Peeper 2017 Golden Brown Algae Study

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Introduction

Figure 1. Example of a diatom on a stalk

In examining data from our golden-brown algae study in 2016[1], a hypothesis was suggested that could explain the increasing visibility of the algae over the course of the summer growing season. Certain diatoms are more tolerant of low nutrient levels because they are able to form stalks which allow them to extend from within the lake floor sediment to on top of the sediment. If the available nutrients in the sediment diminish as the season progresses, this could encourage the stalk-forming diatoms to grow in preference to those requiring higher nutrient levels, thereby becoming the most predominant species. And movement of the diatoms from within the sediment to on top of it would make them more visible.

The purpose of this study was to test this hypothesis by examining the seasonal progression of nutrient levels in the lake water and in the sediment, assessing seasonal progression of types of diatom species, and examining the relationships between these observations.

Because of the widespread presence of the golden-brown algae (GBA, the benthic diatoms), four lake associations, Elk-Skegemog Lakes Association, Lake Leelanau Lake Association, Torch Lake Protection Alliance, and Three Lakes Association, participated in this study.

Materials

Dialysis tubing (DT, Sigma-Aldrich cellulose membrane, item D9652) was used to make sampling baggies. Commercial Spartan Brand distilled water, dispensed with laboratory grade squirt bottles, was used to fill the sampling baggies. PVC pipe with ¼ inch holes drilled for water passage, with a capped 7-inch sample chamber at each end was used to house the DT baggies. The combination of DT baggies and PVC pipe housing are referred to as peepers. A thin-bladed hoe was used for positioning the peepers. Concrete patio blocks served to secure the peepers on the lake floor. A Petri dish and spatula were used for harvesting benthic diatom samples. Acid-washed, triple-rinsed with distilled water, pre-labeled sample bottles from the University of Michigan Biological Station (UMBS) laboratory were used to collect surface lake water grab samples. Pre-labeled zip-lock sandwich bags served to individually store DT samples as they were harvested. Pre-labeled zip-lock sandwich bags served to individually store benthic diatom samples as they were harvested. Copies of the study protocol, pre-labeled field notes, scuzziness scale, sampling equipment, and color chart were provided and explained to all field work volunteers.

Figure 2. Example of a peeper with sample chambers at each capped end

Methods

Training:

The teams responsible for the field work were trained on the construction of the DT baggies, sampling techniques for lake water grab samples and for benthic diatom samples, deployment and securing of the peepers, and use of the field notes at the time of the first deployment. They were also instructed on techniques for retrieval and storage of samples.

DT Baggies:

Eight inch lengths of DT were cut and soaked in distilled water to soften the material. One end of the tubing was sealed by tightly twisting and folding it and then securing it with a tightly-applied approximately 1 ½ inch length of plastic-coated twist-tie (TT). The tubing was then filled to overflowing with distilled water and the other end similarly secured with another piece of TT, producing a hot dog-shaped 1 inch by 7 inch sampling device.

Peepers:

Filled DT baggies were inserted into the sample chambers at each end of the PVC housing and kept in place with screw-in caps.

Sites:

One study site was selected for each of four lakes.

Lake	Site	N Latitude	W Longitude
Leelanau	Green	45.0257	85.7330
Elk	Hamilton	44.8954	85.3505
Torch	Gourley	44.94500	85.28130
Bellaire	Southworth	44.94750	85.23300

Table 1. Study sites included in this research.

Deployment:

A total of 3 peepers (6 sampling chambers) were used for each site for each of three sampling intervals. Two peepers were placed horizontally into the lake floor sediment, buried so that they were fully covered by approximately ¼ inch of sand (sub-benthic DT). One peeper was placed horizontally on the lake floor (benthic DT0 in close proximity to the two buried peepers. Thus, there were planned to be duplicate samples for the lake floor peepers and quadruplicate samples for the buried peepers at each sampling interval. A sketch of the deployment was to be included on the deployment field note sheet. An approximate match of the color chart to the appearance of the lake floor was to be recorded. The deployed equipment was secured with a concrete patio block and left in place for approximately 30 days.

Retrieval:

Peeper samples were retrieved one at a time and placed into pre-labeled zip-lock sandwich bags. Any relevant observations were to be recorded on the retrieval field note sheet. Surface lake water grab samples were collected in duplicate into pre-labeled bottles. Diatom samples were harvested from the lake floor using a small Petri dish: the dish was placed upside down into the benthic surface, a spatula inserted underneath to enclose the material in the dish, the dish and spatula combination lifted from the lake floor and washed with the distilled water squirt bottle into a pre-labeled zip-lock sandwich bag; this procedure was carried out three times and the material combined in a single zip-lock bag to comprise the sample.

Peeper, grab, and diatom samples were placed on ice in coolers on site and transferred to freezers upon completion of each field trip. Labeled, frozen, diatom, benthic and sub-benthic DT, and lake water grab samples were delivered to Becky Norris and stored frozen until delivery to the UMBS laboratory (water samples) and to Dr. Jan Stevenson (diatom samples). The water samples were assayed for concentration of PO_4-P (biologically available phosphorus: soluble reactive phosphorus, SRP). The diatoms in the benthic samples were identified and counted.

In addition to the study activities, Art Hoadley generously took aerial photographs of the study sites during the study, providing a historical record of the appearance of the GBA at and surrounding the sampling sites.

<u>Results</u>

SRP:

The SRP (PO_4-P) results for each site are shown in tables 3 – 6 in Appendix A. Some of the duplicate or quadruplicate samples that were expected to be the same, within laboratory analytical variability, were quite different from one another. None of the analytical results has been omitted from these tables although some of them are suspected to be invalid. Possible reasons for this variability are addressed in the Discussion section, below. With or without including the likely outliers, the following observations and interpretations were made:

1. As expected, based upon prior study results[2], surface lake water samples had the lowest SRP values at all four study sites.
2. The benthic (lake floor) sample SRP values were substantially higher than surface lake water for all four sites.
3. The sub-benthic (buried in the lake floor sediment) SRP values were also substantially higher than surface lake water.
4. The SRP values of benthic and sub-benthic samples varied such that no consistent pattern was shown. This variability in SRP values may have obscured a true relationship.
5. There was a downward trend of SRP over the observation period from July to September.

Scuzzy Score and Percent Coverage:

In order to attempt a visual assessment of the amount of GBA at each sampling event, we estimated the percentage of the lake floor site with visible GBA and scored the appearance of the lake floor on a published scuzziness scale[3]:

0	Substrate rough with no visual evidence of microalgae
0.5	Substrate slimy, but no visual accumulation of microalgae is evident
1	A thin layer of microalgae is visually evident
2	Accumulation of microalgal layer from 0.5 – 1 mm thick is evident
3	Accumulation of microalgal layer from 1 mm to 5 mm thick is evident
4	Accumulation of microalgal layer from 5 mm to 2 cm thick is evident
5	Accumulation of microalgal layer greater than 2 cm thick is evident

Table 2. Scuzzy score descriptions from reference 3.

The scuzzy scores and percent coverage estimates are shown in tables 3 – 6 in Appendix A along with the SRP results. They are also shown along with the available aerial photographs in Figure 8 in Appendix C, part 6. In considering the scuzzy scores and percent coverage estimates, it is worth recognizing that they were made by observations limited to the immediate environment of the sampling sites, not from the aerial photographs which displayed a much larger area.

The Lake Bellaire site had the highest percent coverage and the highest scuzzy scores, consistent with observations at this site in prior years. The coverage increased from July to August; the drop in coverage in September appeared to be due not to a diminution of GBA but rather to rips in the GBA mat produced by rough wave action against the lake floor.

The Torch Lake site showed a sharp increase in both percent coverage and scuzzy score from August to September. This result was in contrast to prior years when the visible GBA was lower in September than in August.

The Elk Lake and Lake Leelanau sites did not show any pattern of change in visible GBA over the period of observation.

Diatom Counts:

The diatom counts are shown in Appendix B. Graphic displays of the counts are shown in Appendix C, part 1. Pie charts by lake and study month display the quantitative distributions of the five most abundant diatom species (*Achnanthidium*, *Cymbella*, *Encyanopsis*, *Fragilaria*, and *Nitzschia*). Also illustrated are the distributions of diatoms from the entire sample counts, lumping all of the other species together as “Other”; this demonstrates that the most abundant species make up only 1/4 to 1/3 of the total population of diatoms present in the samples. In contrast, in the 2016 study[1], *Cymbella* increased with time and became the most abundant diatom species by August.

Aerial Photographic Appearance:

Aerial photographs of the sites were obtained in June, July, and August; these are shown in Appendix C, part 6.

Discussion

This study was undertaken primarily to address the hypothesis generated by the data of the GBA work done in 2016[1]. This hypothesis suggested that a declining availability of SRP over the summer months occurred and thereby stimulated a progressive increase in the types of diatoms that are low-nutrient-tolerant. The low-nutrient-tolerant diatoms can become more visible due to the growth of stalks that allow them to rise from within the lake sediment to its surface and to extend above other diatoms coating the lake floor. In the 2016 study the diatom mix showed a change in composition over the growing season with one low-nutrient-tolerant type, *Cymbella* species, becoming the most prevalent. Interestingly, the diatom mix seen in the current study did not reproduce the increase seen in *Cymbella* species that occurred in the prior study. In this study, of the five most abundant species, we observed a fairly large relative proportion of *Nitzschia*, *Fragilaria*, and *Encyanopsis*, and only modest amounts of *Cymbella*, with minor variation and no clear pattern of change throughout the study period.

To ensure that there was adequate time for equilibration across the dialysis membrane of our peepers and to keep the effort required of the volunteers performing the field work to a reasonable level, we left the peepers in the lakes for approximately 30 days for each of the three months of data collection. The peeper samples remained in the DT baggies when harvested, were stored individually in pre-labeled zip lock sandwich bags, and were delivered to the laboratory frozen. In retrospect, we believe both of these procedures contributed to the remarkable assay variability in our SRP data. The long exposure of the dialysis membranes to the environment likely allowed the formation of a biofilm consisting of bacteria and/or fungi on the exterior of the membranes, with unknowable effects on osmotic equilibration and delivery or consumption of SRP. Additionally, it turned out that the dialysis membrane was altered in some way by freezing; when thawed, the DT baggie samples oozed into the zip-lock bags in which they were stored. This in all probability resulted in contamination of the samples with whatever material was adherent to the outside of the DT baggies and could have resulted in spuriously elevated SRP values in the affected samples. The experience we had with the peepers in this study stimulated us to design better peepers for future study, to limit the environmental exposure of the peepers to the shortest time necessary for equilibration, and to transfer the samples from the DT baggies into standard laboratory sample bottles prior to freezing.

Conclusion

Despite the difficulties we encountered with our peeper samples, we concluded that there is credible evidence of a modest decline in SRP over the growing season. However, given the results of the diatom counts in the current study, we were unable to establish whether the SRP decline materially affected the relative proportions of diatoms over the same time period. Additional efforts to address the effects of nutrient availability on diatom species distribution are planned for 2018 and 2019.

Appendix A, Analytical Results

Peeper Nutrient Study Torch Lake: PO4-P µg/L				
Lake Water Grab	**July**	**August**	**September**	**Mini-graph of data**
Sample 1	0.519	1.0650	1.022	
Sample 2	7.975	2.1710	0.7896	
Average	**4.247**	**1.618**	**0.906**	
Sub-Benthos DT				
Sample 1	18.953	29.6550	5.6617	
Sample 2	130.370	8.8430	6.1597	
Sample 3	13.379	15.8560	1.8354	
Sample 4	41.241	16.8990	3.3211	
Average	**50.986**	**17.813**	**4.244**	
Benthos DT				
Sample 1	19.929	78.8430	4.923	
Sample 2	40.557	20.9340	4.4084	
Average	**30.243**	**49.889**	**4.666**	
Scuzzy Score	1	1	3	
% Coverage	10	10	50	

Table 3. SRP (PO_4–P) levels and averages by sample type and study month at the Torch Lake site. Sub-Benthos DT stands for the buried peepers. Benthos DT stands for the lake floor peepers.

Peeper Nutrient Study Lake Bellaire: PO4-P µg/L				
Lake Water Grab	**July**	**August**	**September**	**Mini-graph of data**
Sample 1	2.781	1.6830	1.0137	
Sample 2	2.124	0.3190	0.5904	
Average	**2.453**	**1.001**	**0.802**	
Sub-benthos DT				
Sample 1	85.418	16.0010	1.7275	
Sample 2	24.072	19.8850	0.5	
Sample 3	17.826	17.4870	2.6488	
Sample 4	25.388	23.8590	2.8729	
Average	**38.176**	**19.308**	**1.937**	
Benthos DT				
Sample 1	18.269	4.1330	4.6076	
Sample 2	36.622	11.8000	1.7026	
Average	**27.446**	**7.967**	**3.155**	
Scuzzy Score	4	4	4	
% Coverage	50	80	35	

Table 4. SRP (PO_4–P) levels and averages by sample type and study month at the Lake Bellaire site. Sub-Benthos DT stands for the buried peepers. Benthos DT stands for the lake floor peepers.

Peeper Nutrient Study Elk Lake: PO4-P µg/L				
Lake Water Grab	**July**	**August**	**September**	**Mini-graph of data**
Sample 1	11.738	1.0730	1.2129	
Sample 2	5.833	1.5700	1.0303	
Average	**8.786**	**1.322**	**1.122**	
Sub-Benthos DT				
Sample 1	42.463	5.4900	2.7235	
Sample 2	38.702	4.1350	2.5575	
Sample 3	38.269	6.1120	2.931	
Sample 4	31.959	10.3550	1.6943	
Average	**37.848**	**6.523**	**2.477**	
Benthos DT				
Sample 1	31.538	49.3710	4.5163	
Sample 2	646.955	6.8530	8.575	
Average	**339.247**	**28.112**	**6.546**	
Scuzzy Score	1	0.5	1	
% Coverage	0	50	na	

Table 5. SRP (PO_4–P) levels and averages by sample type and study month at the Elk Lake site. Sub-Benthos DT stands for the buried peepers. Benthos DT stands for the lake floor peepers.

Peeper Nutrient Study Lake Leelanau: PO4-P µg/L				
Lake Water Grab	**July**	**August**	**September**	**Mini-graph of data**
Sample 1	0.700	1.6830	0.5	
Sample 2	0.700	0.3190	1.3789	
Average	**0.700**	**1.001**	**0.939**	
Sub-Benthos DT				
Sample 1	132.846	16.0010	13.3641	
Sample 2	92.146	19.8850	30.6198	
Sample 3	390.630	17.4870	9.3552	
Sample 4	167.016	23.8590	54.142	
Average	**195.660**	**19.308**	**26.870**	
Benthos DT				
Sample 1	261.216	na	25.3825	
Sample 2	145.586	na	14.8581	
Average	**203.401**	**na**	**20.120**	
Scuzzy Score	3	0	na	
% Coverage	50	0	30	

Table 6. SRP (PO_4–P) levels and averages by sample type and study month at the Lake Leelanau site. Sub-Benthos DT stands for the buried peepers. Benthos DT stands for the lake floor peepers. The Benthos DT peeper for August was lost so there are no data available for those samples.

Appendix B, Diatom Results

		Achnanthidium sp	*Cymbella sp*	*Encyanopsis sp*	*Fragilaria sp*	*Nitzschia sp*	Other	Total
Torch Lake	July	159	279	1100	1016	2072	8987	13613
	August	155	569	1185	1001	3496	17376	23782
	September	167	277	1544	1014	2075	10798	15875
	Alt Sep	180	536	1111	2015	2858	10227	16927
Lake Bellaire	July	350	537	1504	2543	2824	13894	21652
	August	218	533	2267	1512	1406	13291	19227
	September	198	838	1877	1022	2097	15650	21682
Elk Lake	July	151	576	1937	2537	4252	10734	20187
	August	186	1071	1954	2062	2810	12699	20782
	September	192	569	1931	1523	3595	12196	20006
Lake Leelanau	July	228	557	1131	1543	1411	5895	10765
	August	na	na	na	na	na	na	na
	September	178	546	773	2016	1425	11691	16629

Table 7. Diatom Counts by Lake and Month. No benthic algae sample was obtained in August at the Lake Leelanau site. na = not available.

		Achnanthidium sp	*Cymbella sp*	*Encyanopsis sp*	*Fragilaria sp*	*Nitzschia sp*	Other	Total
Torch Lake	July	1.17	2.05	8.08	7.46	15.22	66.02	100
	August	0.65	2.39	4.98	4.21	14.70	73.06	100
	September	1.05	1.74	9.73	6.39	13.07	68.02	100
	Alt Sep	1.06	3.17	6.56	11.90	16.88	60.42	100
Lake Bellaire	July	1.62	2.48	6.95	11.74	13.04	64.17	100
	August	1.13	2.77	11.79	7.86	7.31	69.13	100
	September	0.91	3.86	8.66	4.71	9.67	72.18	100
Elk Lake	July	0.75	2.85	9.60	12.57	21.06	53.17	100
	August	0.90	5.15	9.40	9.92	13.52	61.11	100
	September	0.96	2.84	9.65	7.61	17.97	60.96	100
Lake Leelanau	July	2.12	5.17	10.51	14.33	13.11	54.76	100
	August	na	na	na	na	na	na	na
	September	1.07	3.28	4.65	12.12	8.57	70.30	100

Table 8. Diatom Counts expressed as percent of total. No benthic algae sample was obtained in August at the Lake Leelanau site. na = not available.

Appendix C, Figures

1. Torch Lake Diatom Distributions

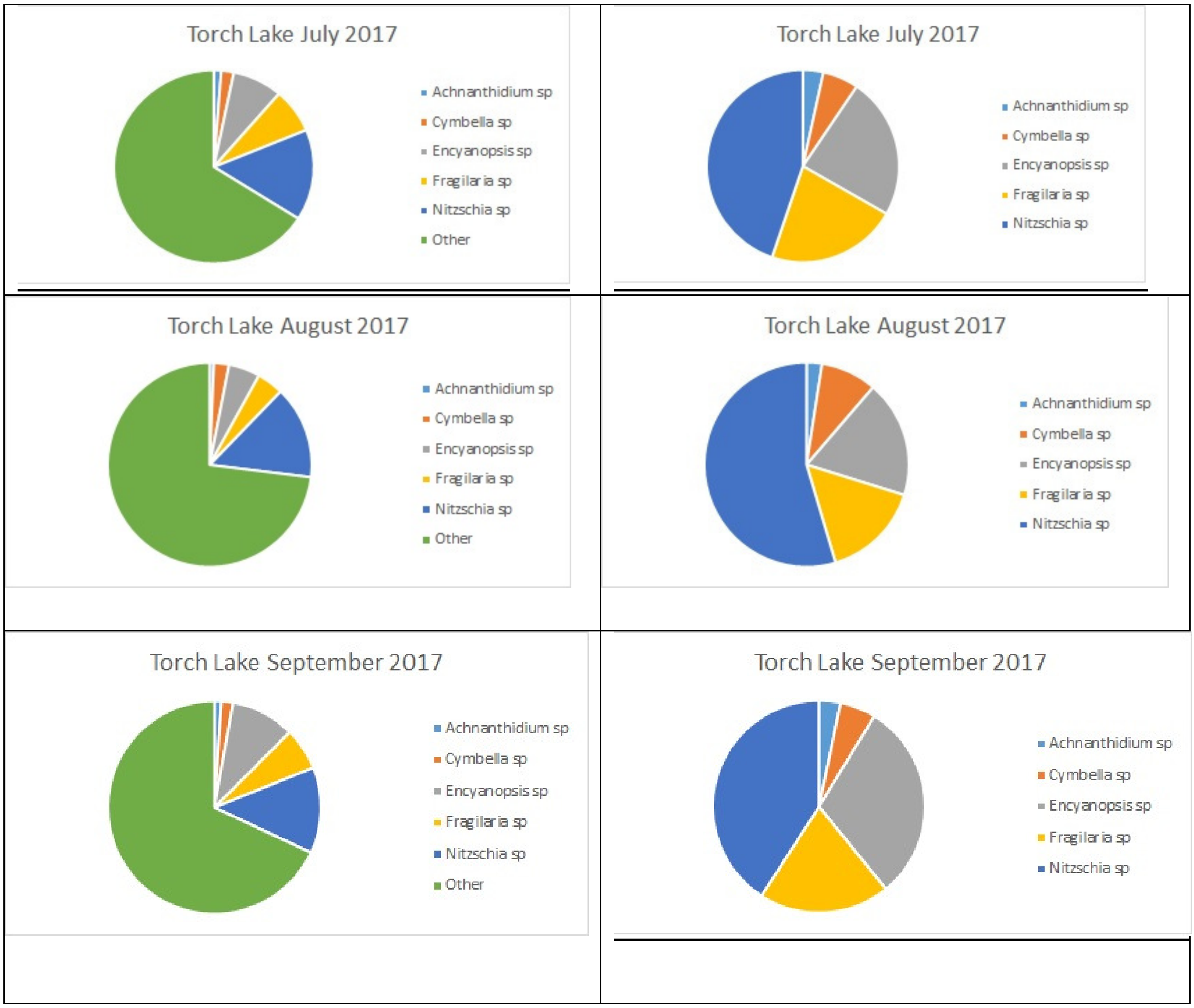


Figure 3. Torch Lake diatom distributions, on the left all diatoms and on the right the five most abundant.

2. <u>Elk Lake Diatom Distributions</u>

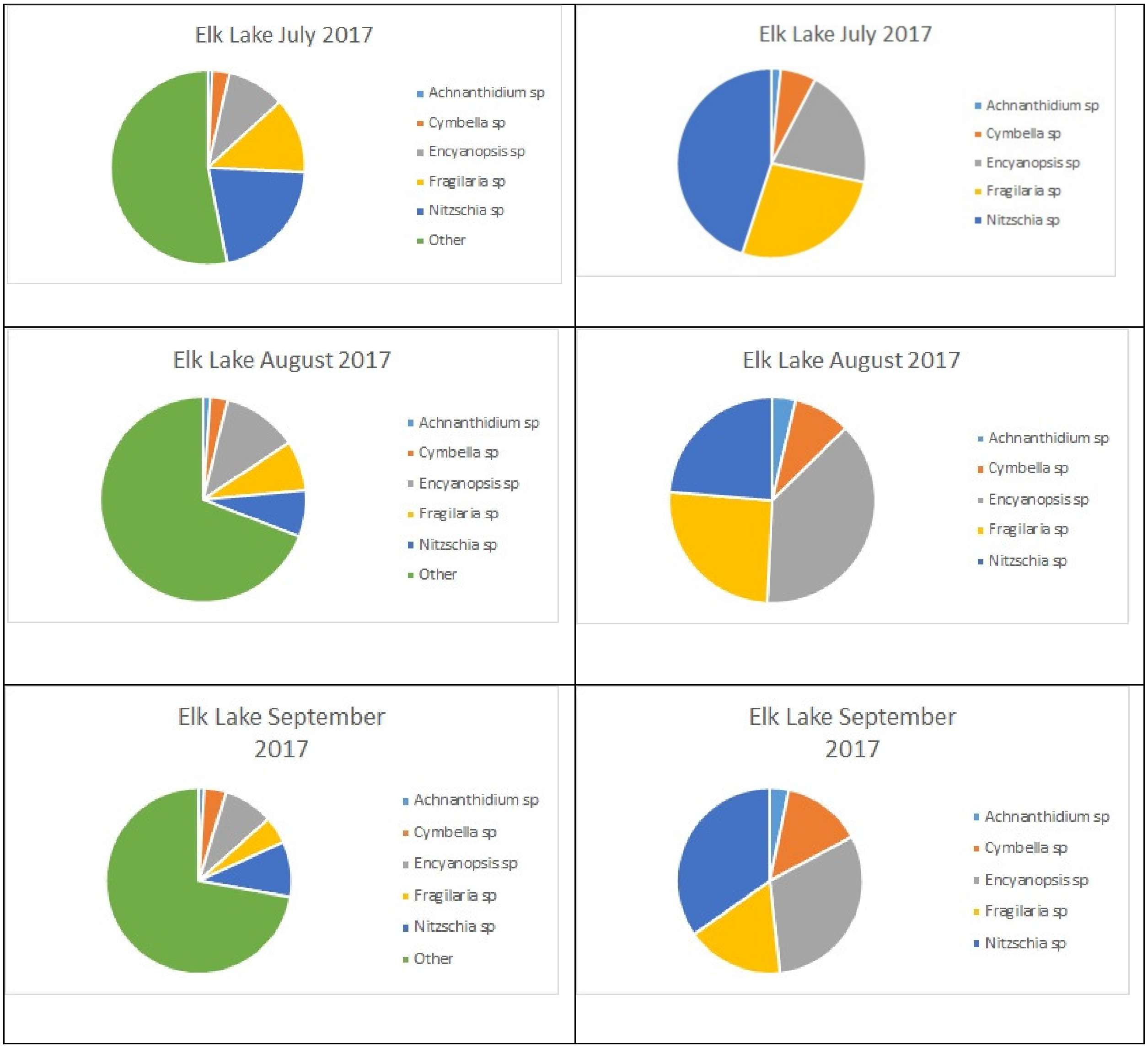


Figure 4. Elk Lake diatom distributions, on the left all diatoms and on the right the five most abundant.

3. <u>Lake Bellaire Diatom Distributions</u>

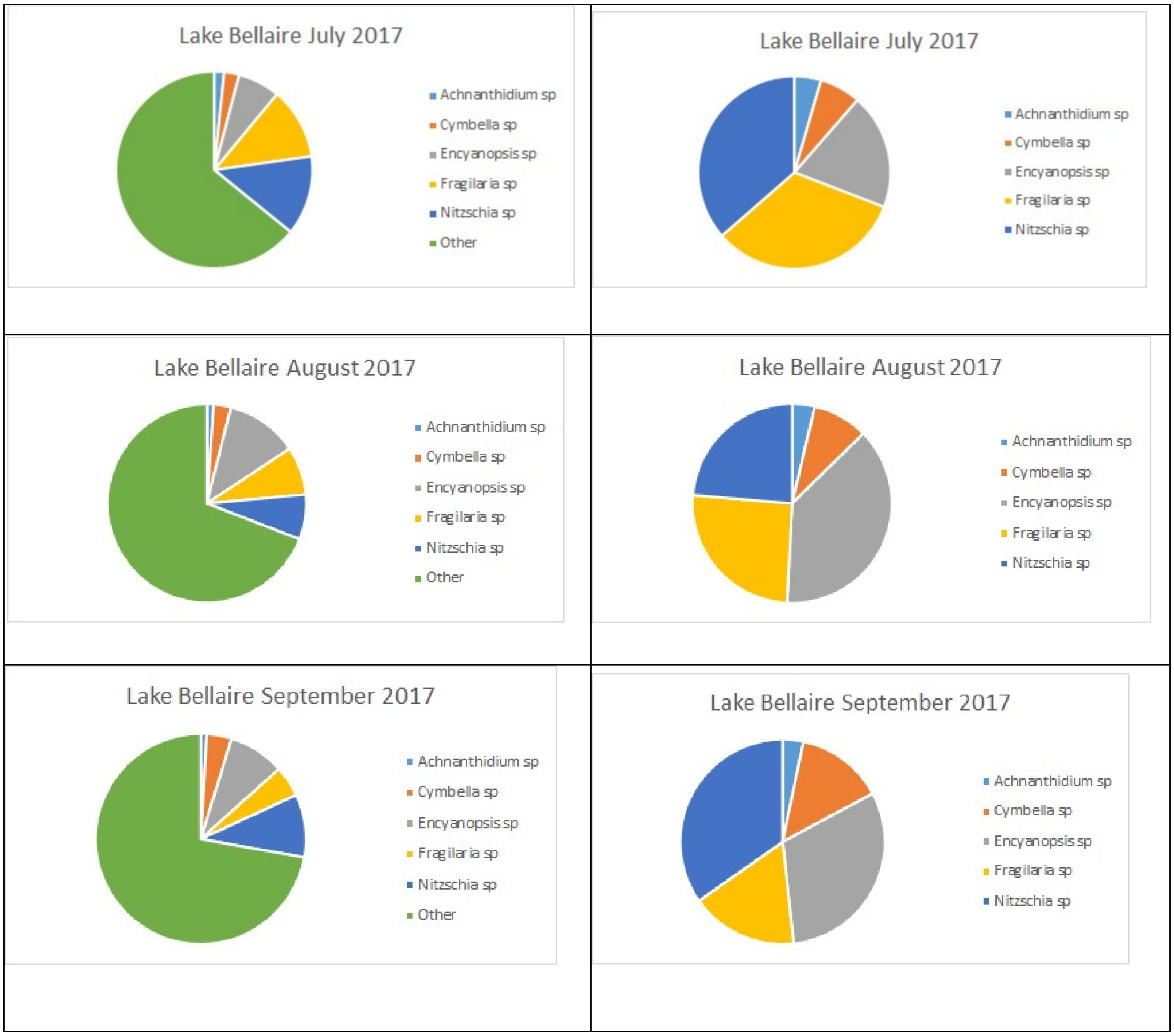


Figure 5. Lake Bellaire diatom distributions, on the left all diatoms and on the right the five most abundant.

4. <u>Lake Leelanau Diatom Distributions</u>

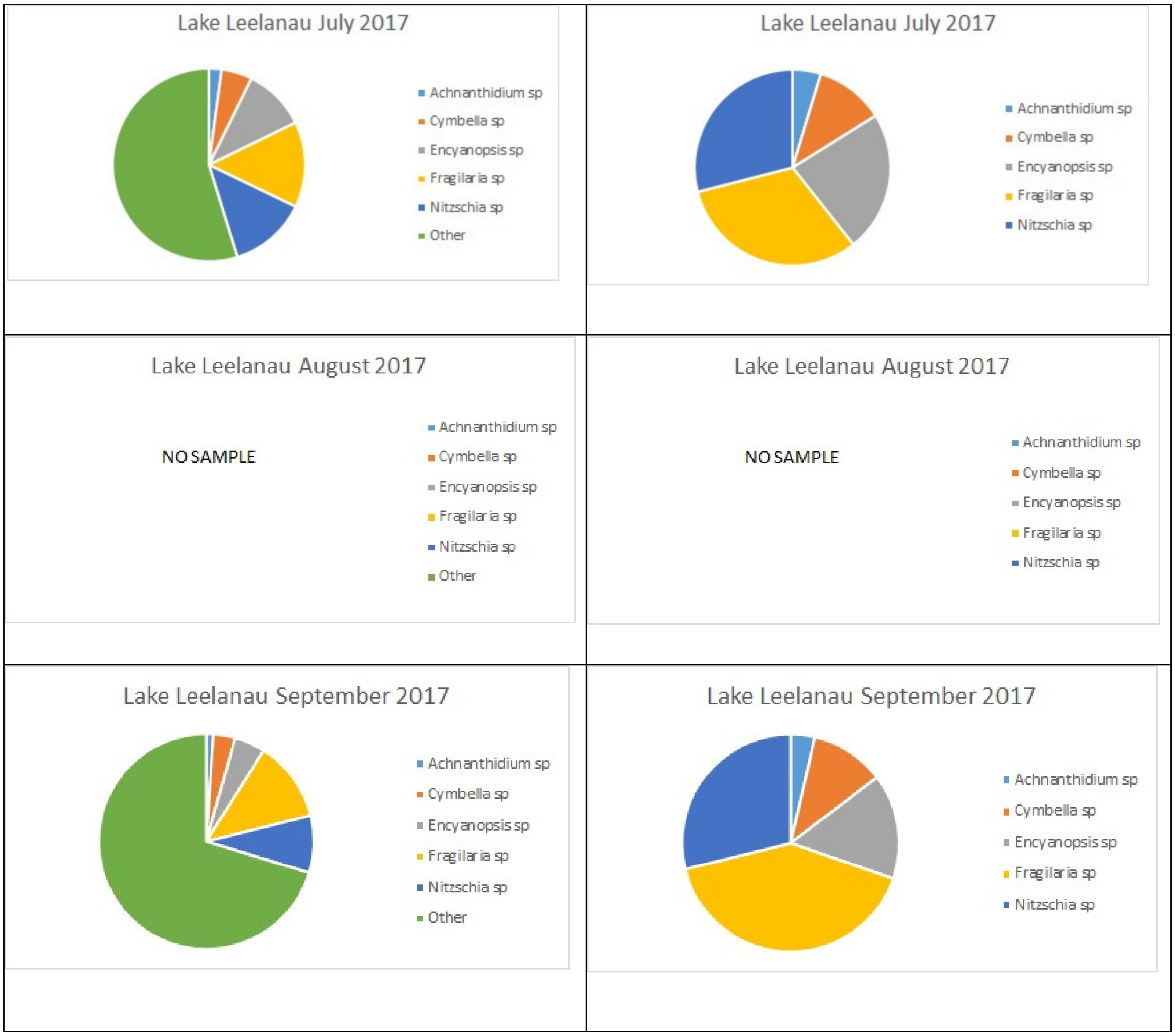


Figure 6. Lake Leelanau diatom distributions, on the left all diatoms and on the right the five most abundant.

5. Field Notes Sheet Design

PEEPER NUTRIENT STUDY FIELD NOTES

Date: ____________ Time: __________ Site ID: ____________ GPS: N ________ W __________

Site Name: ____

Study Visit: □ June (first) □ July (second) □August (third) □ September (fourth)

Sample ID	Item	Action/ Appearance
	Grab 1	
	Grab 2	
	Peeper A	
	Peeper B	
	Peeper C	
	Peeper D	
	Peeper E	
	Peeper F	
	Benthic algae	
	Pictures	

Water depth: Scuzziness score: Color score: % coverage:

Algae appearance:

Other:

Site sketch:

Figure 7. Design of the field notes sheet. The site information, date, and sample IDs were entered prior to each field trip to reduce the chance of misidentification.

6. <u>Aerial Views of Study Sites</u>

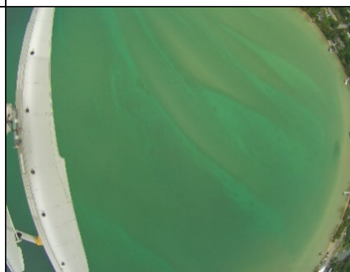
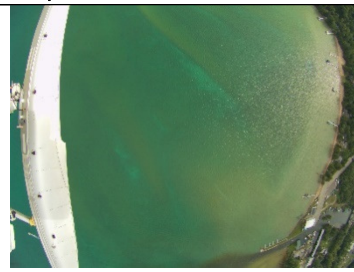
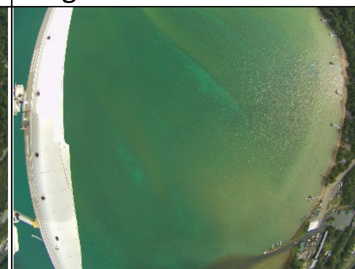
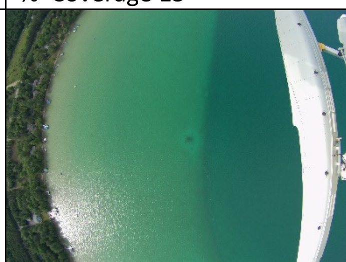
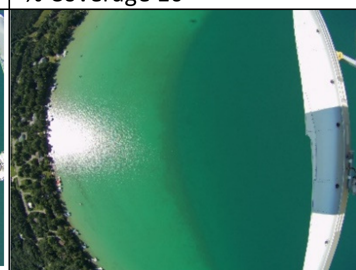
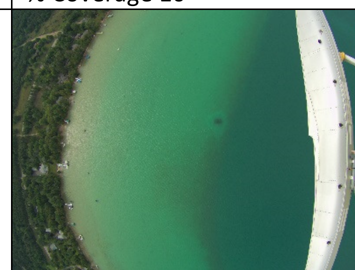
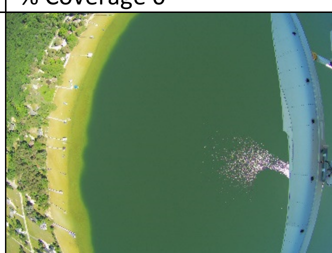
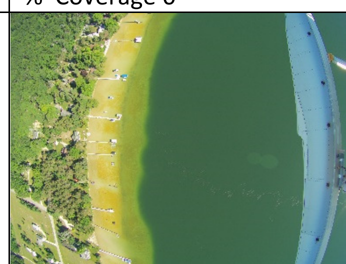
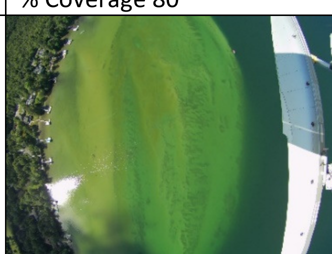
Site	June	July	August
Torch Lake	 Scuzzy Score 1 % Coverage 15	 Scuzzy Score 1 % Coverage 10	 Scuzzy Score 1 % Coverage 10
Elk Lake	 Scuzzy Score 0 % Coverage 0	 Scuzzy Score 1 % Coverage 0	 Scuzzy Score 0.5 % Coverage 50
Lake Bellaire	 Scuzzy score 4 % Coverage 80	 Scuzzy Score 4 % Coverage 50	 Scuzzy Score 4 % Coverage 80
Lake Leelanau	 Scuzzy Score 3 % Coverage 40	 Scuzzy Score 3 % Coverage 50	 Scuzzy Score not done % Coverage not done

Figure 8. Aerial views of the study sites in June, July, and August. Aerial photographs for September are not available. Scuzzy scores and percent coverage were not done in June.

Acknowledgments

The Three Lakes Association wishes to thank the property owners who have generously provided access to their properties, the volunteers who carried out the field work, Dr. Stevenson of MSU for his guidance and the diatom counts, and Tim Veverica of UMBS for the water chemistry data. We also wish to thank the Elk-Skegemog Lake Association, the Lake Leelanau Lake Association, and the Torch Lake Protection Alliance for their generous financial support.

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