Golden Brown Algae Repopulation Experiment

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Introduction

Over the past few years, residents of Torch Lake (Michigan) have observed a significant increase in the levels of benthic Golden Brown Algae (GBA) on their shores. GBA is not currently seen as a threat to humans or the local environment, however it is a visual nuisance to homeowners. This experiment will determine the repopulation rate of the Golden Brown Algae found in Torch Lake by subjecting areas of the algae to disturbance treatments. The experiment will also determine the repopulation rate of GBA after disturbing the mats once and multiple times.

The results of this experiment will hopefully give an indication of how to efficiently remove the GBA from Torch Lake's shores by showing the effect of multiple disturbances versus individual disturbances on GBA mats. Alternatively, the experiment may indicate that disturbing the GBA via raking isn't an efficient means of preventing repopulation.

Research & Hypothesis

The benthic GBA in Torch Lake could be accumulating due to an increase of phosphorus at the bottom of the lake. According to Dr. Jan Stevenson, increases in nutrient concentrations have been shown to support the growth of algae, with phosphorus being one of its main nutrients. One of the several hypotheses presented by Stevenson involves the proliferation of the benthic GBA because of phosphorus in the groundwater from fertilizers and septic systems seeping into the lake bed. This is primarily supported by the fact that there has only been a significant increase in the benthic algae (algae anchored to a surface, in this case the lake floor) rather than planktonic algae (algae that floats freely in the water). Thus the unusual growth of benthic GBA may be correlated with the high amount of phosphorus entering the lake via groundwater.

Another likely contributor to the unusually high GBA growth in Torch Lake is that the natural consumers of GBA are unable to regulate the population for some reason. Stevenson states that increases in algal consumers (examples being microscopic protozoa, macroscopic snails, and insect larvae) can slow or completely stop the growth of algal blooms. However, when nutrient pollution causes the algal blooms to grow at a faster rate. This limits the consumer's regulating effect on the algae, because they are unable to keep up with the growth. So the current phosphorus concentration in Torch Lake has left the natural regulators unable to control the growth of benthic GBA.

Hypothesis:
If the benthic GBA is disturbed multiple times via removing the algae from its substrate (surface), then the repopulation rate will be reduced more than it will with single disturbances. Removing GBA will likely slow the rate of repopulation due to the lack of existing algae available to repopulate. Repeatedly removing algal mats will ensure that small populations exposed to phosphorus nutrient pollution will not be able to grow into a large population that is difficult to control. Disturbances will also allow natural populations of consumers to adequately regulate the algae.

Method

Materials
Underwater camera or underwater viewer
Bricks for marking top right corner (established by frame) - Total of 9
PVC Pipe Frame for a guide

Experiment Setup
A property on Torch Lake was required to run the experiment which was provided by Ed Gourley, a TLA member. A big thank you to him for allowing use of his lakefront. Additionally, Dr. Jan Stevenson (his work mentioned previously) assisted with the experiment design and how to accurately process the results.

The experiment included two disturbance treatment groups and a control treatment group, each with three replicates. The PVC pipe frame was used to measure a $2ft^2$ area. A brick was placed in the top right hand corner to act as a marker for the plot. Each treatment was randomly placed somewhere in a 3x3 plot region with 3 feet between each plot. This is to account for spatial variability within the area. The plots were placed in four foot deep water to reduce wave induced disturbances to the algal mats.

Key to Plot Treatment Type

Group Type	#	Group Type	#	Group Type	#
Multi-Disturbance Group	7	Single-Disturbance Group	8	Control	9
Single-Disturbance Group	6	Control	5	Multi-Disturbance Group	4
Control	1	Multi-Disturbance Group	2	Single-Disturbance Group	3

Dock

The control groups were set up and measured weekly to provide insight into the natural conditions of GBA with no experimental variation. Thus these plots were not interacted with except for collecting data. The first of the disturbance treatment groups to be set up were the multi-disturbance groups. After taking an initial measurement for these plots, they were disturbed once every two days for five days. On the third day of disturbances for the multi-disturbance groups, three more plots were set up, comprising the single-disturbance groups. The single-disturbance groups were introduced on the last day of disturbances for the multi-control group so both groups will have started growing from scratch on the same day. Disturbance treatments consisted of simply stomping on the algal mat, which resulted in compromised mat integrity, and the algae being released into the surrounding water rather than being on a substrate. After the single-disturbance treatments were set up, no more disturbances were performed for the remainder of the experiment.

For the next four weeks post disturbance treatments, every section was measured once a week. A photo was taken of each plot in its entirety, and a zoomed in photo. These photos were later referenced for calculating the scuzziness score. Some of the photos were difficult to view because of the weather creating waves that refracted light onto the lake floor, which showed up in the pictures. Lowering the photo's light value did help a bit, however the algae is still difficult to see. So, ideal weather conditions would be days with low wind and or cloudy days, which is something that should be considered in setting up a schedule for any future projects with similar designs.

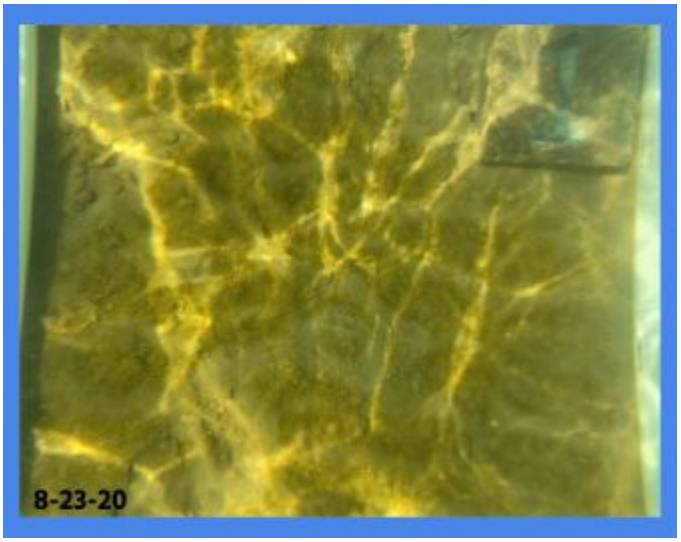
Schedule

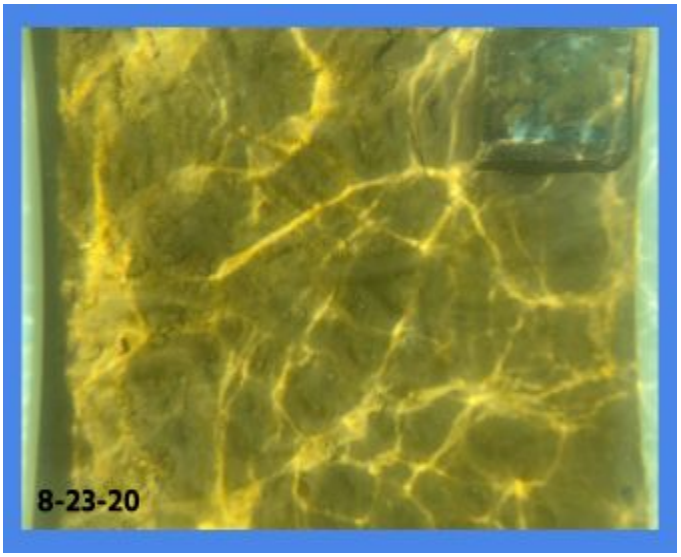
Date	Multi-Disturbance Groups	Single-Disturbance Groups	Control Groups
7/21	Set Up Initial Measurements Disturbance Treatment		Set Up Initial Measurements
7/23	Disturbance Treatment		
7/26	Disturbance Treatment	Set Up Initial Measurements Disturbance Treatment	

Dates for Measurements - (All Groups)
8/2
8/9

8/16
8/23

GBA Photo Examples



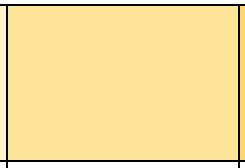
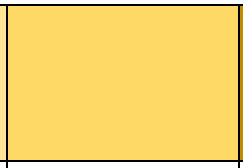
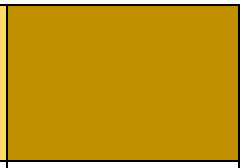


Scuzziness Determination

Each plot on each day measured was assigned a scuzziness score using the Scuzziness Determination Scale below. The scale was developed by TLA member Becky Norris for determining the intensity of the algae present.

Algal mats in the plots were scored for three data points: the mat's thickness, the coverage of the mat (percent of the area covered), and the shade of the algae's color. Together these measurements were used to calculate a total scuzziness score.

Select the score of the block with the closest match to the field observation from each of the following scales.

Shade scale				
	1	2	3	4

Coverage scale	<20%	20 - 39	40 - 59	60 - 79	80 - 100
	1	2	3	4	5

Matt thickness scale	None apparent	1 – 2 mm	3 – 4 mm	5 – 9 mm	≥ 10 mm
	1	2	3	4	5

Add together scores for each scale.

Example: Shade 2, Coverage 4, Matt thickness 3 yields a score of 2+4+3 = 9

Example: Shade 3, Coverage 5, Matt thickness 5 yields a score of 3+5+5 = 13

Scuzziness score is the score resulting from the addition; the possible scores run from a low of 3 to a high of 14.

Note: The original scuzziness scale multiplied the different scaled values together to determine the total scuzziness score, rather than adding them. However, when observing the GBA and calculating its regrowth rate, there was some concern that the multiplication method would skew the apparent rates when the scaled numbers changed. For example, say a plot with the initial scaled data set of 2 coverage, 2 thickness, and 1 color (2*2*1 = 4) changed over the next week to 2

coverage, 2 thickness, and 2 color (2*2*2 = 8). While only one data point (color) changed, the total score doubled (4 to 8). Thus, when working with higher scaled values, the change between total scores reflects a higher value than it should.

Data

See link to data sheet to view the individual scuzziness scores and their components listed by plot and per week as well as full statistical analysis.

Link to Data Sheet

A weekly scuzziness score was given to each of the nine plots. ANOVA and tTest statistical analysis was performed on the week four scuzziness scores that were separated into groups by plot treatment type. This was to determine if the scuzziness scores resulting from the different treatments were statistically different from each other. A single-factor ANOVA test was run on all three groups together, and three tTests were run comparing each of the plot types to one other (control to multi-disturbance, control to single-disturbance, and multi-disturbance to single-disturbance). ANOVAs take the series of three groups and find the mean for each (which can be used to represent the whole data set and plot type). With ANOVAs the null hypothesis (a hypothesis that may or may not be rejected) is that the means of all three groups are statistically the same. ANOVAs generate an F value and an F-critical (F-crit) value. If the F value is greater than the F crit value, the null hypothesis must be rejected, and at least one of the means is different from the others.

tTests are similar in design, except they compare the means of two groups rather than three. The null hypothesis is the same as ANOVA's (the means of all groups are statistically the same). Whether the means are statistically similar is determined by the calculated P value compared to an input alpha value (0.05 is a standard alpha value and was used in this case), and the calculated t stat with the t-critical (t-crit) value. Two conditions must be met to reject the null hypothesis: the alpha value is greater than the P value, and the t stat is larger than the t-crit value. In this case, there are two t-crit values. The second t-crit value (or two-tail values opposed to the one-tail values) is used. Because there are only two groups being compared, specific relationships between groups can then be determined using tTests, unlike through an ANOVA test. For example, if Group A and Group B had statistically different means, but Group C had the same mean as group B, it cannot be determined that Group A is the group with the differing mean. However, if each group were to be individually tested with another, it can be determined that Group A has a different mean than both Group B and C. The type of tTest used for the control group paired with the single and the single group paired with the multi group was the Two-Sample Assuming Unequal Variances test, as this type should be used when it is known that the variances are not the same (their variances were determined to be unequal from the preceding ANOVA test). The tTest for the control group paired with the multi group was a Two-Sample Assuming Equal Variances test because it was known that those groups had equal variances.

ANOVA and tTest Results for Week Four Scuzziness Score Comparison

Conditionals for Rejecting Null Hypothesis - ANOVA

1. F value is greater than F-crit value

Test	ANOVA	F > F-crit 32.16667 > 5.143253
F	32.16667	
F-crit	5.143253	
Reject	True	

Conditionals for Rejecting Null Hypothesis - tTest

1. Alpha value (0.05) is greater than the P value
2. t Stat is larger than the t-crit value

	Control & Multi Groups Two-Sample Assuming Equal Variances tTest	
P Value	0.000348	Alpha value > P value 0.05 > 0.000348 t Stat > t-Crit 11.31371 > 2.776445
t Stat	11.31371	
t-Crit	2.776445	
Reject	True	

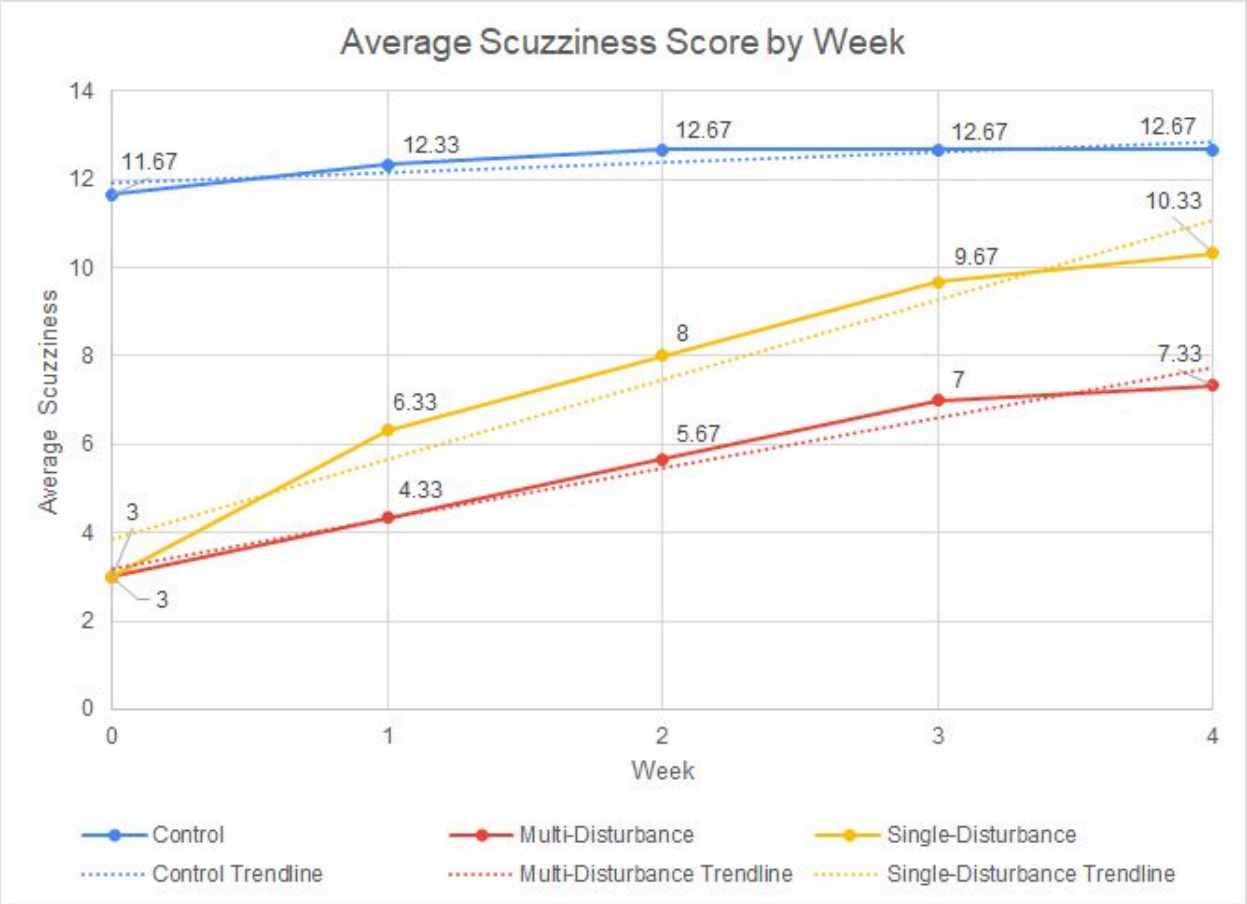
	Control & Single Groups Two-Sample Assuming Unequal Variances tTest	
P Value	0.052046	Alpha value ⊁ P value 0.05 ⊁ 0.052046 t Stat ⊁ t-Crit 3.130495 ⊁ 3.182446
t Stat	3.130495	
t-Crit	3.182446	
Reject	False	

	Multi & Single Groups Two-Sample Assuming Unequal Variances tTest

P Value	0.027556	Alpha value > P value 0.05 > 0.027556 t Stat > t-Crit 4.024922 > 3.182446
t Stat	4.024922	
t-Crit	3.182446	
Reject	True	

The results of the ANOVA determined that there is statistical difference between the means, and therefore the scuzziness scores in the different groups. This means that there is a significant difference in GBA regrowth between treatments. The tTest results corroborated and specified the result of the ANOVA test. The tTests showed that the control group paired with the multi group and the multi group paired with the single group were statistically different (rejected null hypotheses) while the control group paired with the single group were not (did not reject null hypothesis). This supports the hypothesis that multiple disturbance treatments will reduce the rate of GBA repopulation. This is because the multi-disturbance and single-disturbance group scuzziness scores were proven to be significantly different as well as the multi-disturbance group and control group scores. However, the single-disturbance group and control group scores not being significantly different shows that the single disturbance group plots regrew enough GBA to be comparable to the control plots which had consistently the highest scuzziness scores throughout the experiment. The multi-disturbance groups are not statistically comparable to the control group scores, meaning they did not regrow enough GBA to be statistically similar and therefore had a slower GBA repopulation rate than the single-disturbance plots.

After determining that the scuzziness scores at the end of the treatment were statistically different by group, the individual plot scores of the same type were condensed into an average weekly scuzziness score (control, multi-disturbance, and single-disturbance plots). These were used to calculate the average repopulation rate for each treatment using a linear regression model. The equation found represents the line of best fit for the data set. The slope of the equation represents the average growth rate for each treatment and is compared to determine how the variation in treatment type affects the GBA repopulation rate. The R^2 (R-squared) values listed represent the amount of the deviation from the mean of the data set that can be explained (deviation that is not error) in its corresponding equation. Essentially, it's an indicator of how well the equation represents the data collected. A higher R^2 value means that its equation represents the data well, and a lower R^2 value means the equation does not represent the data well. For reference of the R^2 scale, all R^2 values are greater than or equal to zero, and lesser than or equal to one.



Average Plot Type Scuzziness Scores

	Av Control	**Av Multi**	**Av Single**
Week 0	11.67	3	3
Week 1	12.33	4.33	6.33
Week 2	12.67	5.67	8
Week 3	12.67	7	9.67
Week 4	12.67	7.33	10.33

	Trendline Equation	**R^2 Value**	**Slope**
Control Groups	y = 0.234x + 11.934	0.7238	0.234
Multi-Disturbance Groups	y = 1.133x + 3.2	0.9695	1.133
Single-Disturbance Groups	y = 1.8x + 3.866	0.9369	1.8

Each of the equations have a high R^2 value, especially the multi-disturbance and single-disturbance equations, those being above 0.9. This indicates that each of the slopes given by the equations are

a good indicator of the actual rate of change in the scuzziness scores, and therefore the rate of repopulation of GBA.

The linear regression analysis supports the hypothesis that multiple disturbances will lower the rate of GBA repopulation. Looking at the slopes of the lines of best fit for the different treatment data points shows that the single-disturbance groups had the highest rate of change in scuzziness score, or rather GBA regrowth over the four week period. Its 1.8 slope is greater than the multi-disturbance group's 1.133 slope. Both are greater than the control group's slope of 0.234. The control plots changed little over the four weeks likely due to the undisturbed GBA mats being close to maximum sustainable density. Too much GBA in one area would increase competition and reduce available nutrient supply.

Discussion

Observing the GBA plots over time post disturbances has shown that the repopulation rate is affected by the number of times disturbed. The plots only disturbed once had a faster GBA repopulation rate (increase of 1.8 scuzziness score per week) than the plots that were disturbed a total of three times (increase of 1.133 scuzziness score per week). The data is corroborated further with statistical analysis (ANOVA and tTests) showing that there is a statistical difference in regrowth based on whether it received one or multiple treatments. Thus the hypothesis that multiple disturbances that remove the algae from its substrate will reduce the GBA recolonization rate is supported. There are many potential reasons for the results of the experiment. One possibility is that when the GBA was removed from its substrate (the lake floor), it was removed from a source of phosphorus entering the lake via groundwater, which caused the GBA population to increase. As mentioned previously, this hypothesis was developed by Dr. Jan Stevenson from his observation that increases in nutrient levels (such as phosphorus) available to GBA increases its growth. Thus, the frequent disturbances of GBA would be more effective at displacing the algal cells from its nutrient source and subsequently reducing the repopulation rate in that area. Based on his findings, Stevenson also suggests that the inability for natural grazers to manage the increased growth of GBA from nutrient pollution may be a contributing factor to its increasing growth. The repeated disturbances then may have been able to clear enough GBA to make the remaining algal cells easily manageable for the grazers. Finally, the disturbances removing algal cells may have simply reduced the starting population size more than the single disturbances did, therefore reducing the amount of algae found post repopulation time compared to the other plots with a higher starting population.

A potential future experiment could be one that tests the most efficient number of disturbances to keep the population of GBA at a minimum. One observation from the experiment was that the areas around the plots (areas that were repeatedly disturbed due to movement) showed no signs of GBA regrowth. While there was minimal observation with no control variables, assuming that the lack of GBA regrowth in that area was due to repeated disturbances (which is implied from the result of the controlled experiment showing that more disturbance treatments does decrease the

recolonization rate) it may be beneficial to design an experiment that observes the change in recolonization rate dependent on how many times disturbed. In other words, the experiment would find the average rate of change in the average rate of change of GBA growth depending on how many disturbance treatments a plot received. For example, say a plot of GBA that received four disturbance treatments had an average rate of change of 1.0 scuzziness score point per week, while a plot that received five disturbance treatments had an average rate of change of 0.99 scuzziness score points per week. The rate of change concerning scuzziness score per week between four and five treatments is only -0.01 scuzziness score per week per disturbance treatment. Therefore, doing four treatments would be a more efficient use of time in controlling GBA population. The information from this experiment could be used to design an ideal controlling technique that manages the overgrowth of GBA in Torch Lake.

This would be of a similar design to this experiment but over a longer time frame and with more disturbance groups. For instance, an experiment with five groups, each group receiving one to five total disturbances plus a control group would give six different repopulation rates. These can be compared to determine the effect of the different number of disturbances on the rate of repopulation.

Bibliography

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