PR1
PR2
PR3