MICHIGAN DEPARTMENT OF ENVIRONMENT, GREAT LAKES, AND ENERGY
WATER RESOURCES DIVISION
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STAFF REPORT

Investigation of a Water Body Following Several Canine Deaths, Osceola County, Michigan, August 2019.

1. Introduction

In July 2019, Michigan Department of Environment, Great Lakes, and Energy (EGLE) staff were contacted by a citizen regarding the recent deaths of four domestic canines and a human illness that occurred on his property. The homeowners suspected that the canine deaths and human illness all occurred as a result of contact with a small pond located on the property. The property owner reported that all the canines were in good health prior to the sudden deaths and that they had daily access to the pond. Some of the canines were found after death had occurred and no early symptoms were observed. However, at least one canine was observed immediately after contacting the pond and displayed the following symptoms: sudden stumbling, weakness, tremors, shallow breathing, and sudden appearance of the third eyelid, followed by death soon after. Another canine on the property that does not regularly interact with the pond has been reported to be healthy. The description of the symptoms exhibited immediately after contacting the pond followed by death soon after was consistent with neurotoxin poisoning from cyanobacterial toxins (Kuiper-Goodman et al., 1999; Sivonen and Jones, 1999; Pegram et al., 2008).

Photographs of the pond that were taken by the property owners shortly after the canine deaths showed dense *Chara* growth on the pond bottom, some floating mats of filamentous green algae and a pile of *Chara* that had been manually removed from the pond and placed onshore. One close-up photograph of an algal mat showed a purple-colored film/sheen around it that may have been cyanobacteria (Figure 1). EGLE staff visited the pond on two occasions and collected several water and sediment samples in an attempt to determine whether any cyanobacterial toxins or metals were present that could cause acute poisoning. EGLE staff also spoke to the homeowner several times. The homeowner stated that the canines would have had access to both the pond and any of the *Chara* and filamentous green algae that were placed onshore. The homeowner also stated that he removed some of the *Chara* and algae by placing the algae into a canoe upon removal and then disposing of the algae onshore. After removing the algae in the canoe one day, the canoe was placed onshore, in an upright position, with some water and algae still remaining in it. He then stated that the next day, the water was an unusual color, and that he then dumped it out of the canoe. The canines also would have had access to the canoe and any material in it.

EGLE and Michigan Department of Human Health and Services (DHHS) staff theorized that benthic cyanobacteria may have been liberated into the water column when the *Chara* was removed. Thus, a sampling arrangement to mimic the events of those days was devised. In addition, the potential impacts of metals in surface water and sediments on aquatic life and/or human health were also assessed.

Figure 1. Photographs of pond, filamentous green algae mats, and *Chara* that had been removed from the pond. The purple sheen is similar to *Oscillatoria* that was later identified in samples.

2. Materials and Methods

The pond was first visited in mid-July immediately after EGLE was contacted, following the canine deaths, and one ambient (containing no filamentous green algae) water sample was collected. The pond was revisited in mid-August for sediment and additional water sampling. The pond is small (0.16 acres) and has a maximum depth of about 10 feet. The pond contains no stream inlets or outlets and water level is maintained by a groundwater well pump.

During the second (mid-August) sampling event, water samples were collected using a staged approach before and after sediment disturbance to mimic water conditions following the manual removal of *Chara*. Water samples and sediments were collected at Site 1 in Figure 2. This site was chosen for the sampling because that was where the majority of the *Chara* removal had taken place according to the homeowner.

The first water samples were collected for nutrients, metals, and cyanotoxins. Cyanotoxin samples included a sample with no filamentous green algae included, and one with an aliquot of filamentous green algae and water. A large sample of clear water was also collected in a 5-gallon bucket for incubation and observation of later blooms at the EGLE Lansing field facility. Finally, Sonde (EXO2 Sonde, YSI Inc., Yellow Springs, Ohio) measurements were collected for physical/chemical parameters. All of this was done prior to any sediment disturbance.

Following collection of the first round of samples, a sediment sample was collected using a PONAR dredge sampler. One additional sediment sample was collected approximately 37 feet away from the first sampling site at the owner's dock (Figure 2). After collection, the sediment samples were homogenized and a subsample was placed in a glass jar and kept on ice until delivery to the EGLE Environmental Laboratory. Sediment samples were analyzed for the "Michigan 10" metals (arsenic, barium, cadmium, chromium, copper, lead, mercury, selenium, silver, and zinc) using standard U.S. Environmental Protection Agency (USEPA) methods for metal analysis.

Following sediment collection, the bottom sediment at Site 1 was vigorously disturbed using a metal bow rake until the entire water column was an opaque, black color from suspended organic matter. The water sampling was then repeated as described above, including a 5-gallon bucket for incubation and observation of blooms at the EGLE Lansing field facility.

Water samples collected for metals analysis were preserved with nitric acid in the field. Water samples collected for ammonia, Nitrate/Nitrite, Kjeldahl nitrogen, and total phosphorus were preserved with sulfuric acid in the field. Samples collected for ortho phosphate, nitrite, nitrate (calculated) were not preserved. All samples were kept on ice until delivery to the EGLE Environmental Laboratory in Lansing that evening for analysis using USEPA standard methods.

All cyanotoxin samples were kept on ice until delivery to the DHHS Chemistry and Toxicology Laboratory or until it was mailed. Cyanotoxin analysis for ten different microcystin congeners, nodularin, anatoxin-a, and cylindrospermopsin were performed with a Shimadzu (Shimadzu Scientific Instruments, Columbia, Maryland) triple quadrupole liquid chromatograph mass spectrometer.

The two buckets of water (pre- and post-disturbance) were collected in an attempt to mimic conditions in the canoe after the algae removal. Upon arrival at the field station in Lansing, the two buckets were placed within an outside, fenced-in parking lot for two weeks and checked by EGLE staff at least every two days. No filamentous green algae had been placed in the buckets.

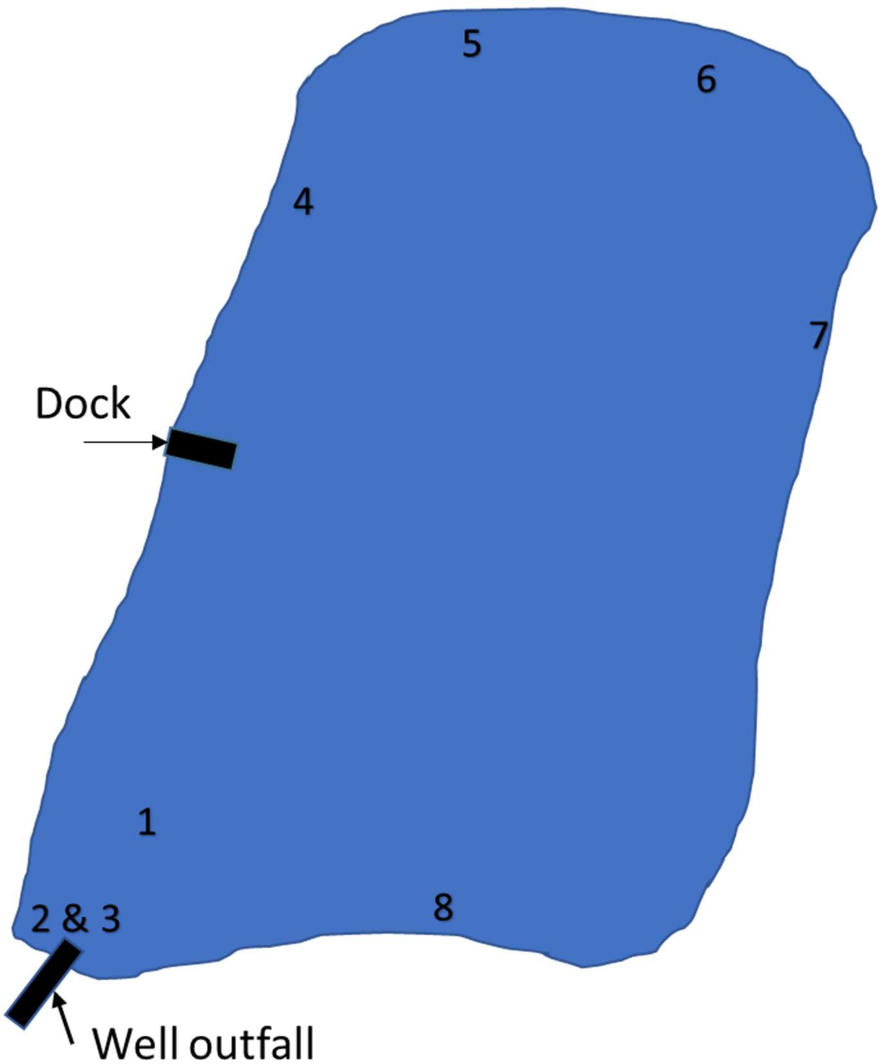

Figure 2. Sonde sampling locations within the pond.

3. Results

3.1 Water

When ambient water samples were collected, six different metals were detected and nutrients were slightly elevated. Ambient total phosphorus concentrations were at eutrophic (productive) levels (Fuller and Minnerick, 2008) and ambient water hardness was at a concentration that would be considered "very hard" (Shaw et al., 2004). After the sediment was disturbed, the nutrient concentrations increased (Table 1), 13 different metals were detected, and water hardness was nearly 5 times the ambient measurement (Table 2).

Table 1. Water nutrient concentrations before and after sediment disturbance (milligrams per liter [mg/l]; ND = nondetect).

	Water Samples Undisturbed Sediment	Water Samples Post-Sediment Disturbance
Parameter	Result	Result
Ammonia - nitrogen (mg/l)	0.05	0.05
Kjeldahl Nitrogen-N (mg)	0.52	6.7
Nitrate/Nitrite-N	0.014	0.016
Nitrate-N-calculated	ND	0.016
Nitrite-N	ND	ND
Ortho Phosphate-P	0.005	0.012
Total Phosphorus-P	0.027	0.42

Table 2. Water metal concentrations before and after sediment disturbance. (micrograms per liter [µg/l]).

Parameter	Pre-Sediment Disturbance Water Results	Post-Sediment Disturbance Water Results
Hardness-calculated (mg/l)	210	960
Antimony (µg/l)	ND	ND
Arsenic (µg/l)	8.9	100
Barium (µg/l)	46	240
Beryllium (µg/l)	ND	ND
Cadmium (µg/l)	ND	ND
Calcium (mg/l)	49	350
Chromium (µg/l)	ND	2.2
Cobalt (µg/l)	ND	13
Copper (µg/l)	ND	18
Iron (µg/l)	340	35000
Lead (µg/l)	ND	1.8
Magnesium (mg/l)	20	23
Manganese (µg/l)	24	2700
Mercury (µg/l)	ND	ND
Molybdenum (µg/l)	ND	8.4
Nickel (µg/l)	ND	4.6
Selenium (µg/l)	ND	ND
Silver (µg/l)	ND	ND
Thallium (µg/l)	ND	ND
Vanadium (µg/l)	ND	ND
Zinc (µg/l)	ND	66

Metals that were (1) detected and (2) have the potential to adversely affect aquatic life and/or human health per Rule 323.1057 (Rule 57) of the Part 4 Rules, Water Quality Standards, promulgated under Part 31, Water Resources Protection, of the Natural Resources and Environmental Protection Act, 1994 PA 451, as amended, are listed in Table 3. Table 3 compares the concentration of metals measured in the pond to surface water values designed to be protective of aquatic life from short-term exposure (Aquatic Maximum Value [AMV]) and long-term exposure (Final Chronic Value [FCV]). The table also compares the concentration of these substances to surface water values designed to be protective of humans from non-cancer effects (Human Non-cancer Value [HNV]) and cancer effects (Human Cancer Value [HCV]) following daily exposure via the incidental ingestion of water and the consumption of fish for a lifetime. All metals were below AMV, FCV, and HNV values. Arsenic was below the HCV value in ambient water and above it after the sediment was disturbed.

Table 3. Comparison of the concentration of inorganics found in water samples collected from pond to surface water values designed to be protective of aquatic life and human health. Note: these tables only include metals with concentrations above the detection limit that have numeric standards as identified in Rule 57.

Water Sample - Undisturbed Sediment					
	Osceola Pond Concentration	Aquatic Maximum Value (AMV)	Final Chronic Value (FCV)	Human Noncancer Value (HNV; non-drinking water)	Human Cancer Value (HCV; non-drinking water)
Ammonia-N (mg/l)*	**0.05**	4.5	1.1	Not applicable	Not applicable
Arsenic (µg/l)	**8.9**	340	150	280	10
Barium (µg/l)**	**46**	2748.5	963	160000	Not applicable
Manganese (µg/l)	**24**	8138	3772	59000	Not applicable
Water Sample - Disturbed Sediment					
	Osceola Pond Concentration	Aquatic Maximum Value (AMV)	Final Chronic Value (FCV)	Human Noncancer Value (HNV; non-drinking water)	Human Cancer Value (HCV; non-drinking water)
Ammonia-N (mg/l)***	**0.05**	3.1	0.8	Not applicable	Not applicable
Arsenic (µg/l)	**100**	340	150	280	10
Barium (µg/l)****	**240**	13825	4845	160000	Not applicable
Chromium (µg/l)****	**2.2**	3632	472.5	9400	Not applicable
Cobalt (µg/l)	**13**	370	100	Not applicable	Not applicable
Copper (µg/l)****	**18**	113.2	61.9	38000	Not applicable
Lead (µg/l)****	**1.8**	655.5	134.3	190	Not applicable
Manganese (µg/l)****	**2700**	30926	14,334	59000	Not applicable
Molybdenum (µg/l)	**8.4**	29000	3200	10000	Not applicable
Nickel (µg/l)****	**4.6**	3173	352.4	210000	Not applicable
Zinc (µg/l)****	**66**	796.5	803	16000	Not applicable

*Ammonia water quality values based on a measured temperature of 16°C and pH of 8.
**Barium water quality values calculated using a laboratory calculated hardness of 210 mg/l $CaCO_3$.

***Ammonia water quality values based on a measured temperature of 16°C and pH of 8.2.
****Metal water quality values calculated using a laboratory calculated hardness of 960 mg/l $CaCO_3$.

3.2 Sediments

Five different metals were found in either one or both sites in the pond (Table 4). Table 5 compares the observed sediment metal concentrations to Threshold Effects Concentration (TEC) and Probable Effects Concentrations (PEC). Note: Table 5 only includes metals with concentrations above the detection limit that have numeric standards as identified in WRD-048 (EGLE, 2018) or MacDonald et al. (2000).

The TEC is the concentration below which adverse effects are not expected to occur in benthic (bottom-dwelling) biological communities (MacDonald et al., 2000). The PEC is the concentration above which adverse effects are expected to occur within benthic communities more often than not (MacDonald et al., 2000). Chromium, copper, and zinc were below TEC values and are not expected to impair benthic ecological communities. However, arsenic was above the PEC value of 33 mg/L at the sample 1 location.

Table 4. Metal concentrations in sediment from two sites in the pond (milligrams per kilogram [mg/kg]).

Parameter (mg/kg)	Pond Sample 1	Pond Sample 2
Arsenic	66	18
Barium	93	100
Cadmium	ND	ND
Chromium	ND	2.2
Copper	13	16
Lead	ND	ND
Mercury	ND	ND
Selenium	ND	ND
Silver	ND	ND
Zinc	26	23

Table 5. Observed sediment metal concentrations compared TEC and PEC values outlined in MacDonald et al. (2000).

Parameter (mg/kg)	Consensus-Based TEC	Consensus-Based PEC	Pond Sample 1	Pond Sample 2
Arsenic	9.79	33	66	18
Chromium	43.4	111	ND	2.2
Copper	31.6	149	13	16
Zinc	121	459	26	23

The first water sample that was collected in mid-July did not contain any cyanotoxins (Table 6). When water samples were collected in mid-August, the two water samples that did not contain any filamentous green algae (both pre- and post-sediment disturbance) did not contain any cyanotoxins. The two mid-August samples (pre- and post-sediment disturbance) that were sampled with an aliquot of filamentous green algae contained anatoxin-a but no other cyanotoxins (Table 6). Anatoxin-a was detected in the pre- and post-sediment disturbance samples at 43 and 24 µg/l, respectively. The two water samples in the buckets were observed for two weeks in Lansing and no cyanobacteria blooms occurred. These buckets did not contain any filamentous green algae, which may explain why no cyanobacteria was observed in them either.

4. Discussion

Previous statewide sampling of anatoxin-a had a maximum concentration of 4.4 µg/l (Parker, 2018). Anatoxin-a is not typically found in Michigan and has only been found in six water bodies (including this one) out of 226 total that have been tested for it, to date, by EGLE. An obvious cyanobacteria bloom was not visually evident while EGLE staff were collecting the samples. However, after the filamentous green algae samples were placed into the sample collection jars and stored in a refrigerator at the laboratory, cyanobacteria separated from the green algae within the sample jars. The cyanobacteria were identified as *Oscillatoria,* which is a known anatoxin-a producer (Figure 3; James et al., 1997; Sivonen and Jones, 1999; Cadel-Six et al., 2009; Du et al., 2019) and has been implicated in similar cases of canine fatalities (Edwards et al., 1992; Hamill, 2001). *Oscillatoria* was also found in the “ambient, post-sediment disturbance” sample; however, no cyanotoxins were detected in that sample (Table 6).

Phycocyanin (pigment that is unique to cyanobacteria) was also measured at various concentrations with the sonde unit, particularly in the filamentous green algae mats. The highest phycocyanin concentration was found in an algal mat near the well water outfall (Figure 4; Table 7). Phycocyanin was detected amongst the green algae mats prior to sediment disturbance by EGLE staff, suggesting that the *Oscillatoria* was already sequestered within it prior to staff arriving. *Oscillatoria* were also identified in a sample collected after the bottom sediments were disturbed, indicating that it was both on the pond sediments and within the green algae mats when EGLE staff arrived in August. *Oscillatoria* is considered a benthic cyanobacteria; however, it can control its buoyancy and is known to migrate up and down in water columns to obtain ideal amounts of sunlight and nutrients (Konopka, 1982; van Rijn and Shilo, 1983).

Typically, when mats of filamentous green algae are sampled by EGLE staff, no cyanotoxins are detected since the green algae itself is not capable of producing toxins. This is only the third water body known to have cyanotoxins detected amongst filamentous green algae mats since EGLE began monitoring statewide harmful algal blooms. In September 2016, low amounts of anatoxin-a (1.3 and 2.4 µg/l) were found amongst filamentous green algae mats in the St. Louis Impoundment of the Pine River, Gratiot County (Parker, 2017). On two separate dates in June 2018, microcystin concentrations of 22.6 and 5.1 µg/l were found amongst a localized, dense, filamentous green algae bloom in Coldwater Lake, Branch County (raw data available in appendix of Parker [2019]). Rediske et al. (2010) found that mats of the filamentous green algae *Cladophora*, collected along Great Lakes beaches, was able to sequester both microcystin toxins and *E. coli* bacteria. In this latest case, it is believed that anatoxin-a producing *Oscillatoria* were sequestered amongst the filamentous green algae mats in the pond. *Oscillatoria* is known to have a purple hue similar to the light sheen of material pictured in Figure 1 shortly after the last canine death.

Concentrations of metals in the surface water with undisturbed sediment were all below the Rule 57 AMV, FCV, HNV, and HCV suggesting that these metals would not pose aquatic or human health concerns. Since it would be expected that the concentrations of metals that would cause acute effects to mammals would be higher than the Rule 57 human health values, it can be presumed that the metals at these levels would not cause acute effects on humans or dogs. Several metals were elevated in surface water following sediment disturbance, with only arsenic exceeding the human health value. However, since people would not be expected to routinely swim in water soon after sediment disturbance, the temporary elevation of these metals in the water would not be expected to pose a human health concern.

Concentrations of metals within the pond sediments were all below the PEC screening values, with the exception of arsenic at the pond sample 1 location. Arsenic at this location was 66 mg/kg. The corresponding PEC for arsenic is 33 mg/kg. Arsenic is a common naturally-occurring element and previous studies of arsenic occurrence in Michigan have shown that concentrations are regionally variable (U.S. Geological Survey [USGS], 2000). Dissolved arsenic has a strong tendency to selectively bind to sediments in surface waters – especially in water bodies with oxidizing conditions (i.e., with high concentrations of oxygen) or under reducing conditions with high concentrations of free sulfides (Smedley and Kinniburgh, 2002; Agency for Toxic Substances and Disease Registry [ATSDR], 2007). Concentrations of arsenic in sediments were within the range reported for natural inland water bodies in the Midwest (National Research Council [NRC], 1977). Therefore, the elevated concentrations of arsenic in the sediments at location 1 are believed to be completely natural and may be related to the inflow/discharge of groundwater and rapid precipitation and concentration of arsenic into the pond's sediments.

Canine deaths from cyanotoxins have been reported throughout the United States and the World (Backer et al., 2013; Wood, 2016). In Michigan, cyanotoxins were the suspected cause of death for a canine in Livingston County in 2007 (Backer et al., 2013). However, no cyanotoxin data were collected and the necropsy results from that case were inconclusive.

Based on (A) good health history of the canines prior to death; (B) contact with the pond shortly before death; (C) observed symptoms prior to death in at least one canine; and (D) the presence of anatoxin-a in the pond, we conclude that cyanotoxins were responsible for the canine deaths. This is the first time such an incident has been verified in Michigan.

Figure 3. *Oscillatoria* from pond sample collected August 2019.

Table 6. Cyanotoxin concentrations from pond samples in July and August.

Sample type	Date	Microcystin-RR	Microcystin-YR	Microcystin-HTYR	Microcystin-LR	Microcystin-LR Asp3	Microcystin-WR	Microcystin-LA	Microcystin-LY	Microcystin-LW	Microcystin-LF	Nodularin	Anatoxin-a	Cylindrospermopsin
Ambient	7/11/2019	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect
Ambient, pre-sediment disturbance	8/21/2019	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect
Algae sample, pre-sediment disturbance	8/21/2019	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	**43**	non-detect
Ambient, post-sediment disturbance	8/21/2019	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect
Algae sample, post-sediment disturbance	8/21/2019	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	non-detect	**24**	non-detect

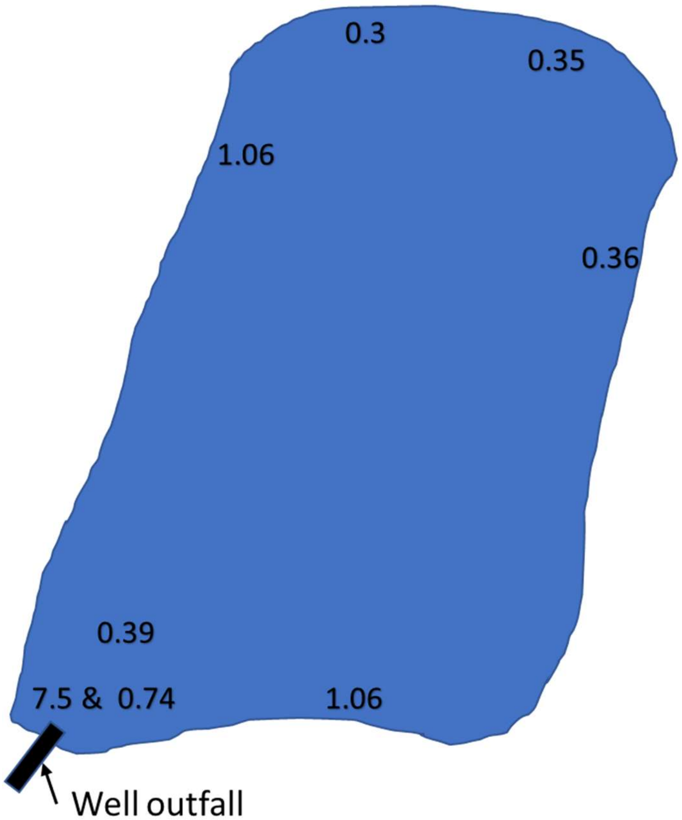

Figure 4. Phycocyanin concentrations (µg/l) measured within and along the pond.

Table 7. Sonde measurements around the pond. See Figure 2 for site locations.

Site	Date	Time	°F	DO %	DO mg/L	SPC-uS/cm	C-uS/cm	pH	BGA-PC RFU	BGA-PC ug/L	Chl RFU	Chl ug/L
1	8/20/2019	10:16:41	60.343	122.1	12.11	458.9	377.8	8.16	0.5	0.4	1.6	5.9
2	8/20/2019	10:34:23	49.079	41	4.68	152.9	107.6	7.66	8.2	7.5	10.2	37.6
3	8/20/2019	10:34:29	49.322	36.7	4.17	525	370.9	7.66	0.8	0.7	0.0	0.0
4	8/20/2019	10:35:05	62.455	138	13.34	443.3	374.9	8.04	1.2	1.1	0.1	0.4
5	8/20/2019	10:35:37	62.586	134.7	13.01	431.2	365.2	8.03	0.4	0.3	0.4	1.5
6	8/20/2019	10:36:13	60.98	118	11.61	453.2	376.2	7.97	0.4	0.4	0.4	1.7
7	8/20/2019	10:36:53	61.27	116.7	11.46	446.2	372	7.96	0.4	0.4	0.5	2.0
8	8/20/2019	10:37:40	63.033	117.3	11.25	450.2	383.5	7.9	1.2	1.0	6.4	23.4

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