SIS.BIO

Leading the Way to
RENEWABLE WATER

2023 Integrated Report

Indian Lake, Cuba, Missouri

HAB Prevention & Lake Remediation Program

We Simplify the Science™

Contents

1. INTRODUCTION

Indian Lake is 320 acres, with an average depth of 15 feet and a maximum depth of 43 feet. For many years, like many lake communities in the USA, the Indian Lake lifestyle has been threatened by the effects of eutrophication on recreational enjoyment, esthetics, property values and the environment in general.

Media and government agencies are increasingly outspoken about their concern with the progressive worsening of the eutrophication of lakes in the USA. It inevitably leads to a tipping point being reached, when toxic HAB (harmful algae bloom) events occur.

That tipping point was reached in Indian Lake with the first confirmation of HABs in summer 2020 (although HABs had likely been present for many years prior), escalating the need for responsible decision making to save the Indian Lake lifestyle.

1.1 STATEMENT OF OBJECTIVES

The higher purpose of the Indian Lake Property Owners' Association's (ILPOA) Lake Committee is to **save the lake lifestyle.**

The first and most urgent objective was to **prevent the persistent toxic cyanobacteria HABs** that have been confirmed to have occurred since 2020. Not only are these a threat to human and pet health, but they also cause property values to drop.

The second objective is to **reverse eutrophication for sustainable remediation and restoration.** This work is the ongoing lake health maintenance program, to ensure that Indian Lake stays healthy for generations to come.

The second year of a 3-year program to achieve the first objective was completed in 2023.

1.2 FINDING A SOLUTION

ILPOA formed its lake restoration committee in late 2018. It was initially focused on the need to dredge the lake, in an attempt to remove the main source of phosphorus loading in the lake.

Scientists, consultants, government agencies and other experts agree on the need for proactive prevention and remediation of eutrophication and HABs but have not given any guidance for doing so. They insist that continuing to treat the symptoms in the conventional way has meant that communities are paying good money to make conditions worse, not better. They have called for an end to the conventional practices of herbicides and other chemical interventions.

In the face of this risk and confusion, it is an onerous task for any volunteer lake committee to responsibly spend funds raised from the community on a lake remediation company/project.

After extensive research, ILPOA's committee identified SIS.BIO and Clean-Flo International, as a unique combination of innovative new technology with a proven, verifiable track record of meeting its stated objectives of preventing HABs and reversing eutrophication for sustainable restoration.

1.3 OBJECTIVE MEASUREMENT & PERFORMANCE MANAGEMENT

The lake committee believed the objectives of the program should be specific, relevant, and quantifiable to ensure objective measurement of performance. Because the SIS.BIO solution is unique, in that it is based on biotechnology and a holistic, systemic, multi-faceted approach, the committee took time to learn from SIS.BIO what was required.

The SIS.BIO biotechnology approach differs from conventional lake treatments that target symptoms of eutrophication and not the root causes. conventional performance metrics are focused on symptomatic relief rather than sustainable solutions.

SIS.BIO defines eutrophication as an "eco-system regime change" which means that instead of inflowing nutrients to the lake being consumed and cleared via the food web, they accumulate in mucky sediments. Continual *nutrient recycling* produces excessive growth of cyanobacteria. The objective is to reverse the regime change from *nutrient recycling*, back to natural *nutrient clearance*. This means shifting from nutrients being primarily used to produce cyanobacteria biomass, to instead produce "good algae" biomass which can be consumed and cleared by animal life in the food web. Among the many benefits to the ecosystem, one especially attractive appeal of this approach is the prospect of better fishing in the lake. SIS.BIO's biotechnology aims to support nature to achieve this change.

The three key requirements and measures of success are:

1. Oxygenation of the whole water column all the way down to the mucky sediment on the bottom throughout the lake.
2. Bio-Dredging - effectively eliminating - the mucky sediment that recycles nutrients to sustain the eutrophic condition and drive invasive weed, algae, and cyanobacteria growth. (Cyanobacteria or "bluegreen algae" being the type of phytoplankton responsible for toxic HABs.)
3. Achieving a shift in the phytoplankton balance away from toxic cyanobacteria in favor of "good" green algae so that HABs are prevented.

No single company submitted a proposal that demonstrated a clear understanding of the SIS.BIO biotechnology and methodology or included all relevant metrics to ensure measurement of performance against these parameters and objectives.

The committee therefore decided to appoint:

- Restorative Lake Sciences (RLS) - to do water quality monitoring.
- PhycoTech – a laboratory that has pioneered high-precision algae species and biovolume analysis - to do phycological (phytoplankton) analysis and reporting in line with a protocol specified by SIS.BIO.
- Clean-Flo - to do bathymetric scans and analysis to quantify sediment reduction by Bio-Dredging.

SIS.BIO was tasked with providing an integrated report incorporating all data, measurement and testing annually.

2. WATER QUALITY

RLS specified testing protocols for water quality. Baseline testing was done in August 2021, October 2021, and March 2022. The remediation program began at the end of April 2022, once these reference baseline data sets had been collected.

RLS completed further tests in August 2022, October 2022, April 2023, and August 2023.

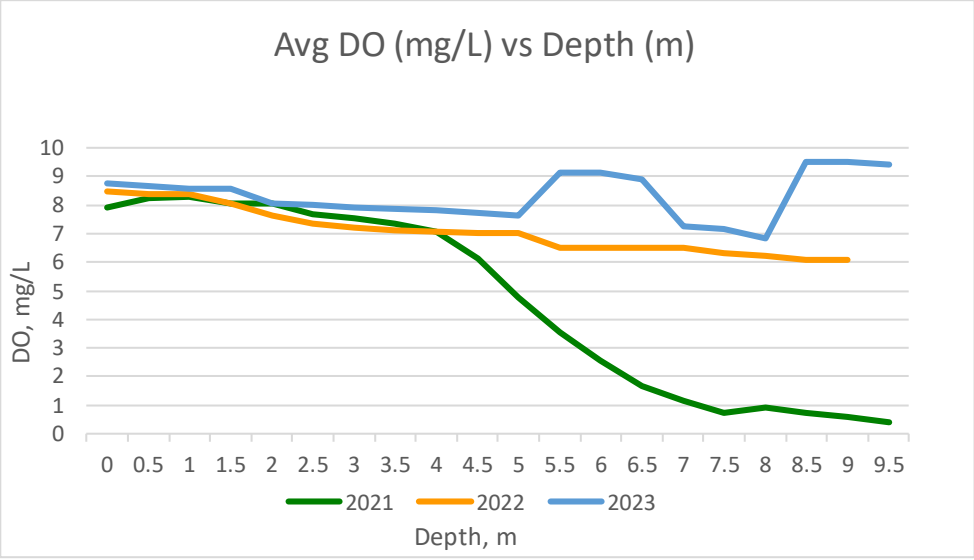
For performance measurement purposes, only the August data allows "like-for-like" comparisons in peak summer, when problems are at their worst. Therefore, this is the data utilized in this report, which is summarized from a more detailed 86-page report submitted by RLS in late September 2023.

RLS selected 4 sampling points at different locations around the lake, from which samples were taken and 11 different water chemistry parameters were tested during each round of tests. The data below shows averaged results from all 4 sampling points in August of each year.

2.1 DISSOLVED OXYGEN

SIS.BIO states that the necessary prerequisite for reversing eutrophication is to create aerobic conditions at the benthic margin, which is the interface between water and sediment, by oxygenating the whole water column, from top to bottom, throughout the lake,

The graph below shows that in August 2022, before beginning the remediation program, dissolved oxygen (DO) fell below 5mg at about 5 meters or 15 feet (green line).



In August 2022 (orange line) the objective of ensuring a minimum of 5mg/l DO was achieved. In August 2023 (blue line) that was improved upon, after SIS.BIO and Clean-Flo made modifications to further optimize the RADOR oxygenation system.

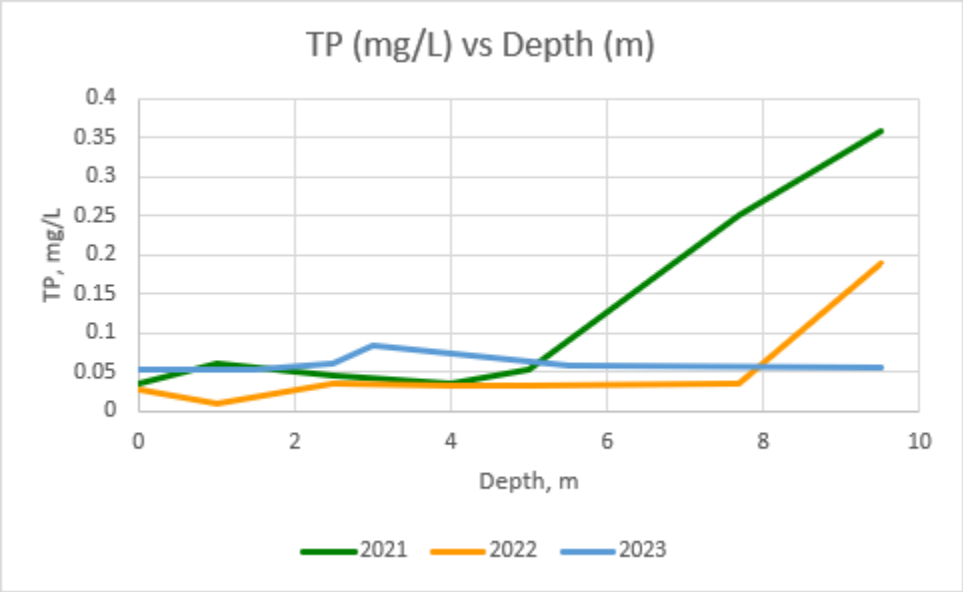
The next three parameters measured address the factors critical to preventing the sediment nutrient recycling that drives toxic cyanobacteria HABs. As shown below, all were inevitably

reduced by ensuring full oxygenation of the water column. Therefore, improvement of these parameters are directly correlated with higher oxygenation.

2.2 TOTAL PHOSPHORUS

Total phosphorus (TP) is a measure of both phosphorus that has been taken up by phytoplankton cells to produce algal biomass, and dissolved phosphorus (ortho-phosphorus). Ortho-phosphorous is available to be taken up by phytoplankton to further produce algal biomass. In other words, ortho-phosphorous concentrations are a component of TP.

The remediation program aims to reduce both phytoplankton biomass and dissolved phosphorus in the water. Therefore, TP is a reflection of both, and an indication of the remediation program's success at reducing the present growth of algae and the dissolved nutrients available for further growth.



In August 2021 (green line) TP increased below 5m, or 15 feet, where dissolved oxygen decreased (see DO graph in the previous section). This illustrates the correlation between the two.

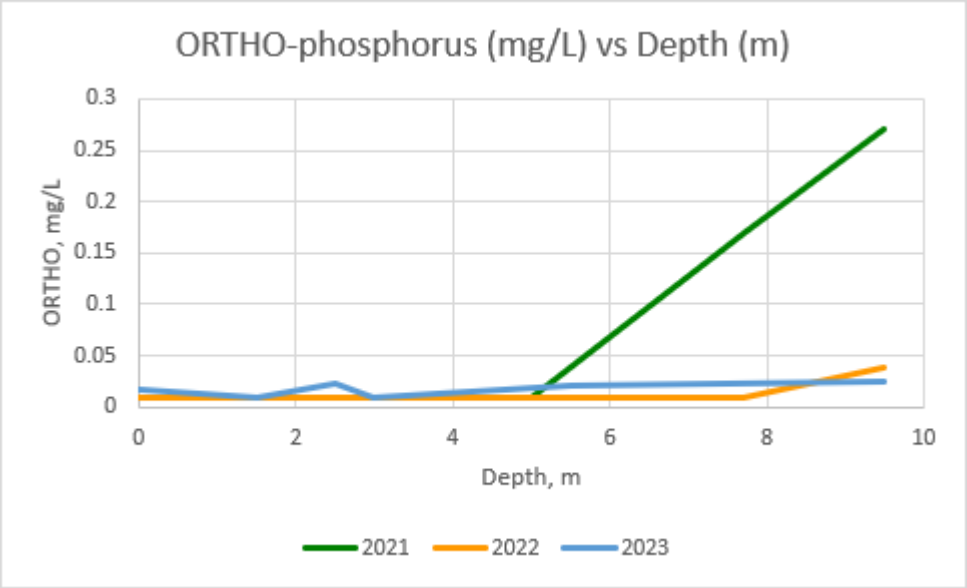
In 2022 (orange line), TP was significantly reduced, but still rose slightly below 8m, or 26 feet, depth. In August 2023 (blue line) that was further improved after SIS.BIO and Clean-Flo made modifications to optimize the RADOR oxygenation system in the deepest zone of the lake. The results prove the expected correlation between full oxygenation and reduction of nutrients and suggest that the level of dissolved oxygen will always be a reliable indicator of the concentration of nutrients either entrained in or available to algae growth.

2.3 ORTHO-PHOSPHORUS

Ortho-phosphorus (OP) is a component of Total Phosphorus, but only measures the dissolved phosphorus in the water that is available for uptake to produce more phytoplankton. This form of phosphorus is less soluble in well oxygenated water. When water is not oxygenated near the

sediment, dissolved phosphorus levels increase because phosphorus is released from the sediment.

Because cyanobacteria can control their buoyancy and descend to the bottom, but "good" green algae cannot, cyanobacteria can access this phosphorus and outcompete "good" green algae to produce HABs. Therefore, reducing the amount of ortho-phosphorus at the bottom is critical to countering the competitive advantages that cyanobacteria have in conditions of low dissolved oxygen and high nutrient concentrations. This is key to preventing toxic cyanobacteria HAB events.



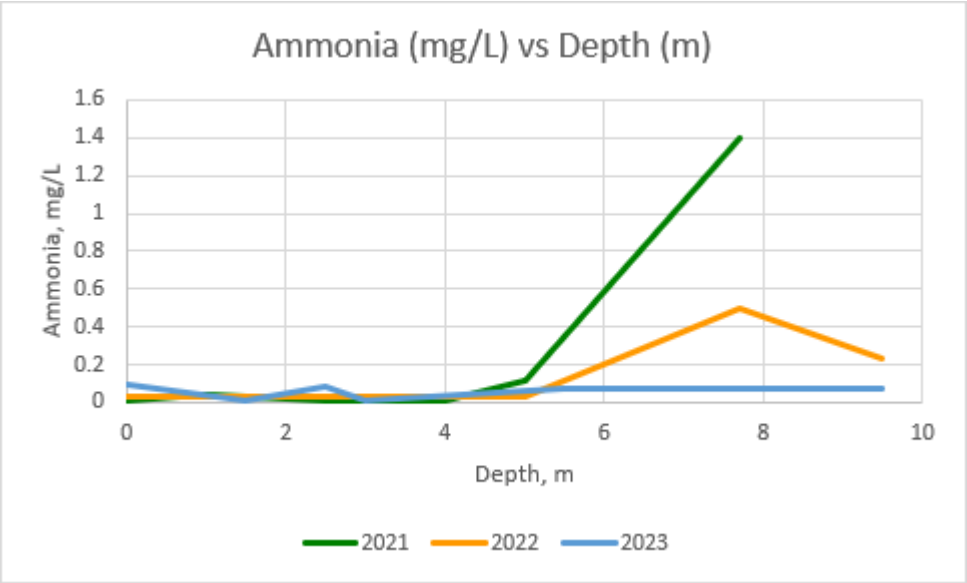
In August 2021, (green line) OP increased below 5m or 15 feet, where dissolved oxygen decreased (see DO graph above). This reflected ideal conditions for cyanobacteria HABs.

In 2022, (orange line) OP reduced significantly. In August 2023, (blue line) low OP levels were maintained. This reduction in benthic (bottom) levels of phosphorus help reduce phytoplankton in general, and cyanobacteria in particular. Measurement of phytoplankton biovolume appears later in this report.

2.4 AMMONIA

Ammonia levels are elevated in deoxygenated water by sediment nutrient recycling at the bottom of lakes. Cyanobacteria can descend in the water column to access this nutrient source, but "good" algae cannot. As with phosphorous, reducing ammonia levels in benthic (bottom) zones is critical to eliminating the competitive advantage that cyanobacteria have, and preventing HABs. Furthermore, ammonia is toxic to fish.

It can be expected that once the water column is fully oxygenated, ammonia levels will reduce through normal water chemistry.



In August 2021 (green line) ammonia increased significantly below 5m, or 15 feet, which is the depth at which DO dropped below 5mg/l. The ammonia further increases at depths where DO dropped to near zero (see DO graph above). This demonstrates the correlation between the two.

In 2022 (orange line) ammonia was further reduced significantly, but there was still a slight peak at about 7.5m, or 24 feet.

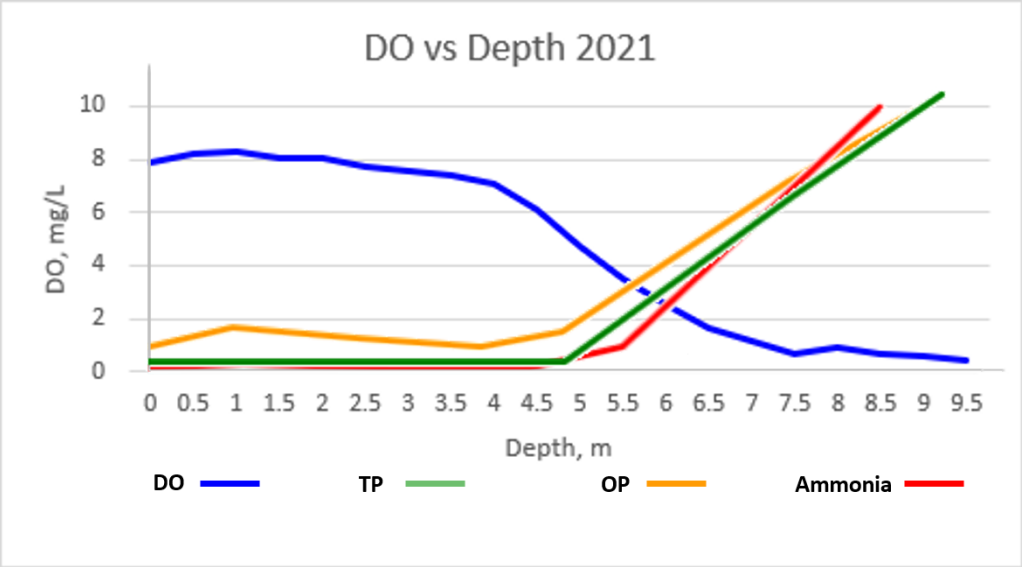
In August 2023 (blue line) – as with phosphorous - the peak in ammonia was eliminated after SIS.BIO and Clean-Flo made modifications to further optimize the RADOR oxygenation system.

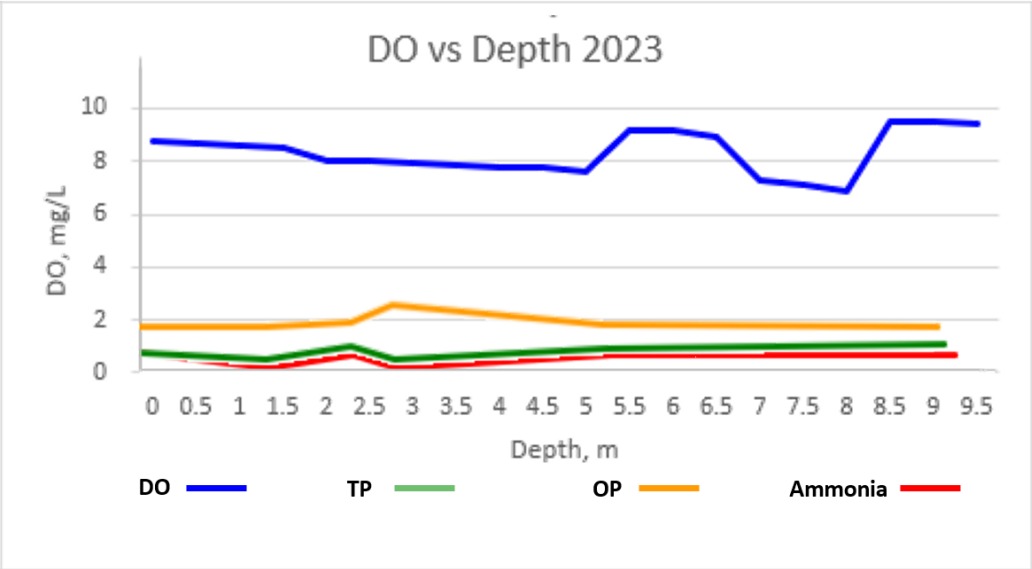
This reduction in benthic (bottom) levels of ammonia translates into a reduction in phytoplankton biomass in general, and cyanobacteria in particular. Confirmation of this appears later in the report.

2.5 OXYGENATION: MANAGING SEDIMENT NUTRIENT RECYCLING

The RLS data confirms that achieving full oxygenation of the water column and the benthic margin ensures that nutrient availability, as measured by TP, OP, and Ammonia, is reduced automatically by virtue of simple water chemistry.

In the two charts below, DO, OP, TP, and Ammonia data from the individual charts above are overlayed to illustrate the correlation between them and to show the positive impact of benthic oxygenation on sediment nutrient recycling. As mentioned, this is the main factor that facilitates cyanobacteria HAB species' domination of eutrophic water bodies.



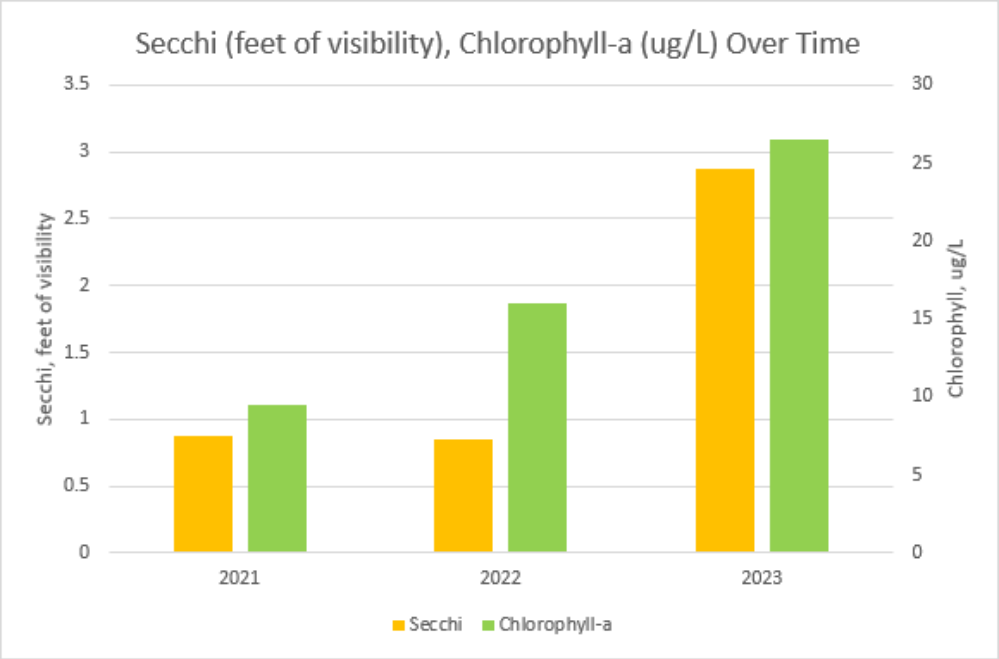


Logically, managing nutrient availability with oxygenation negates the competitive advantages that cyanobacteria have in eutrophic lakes, and prevents HABs.

These results demonstrate that the first key requirement for solution delivery: **Oxygenation of the water column throughout the lake**, and the ensuing suppression of nutrient recycling, has been achieved.

2.6 SECCHI DEPTH OF WATER TRANSPARENCY & CHLOROPHYLL-α

As the lake remediation program progresses, and phytoplankton biomass is reduced, Secchi readings of the transparency of the water should increase. The data shows that this is in fact happening as expected (orange columns).



Chlorophyll-a is a proxy measure of the amount of algae biomass present in the water. Chlorophyll-a is a photosynthetic chromophore – a biochemical that algae use to capture the sun's energy to create biomass.

As the lake remediation program progresses, it would be expected that Chlorophyll-a levels would decrease. However, the data shows that they have increased each year (green columns). This is contrary to expectations and appears to suggest a contradiction in this data. This is explained and clarified in the analysis of phytoplankton data below.

3 PHYCOLOGY & HAB PREVENTION

Phycology is the study of seaweed and phytoplankton, the most basic form of "primary" biomass in aquatic ecosystems. Phytoplankton belongs in the vegetable kingdom and like all plant life, uses photosynthesis to capture sunlight as an energy source to produce biomass.

The main categories of freshwater phytoplankton are cyanobacteria, algae, and diatoms. Cyanobacteria produce toxins and harmful algae blooms, or HABs. "Green" algae and diatoms can be considered "good algae" because they do not produce toxins and provide better nutrition for animal life in the food web. Animals consume them and in turn clear the nutrients from the water.

It was confirmed that Indian Lake was plagued with cyanobacteria HABs in 2020 and throughout 2021. This means that cyanobacteria species were dominant, and the level of phytoplankton biomass was too high.

Preventing cyanobacteria HABs and remediating the lake's eutrophic condition is a three step process.

Step 1: Increase biodiversity –re-establish many "good algae" species that can compete against the cyanobacteria to displace their dominance.

Step 2: Enable the "good algae" to increase in quantity and outcompete the cyanobacteria so that the cyanobacteria are marginalized and displaced from dominance.

The achievement of these two steps would ensure the prevention of cyanobacteria HABs.

Step 3: Reduce the overall total phytoplankton biovolume.

This will become the objective as the program shifts focus to reversing eutrophication and sustaining good water quality and lake health.

The phytoplankton data therefore focuses on two critical factors:

1. **Biodiversity** – measuring how many cyanobacteria HAB taxa are present and how many competitor algae taxa are present to compete against them.
2. **Biovolume** – measuring to what extent cyanobacteria dominate nutrient uptake to exceed the biovolume of competitors.

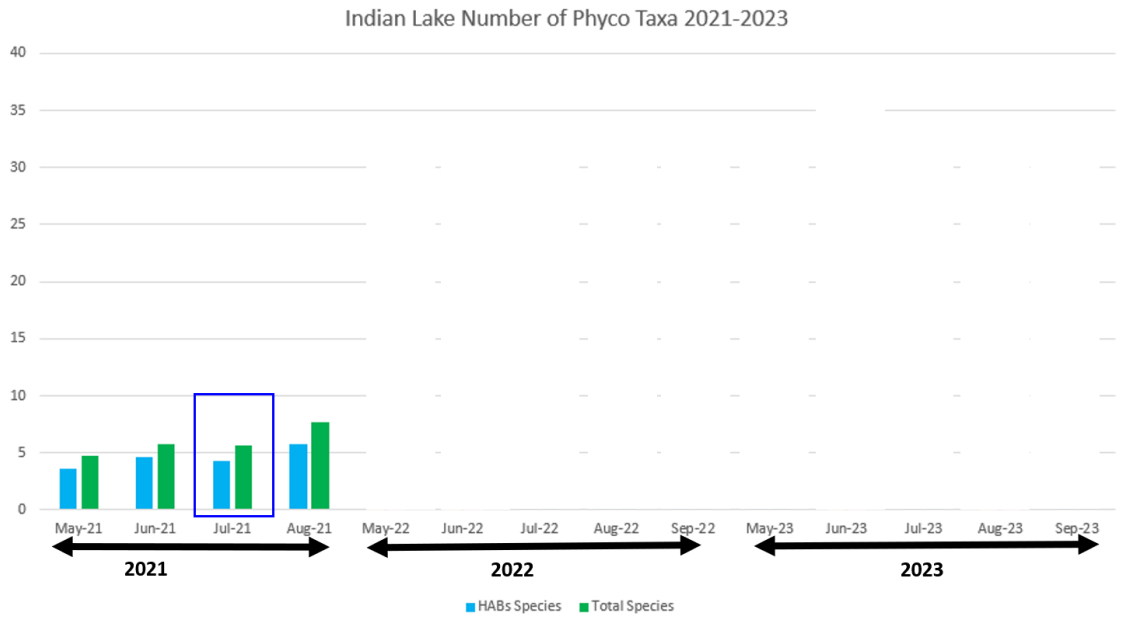
3.1 RESULTS - BIODIVERSITY

Biodiversity measures how many different taxa or types of phytoplankton are present (two taxa are elephants and ants). It does not measure what biomass or biovolume of each taxa there is – one elephant is equivalent in biovolume to millions of ants. Biodiversity just identifies the presence of elephants and ants,

If you are a farmer producing meat, biodiversity just says "in my field I have cows, pigs, sheep and chickens", but it doesn't tell you how many of each you have – i.e., how much biomass or meat you have to sell.

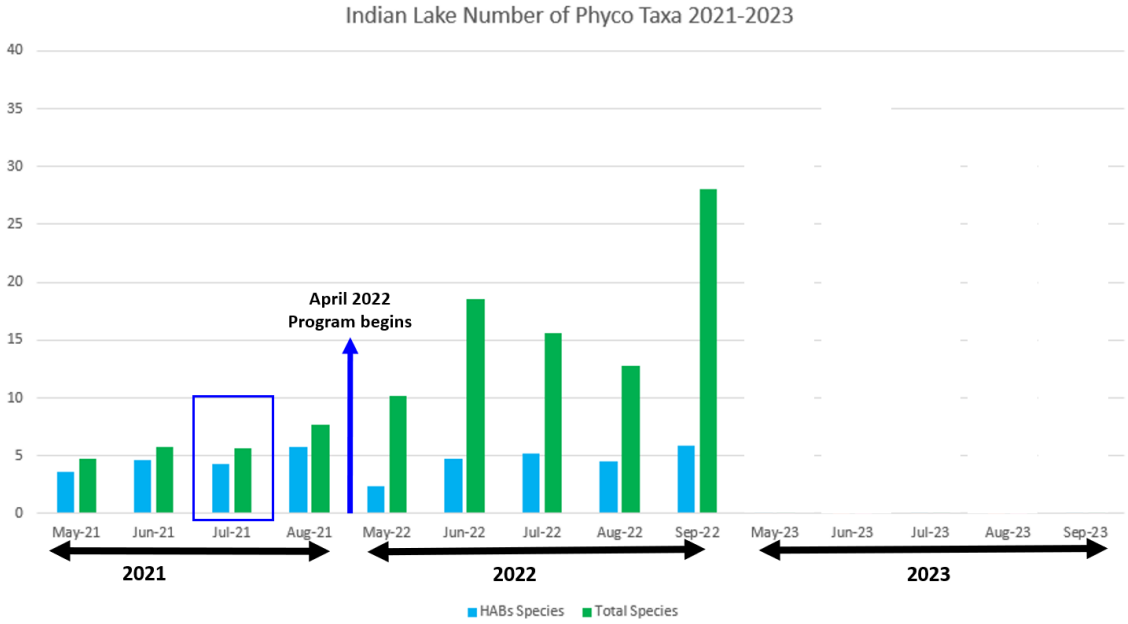
The chart below shows averaged phytoplankton data for 2021, (the year before the SIS.BIO program commenced). For each month it shows the average total number of taxa identified and the number of those taxa that were cyanobacteria HABs. In the highlighted month of July 2021, on average 6 taxa were identified of which 5 (83%) were HABs.

The remediation program commenced in late April 2022, after RLS had collected the final set of benchmark data in late March 2022.



In the chart below, data from 2022 is added.

It shows an increase in total taxa identified from 10 in May 2022 to 27 in September 2022. Of these there were 2 HABs in May 2022 (20%) and 5 in September 2022 (18%).



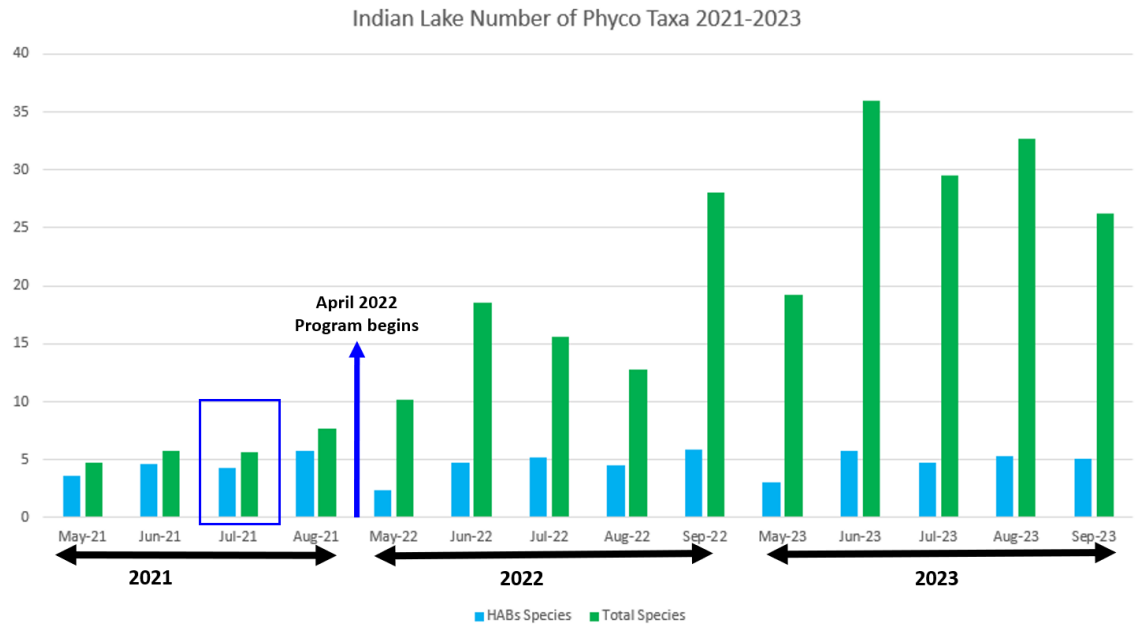
The most important fact is that <u>by September, the total number of different taxa (or biodiversity of phytoplankton) increased 170%</u> and there were many more types of algae to compete against the cyanobacteria.

This demonstrates the achievement of the first step in the process:

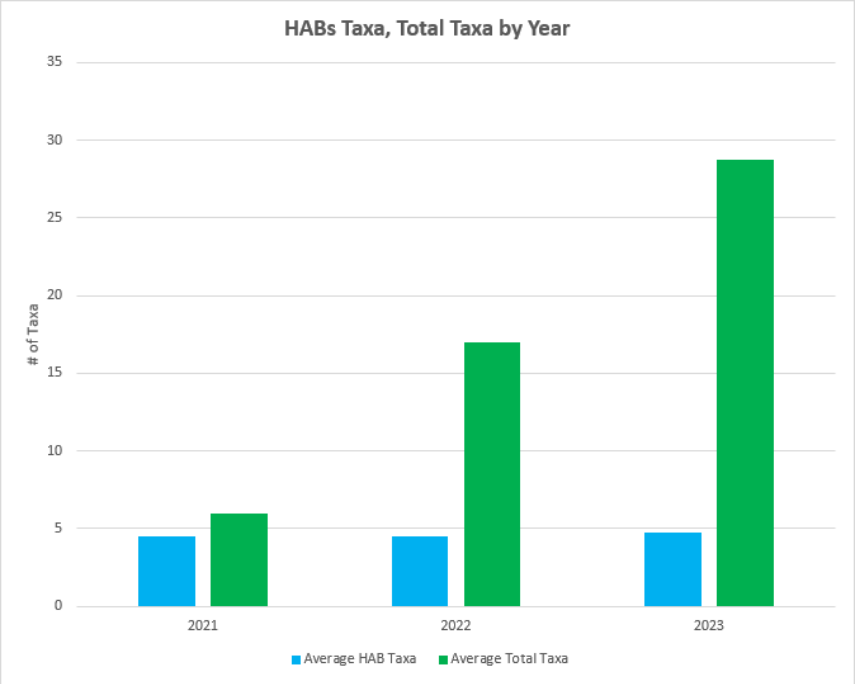
Step 1: Increase biodiversity – in other words re-establish many "good algae" species that can compete against the cyanobacteria in order to displace their dominance.

This created a solid platform for the program to pick up from in 2023.

2023 data is added below. It shows that the total number of different taxa identified increased even more, and these increased levels were maintained throughout the year.



Below this data is summarized into a simple annual average of monthly measures of the number of Total taxa and HAB taxa identified.



This again demonstrates the successful achievement of the first step in the process:

Step 1: Increase biodiversity – in other words re-establish many "good green algae" species that can compete against the cyanobacteria in order to displace their dominance.

3.2 RESULTS - BIOVOLUME

The second aspect of the phytoplankton data measures to what extent the competitors are competing by increasing their nutrient uptake and biovolume at the expense of the cyanobacteria HAB species biovolume.

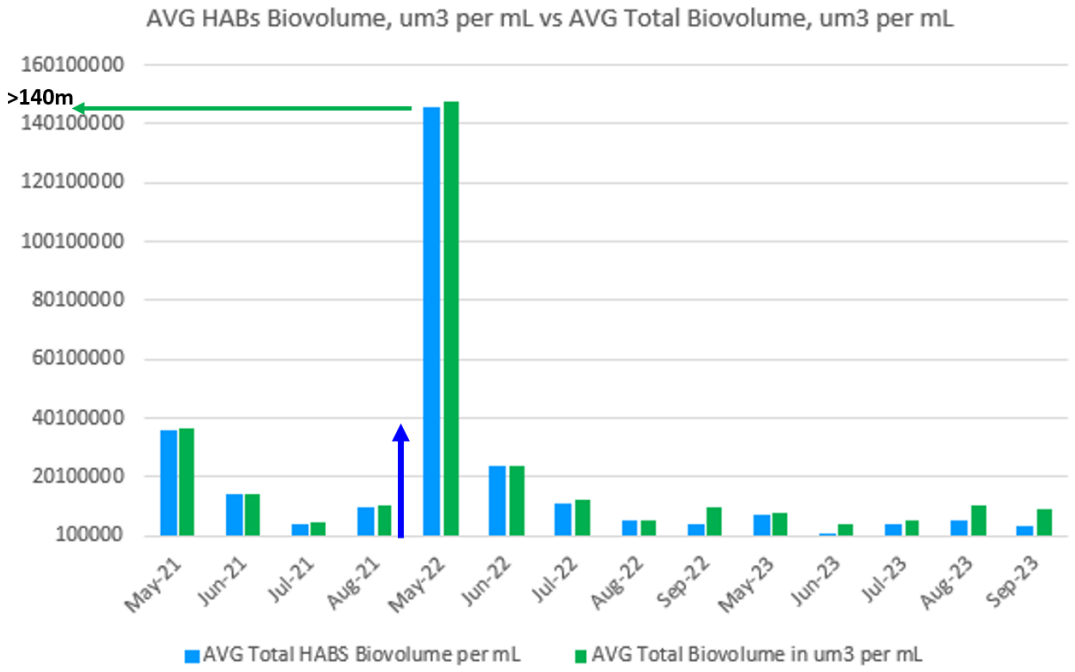
To use the farming analogy again, let's say cows are the equivalent of cyanobacteria, and we started with 100 of them, and the objective was to get rid of the cows (because the price of beef has dropped to nothing). We now have cows, pigs, sheep, and chickens. Then the question is "how much alternative meat do I have to sell?" or "what is the biomass of each type of animal?"

If I now have 99 cows and only a single pig, sheep and chicken, then despite increasing biodiversity or number of taxa, I have not achieved much in terms of shifting biomass away from being primarily composed of beef. However, if I have 50 pigs, 50 sheep, 100 chickens and only 1 cow remaining, then I have accomplished my objective.

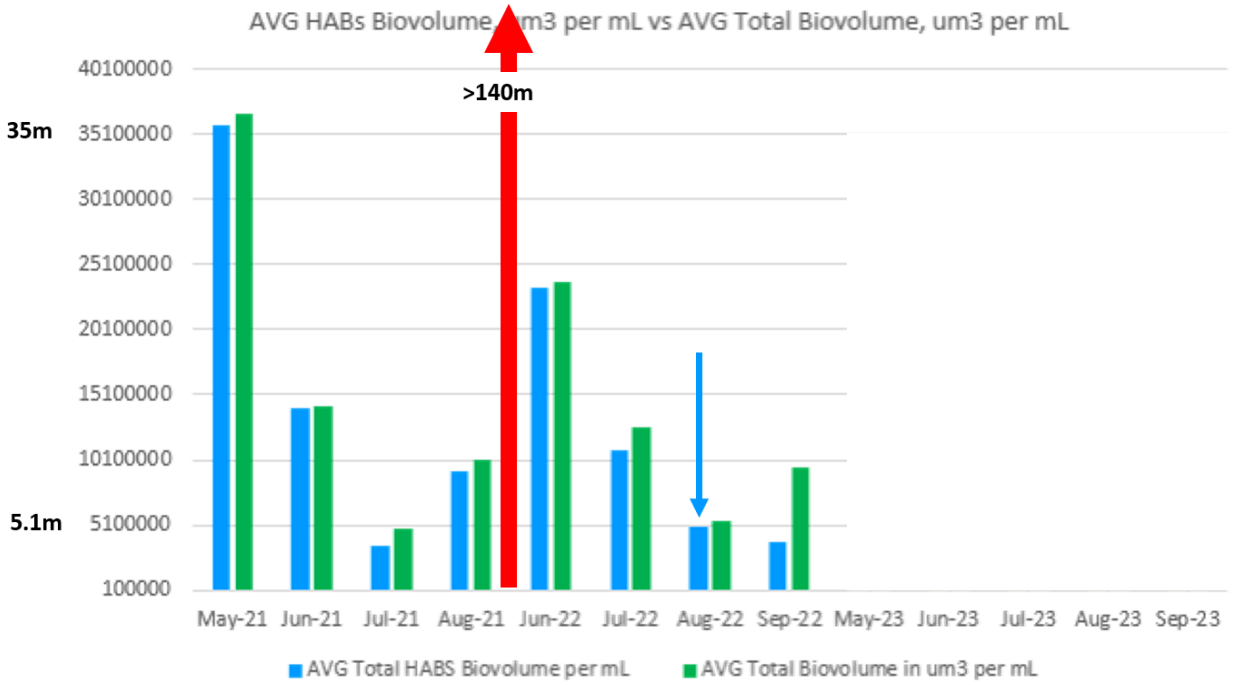
The chart below shows Cyanobacteria HAB biovolume in green and Total phytoplankton biovolume in blue.

The blue arrow shows that when the SIS.BIO program began at the end of April 2022, an intense HAB bloom had already taken hold, with a biovolume of over 140 million units. In comparison, in May of the previous year, the biovolume was about a quarter as much at just over 35 million units.

It is clear that after May 2022, biovolume was significantly reduced, but because the vertical scale in this chart has to allow for the massive bloom in May 2022, the columns after that are so small that an objective perspective for analysis cannot be obtained.



Therefore, in the chart below, May 2022 data is considered an outlier and has been removed to allow a change of vertical scale and a more detailed analysis and scrutiny of performance.

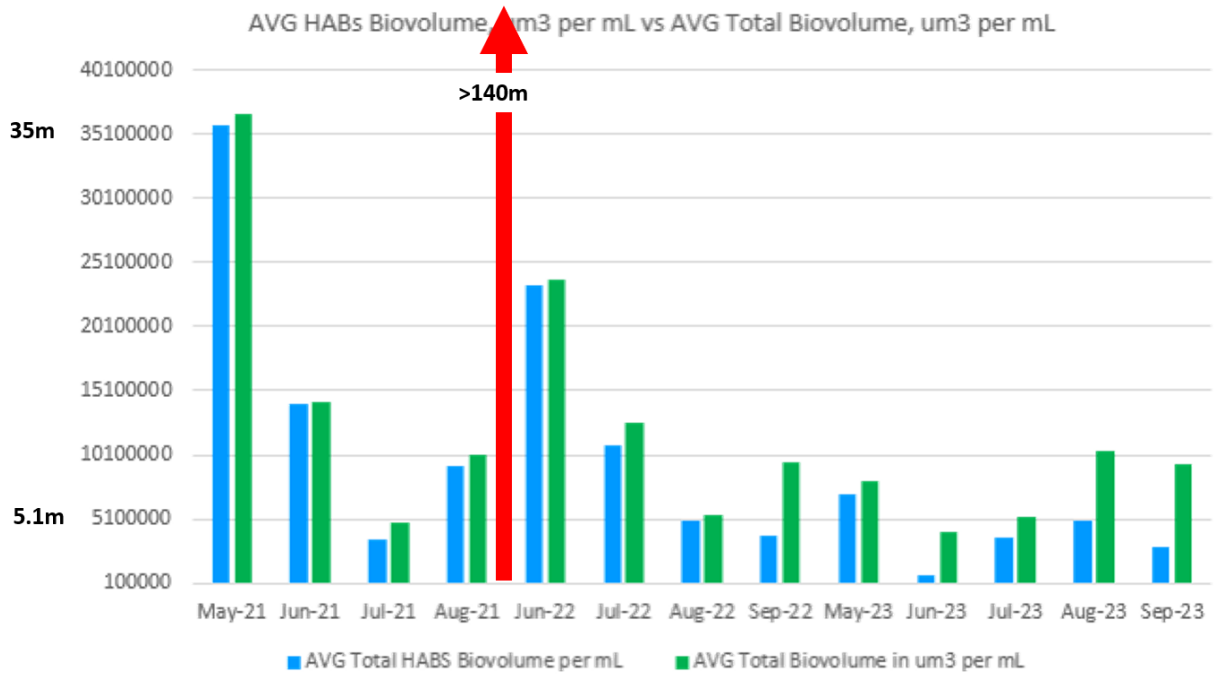


With the outlier HAB bloom of May 2022 removed, we can see that the vertical scale now only ranges up to about 35 million units (the level in May 2021), and it was over 95% cyanobacteria HABs. By comparing June and July 2021 to 2022, we can see that it took until August 2022 before the effects of that bloom had been overcome and the total biovolume in the lake was lower than

the equivalent month the previous year (blue arrows pointing down). But the cyanobacteria HAB percentage of total biovolume remained above 90%.

However, with reduction in total biovolume accomplished, in September 2022 the competitor "good green" algae began to compete very effectively and the cyanobacteria HAB biovolume percentage dropped to 40% of the total. This laid a good foundation for 2023.

The chart below adds 2023 biovolume data and shows that in May 2023, total phytoplankton biomass was about 8 million units compared to 140 million in 2022 and 35 million in 2021. As the year progressed, cyanobacteria HAB biovolume was kept below 5m units as total biovolume increased toward 10m units as "good green" algae continued to compete effectively and displace the cyanobacteria from their dominant position.



Worthy of note is the fact that after several big stormwater (and nutrient) inflows in August 2023, the subsequent surge in biovolume was predominantly Non-HAB "good green algae", showing that these species are competing effectively against the cyanobacteria HAB species.

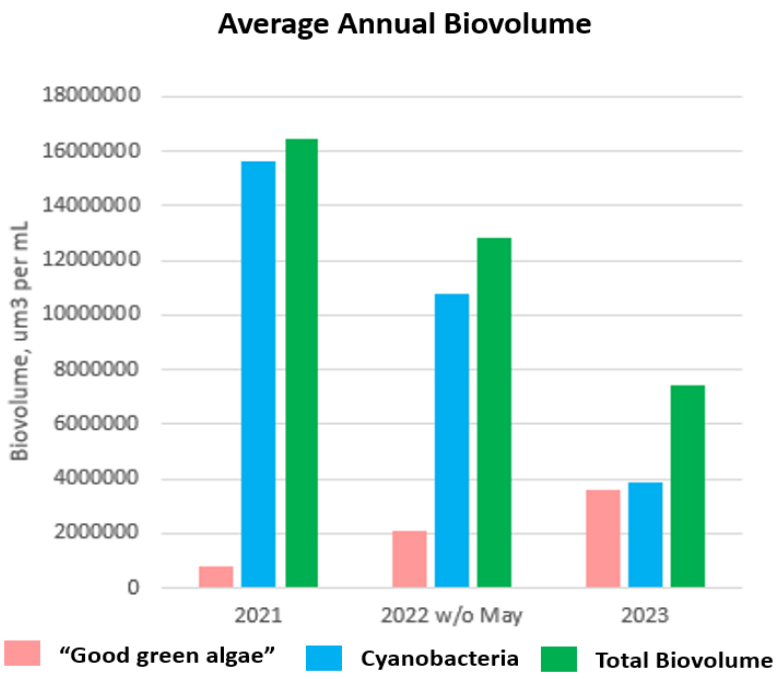
The chart below shows the average biovolume calculated across all samples taken each year. The HAB bloom in May 2022 at the start of the program has a disproportionate impact on the annualized data, so again this one data set has been removed in order to provide a more meaningful understanding of the trends.

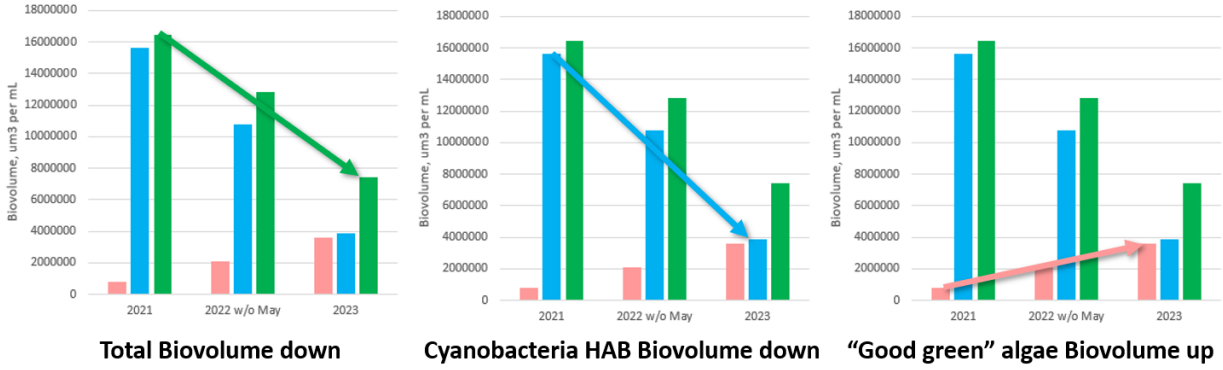
It shows the Total Phytoplankton biovolume (green) and breaks this down into Cyanobacteria biovolume (blue) and "good green" algae biovolume (pink).

What we want to see is

- Reduction in total biovolume
- A shift in the balance away from cyanobacteria towards competing "good green" algae.

This is what the data in the chart shows.





3.3 RESOLVING THE CHLOROPHYLL-α / SECCHI DEPTH ANOMALY

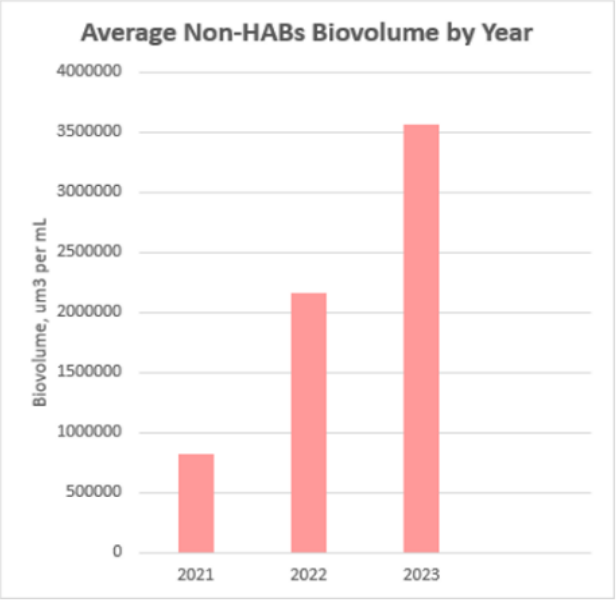
Returning to the apparent anomaly of the increase in Chlorophyll-a highlighted in the RLS water quality report: If the Total Biovolume of phytoplankton is going down, why are the chlorophyll-a levels going up?

Dr Jennifer Jermalowicz-Jones of RLS has explained that chlorophyll-a is primarily a measure of "good green" algae. Cyanobacteria's equivalent photosynthetic chromophore is called phycocyanin. That was not included in the parameters measured.

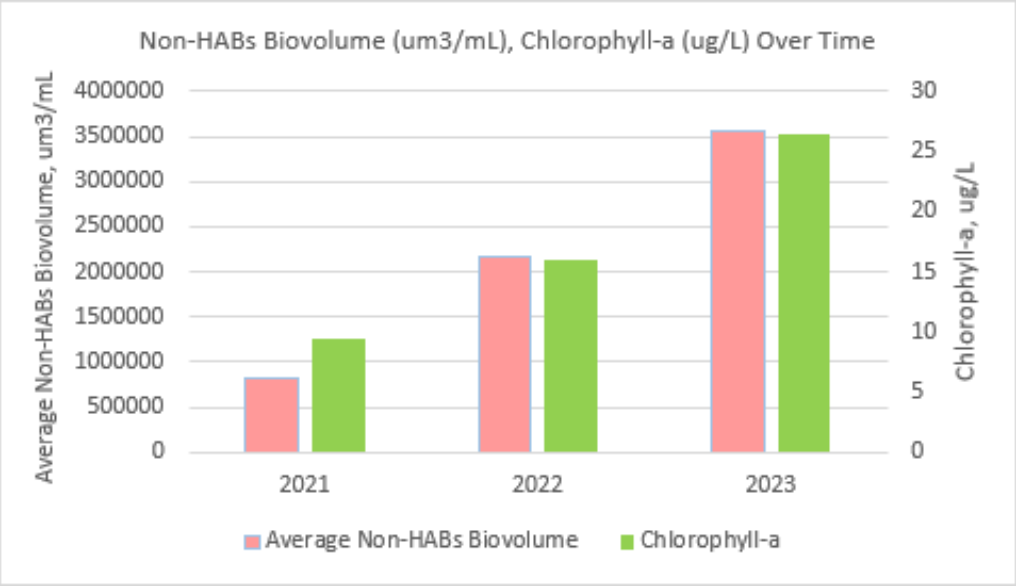
To understand the apparent anomaly, we need to examine how much non-cyanobacteria HAB (ie "good green") algae there is. For a given biovolume of phytoplankton, if 99% of it is cyanobacteria

and only 1% of it is "good green" algae, (as was the case at the start of the program), then the chlorophyll-a level will be low. But if we have exactly the same phytoplankton biovolume, but 1% is cyanobacteria and 99% is "good green" algae, then we would expect the chlorophyll-a level to be 99 times higher.

The chart below shows the Non-HAB or "good green" algae biovolume. It has increased fivefold since 2021. The "good green" algae is now competing effectively against the cyanobacteria.



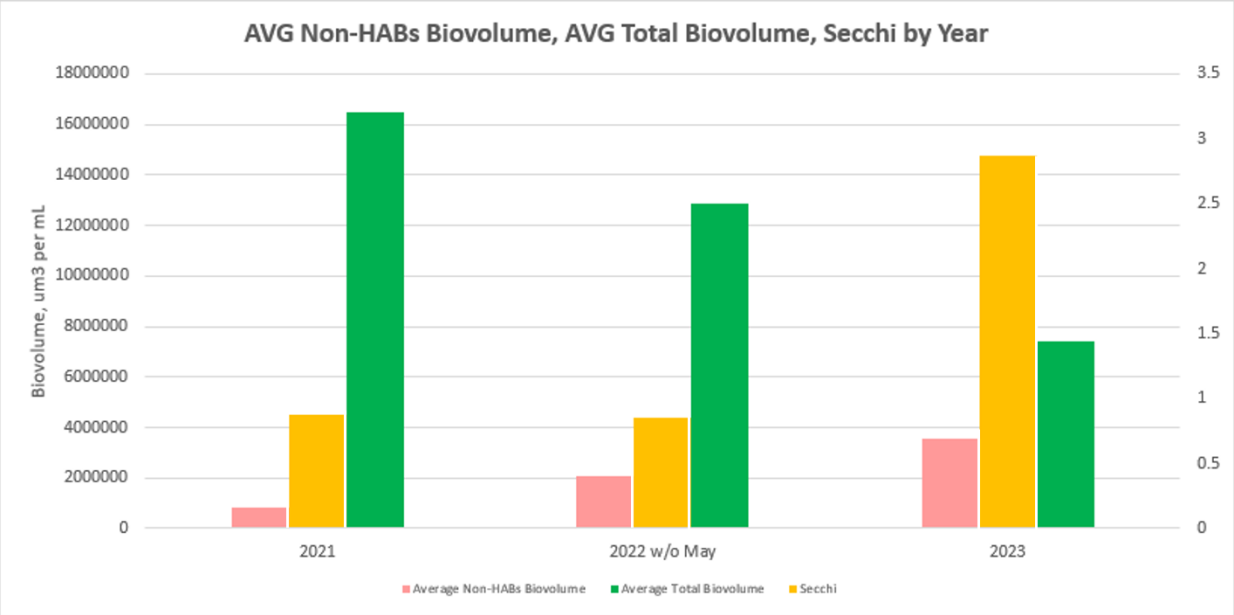
The chart below shows the average chlorophyll-a levels each year alongside the "good green" algae levels from the previous chart, and there is a high correlation between the two.

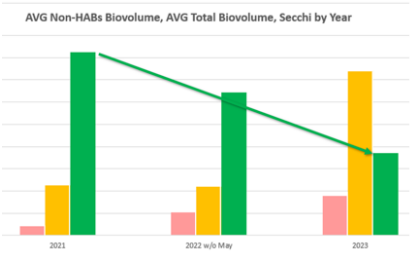


The increase in chlorophyll-a levels is a positive change because it reflects the shift away from cyanobacteria HAB taxa in favor of "good green" algae competitors. This demonstrates the successful achievement of the second step in the process:

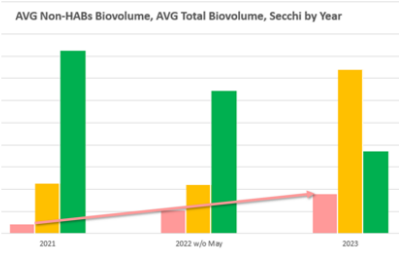
Step 2: Enable the "good algae" to increase in quantity so that the cyanobacteria are marginalized and displaced from dominance.

The chart below shows that although Non-HAB or "good green" algae biovolume has increased, Total Biovolume has decreased, which means the clarity of the water has improved as reflected in the increased Secchi depth visibility. Hence the apparent anomaly is resolved.

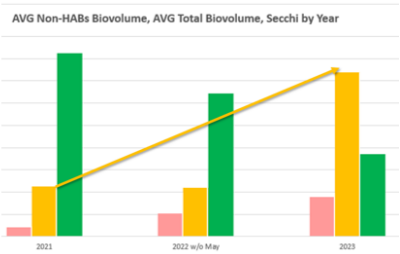




Total Biovolume decrease



Non-HAB Biovolume increase



Secchi visibility increase

This confirms progress in terms of the third step in the process:

Step 3: Reduce the overall total phytoplankton biovolume.

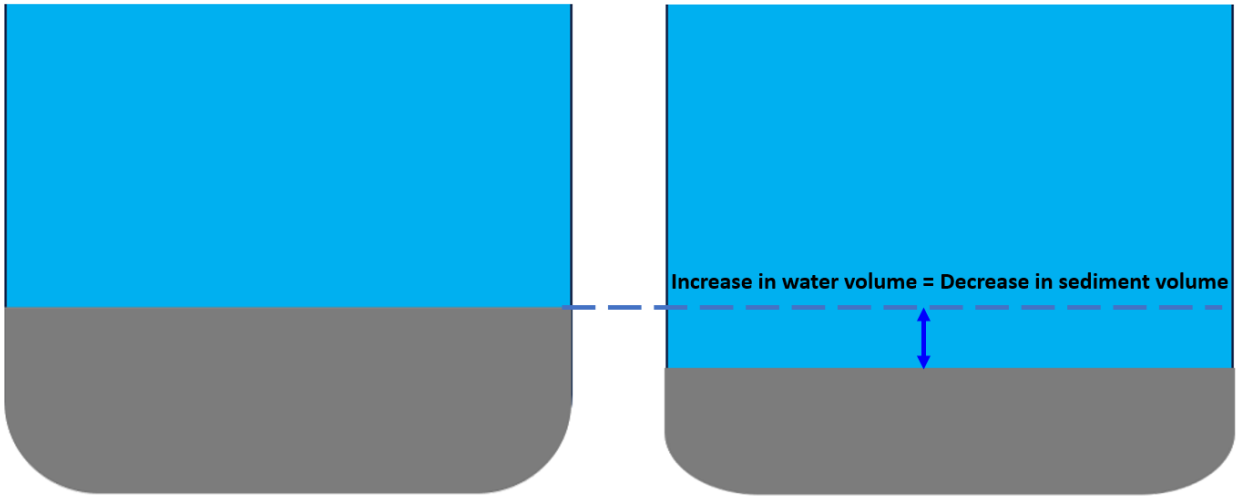
4. BIO-DREDGING OF NUTRIENT-RICH ORGANIC SEDIMENT

Bio-Dredging is the process of deepening a lake by removing organic sediment from the bottom through accelerated aerobic digestion. The objective of Bio-Dredging is to "level the playing fields" between "good green" algae and toxic cyanobacteria by depleting the sediment nutrient stockpiles that cyanobacteria are adapted to exploit. Using their competitive advantage to access the nutrient stockpile, they outcompete "good green" algae and produce HABs.

Bathymetry allows a detailed depth profile to be created and the total volume of water in the lake to be calculated and compared year to year. This provides an objective measure of the reduction of organic sediment achieved through Bio-Dredging.

If the lake level differs at the time of the bathymetric scan from year to year, the results are equalized to a fixed benchmark recorded at the time of the original scan. The software that analyses the depth and volume takes into account this benchmark.

The key metric to evaluating the result of Bio-Dredging is the increase in the lake's volume. The volume that the lake gains is equal to the amount of sediment that has been removed.



Volume: Increase in Volume of Water = Decrease in Organic Sediment Volume

The initial scan of Indian Lake took place on July 10, 2019. Data from this scan established baseline conditions against which data from subsequent scans during the project would be compared. In 2019, the lake had an average depth of 15.4 feet and maximum depth of 43.8 feet. The volume was 4,619 acre-feet (1.505B gallons, or 7,451,987 cubic yards).

By 2021 when the RADOR system was installed, an additional 130,680 cubic yards had accumulated since 2019, reducing the water volume by 26,392,133 gallons.

At the time of the October 2022 survey, 551,760 cubic yards of sediment had been Bio-Dredged away compared to 2019, and 421,080 cubic yards compared to 2021, as shown below.

INDIAN LAKE, CUBA, MO				
Description	2019	2022	2023	2019-2023 Change
Volume (ac-ft)	4,619	4,880	4,857	238
Volume (gal)	1,505,003,227	1,590,044,544	1,582,550,482	77,547,255
Cu.yd Sediment Added (Removed)		(421,080)	37,107	(383,973)
Avg Depth (ft)	15.4	15.7	15.6	0.20
PAC	4.80%	1.50%	1.00%	-3.80%
BVw	0.40%	0.10%	0.10%	-0.30%

The 2023 scan took place on October 18. The lake as a whole has shown no significant change when compared to 2022, although the improvements compared to 2019 and 2021 have been maintained.

The lake currently has an average depth of 15.6 feet and maximum depth of 43.3 feet. The volume is 4,857 acre-feet (1.582B gallons).

The changes between 2019 and 2023 reflect an increase in average depth of the whole lake of 0.3 feet (4 inches), and an increase in volume of 238 acre-feet (66.6M gallons or 383,973 cubic yards).

The increase in water volume is due to the digestion and Bio-Dredging of nutrient-rich organic sediment and is equivalent to the removal by physical dredging of 383,973 cubic yards.

In August there were severe storm events and large volumes of water inflow that saw lake levels reportedly rise over 12 inches in a matter of 48 hours or so. Our lake management team undertook spot scans of some coves and noted that there appeared to have been an inflow of new sediment. A surge in phytoplankton growth was noticed, but as noted above, this seems to have had the positive effect of giving the “good green algae” the opportunity to turn the tables on the cyanobacteria. The growth in biovolume of good algae showed that conditions favor their ability to outcompete cyanobacteria and take a significant step towards undermining their dominance.

What was not immediately apparent from the spot checks in August this year was to what degree the additional sediment from stormwater was organic or inorganic in nature.

In 2023 the dosing program for digestive enzymes that achieve the Bio-Dredging was targeted at the cove areas, since this is where community members are most affected by mucky, foul smelling sediment.

Community members living on coves have reported the reduction in mucky sediment this year and commented that when wading around docks bare footed, sandy bottom can now be felt again rather than slimy black sludge.

4.1 ANALYSIS OF BATHYMETRY OF COVES

In this year's analysis, the lake was broken down into ten different areas so that the impact on the coves could be more specifically analyzed. (Cove 3 is included with Star Dock as it is very small).

By way of explanation of each table below, with reference to the Cove 1 table, it shows, when comparing 2019 with 2023:

- Increase in water volume (decrease in sediment) of 8.5 acre-feet, a 22.2% improvement.
- Increase in average water depth of 1.34 feet, a 22.3% improvement.
- Reduction in vegetative biovolume % (percentage of water column occupied by aquatic plants and weeds) from 11.32% to 5.24%, a 46.3% improvement.
- Reduction in the area of the bottom covered by aquatic plants and weeds from 9.03% to 0.46%, a 94.9% improvement.

The fact that the vegetation biovolume has reduced by more than the vegetation cover shows that there has been a net shift from taller plants (which tend to be invasive species such as *Curly Leaf Pondweed* or *Eurasian milfoil*) to shorter plants, which tend to be indigenous, more desirable species.

COVE 1 - 6.35 Acres				
Description	2019	2023	2019-2023 Change	% Change
Water Volume (acre ft)	38.21	46.71	8.50	22.2%
Water Depth Avg (ft)	6.01	7.35	1.34	22.3%
Vegetation Avg BV (%)	11.32	6.08	-5.24	-46.3%
Vegetation Cover (%)	9.03	0.46	-8.57	-94.9%

COVE 2 - 5.55 Acres				
Description	2019	2023	2019-2023 Change	% Change
Water Volume (acre ft)	47.27	54.44	7.17	15.2%
Water Depth Avg (ft)	8.52	9.81	1.29	15.1%
Vegetation Avg BV (%)	5.79	5.61	-0.18	-3.1%
Vegetation Cover (%)	4.62	0.42	-4.20	-90.9%

COVE 4 - 1.71 Acres				
Description	2019	2023	2019-2023 Change	% Change
Water Volume (acre ft)	9.00	9.67	0.67	7.4%
Water Depth Avg (ft)	5.27	5.66	0.39	7.4%
Vegetation Avg BV (%)	5.02	5.63	0.61	12.2%
Vegetation Cover (%)	0.05	0.68	0.63	1260.0%

COVE 5 - 2.67 Acres				
Description	2019	2023	2019-2023 Change	% Change
Water Volume (acre ft)	15.87	16.89	1.02	6.4%
Water Depth Avg (ft)	5.95	6.33	0.38	6.4%
Vegetation Avg BV (%)	8.04	5.57	-2.47	-30.7%
Vegetation Cover (%)	1.67	0.37	-1.30	-77.8%

COVE 6 - 2.84 Acres				
Description	2019	2023	2019-2023 Change	% Change
Water Volume (acre ft)	16.73	18.52	1.79	10.7%
Water Depth Avg (ft)	5.90	6.53	0.63	10.7%
Vegetation Avg BV (%)	10.66	5.45	-5.21	-48.9%
Vegetation Cover (%)	16.18	0.39	-15.79	-97.6%

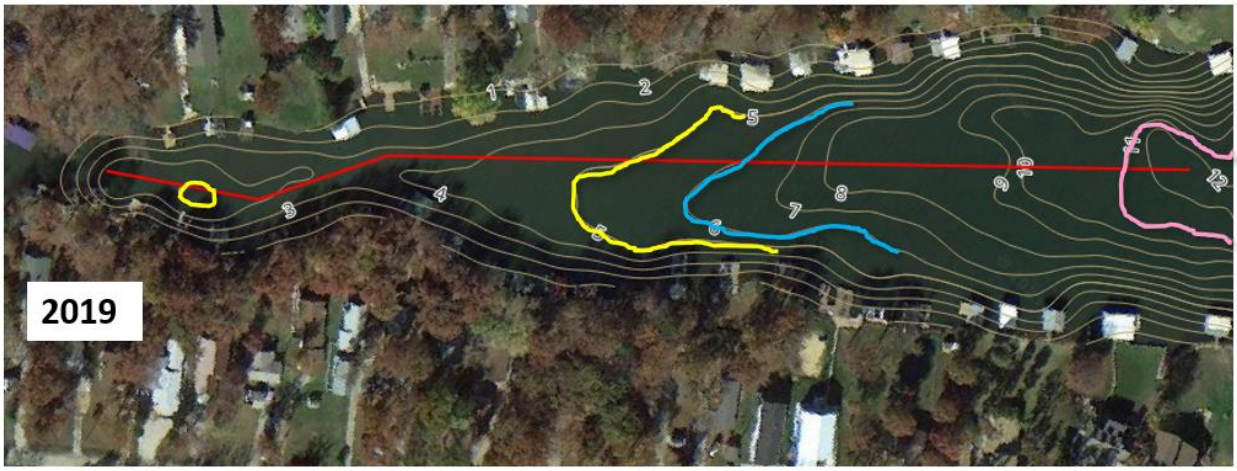
COVE 7 - 9.84 Acres				
Description	2019	2023	2019-2023 Change	% Change
Water Volume (acre ft)	59.37	68.77	9.40	15.8%
Water Depth Avg (ft)	6.03	6.99	0.96	15.9%
Vegetation Avg BV (%)	7.03	5.78	-1.25	-17.8%
Vegetation Cover (%)	39.39	0.67	-38.72	-98.3%

Cove 7 Detail

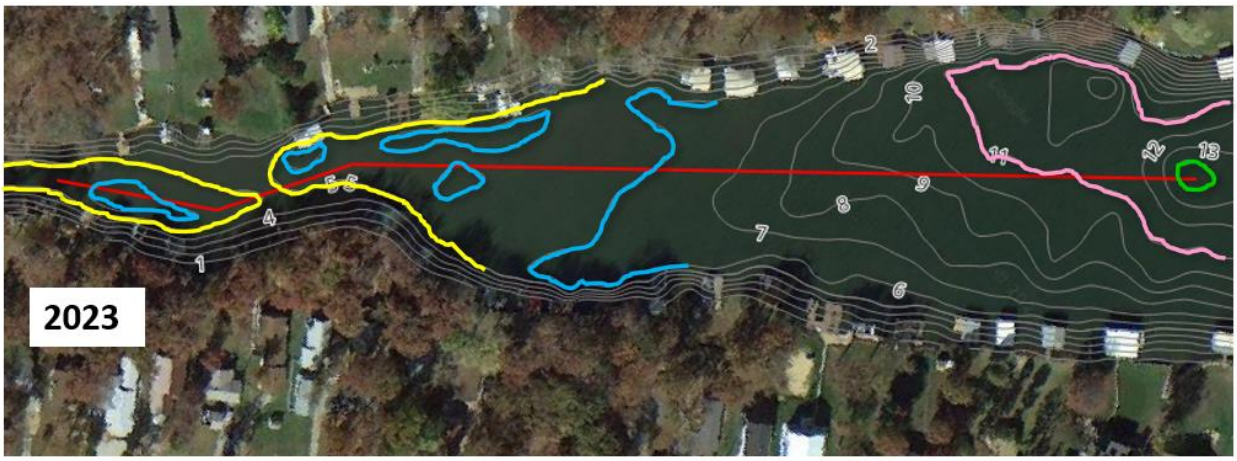
To illustrate how the data in the tables above and the depth profiles generated from bathymetric scanning are related to each other, below are the depth charts for Cove 7 from 2019 and 2023.

The 5 -foot contour (yellow) has pushed well up into the cove and the 6-foot contour (blue) has followed it, opening up new contours. The way that contours of 5 feet or more have opened up more widely in the cove explains why people are observing and experiencing the difference in the feel of the bottom when wading bare foot near their docks.

The 11-foot contour (pink), which just barely reached into the cove in 2019, has also expanded significantly and 12, 13 and even 14-foot (green) contours are now present.

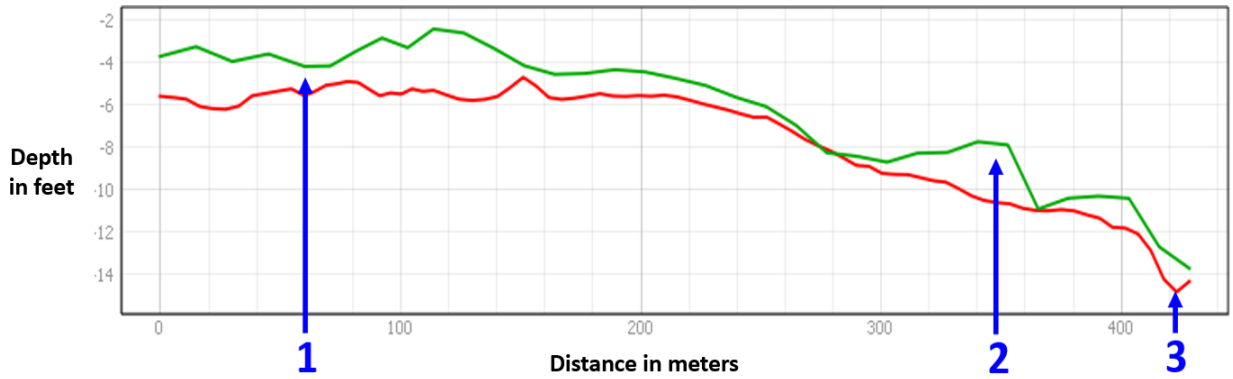


5 feet 6 feet 11 feet



5 feet 6 feet 11 feet 14 feet

Below, the cove is shown in profile along the red transect line in the contour maps above. The green line shows the profile of the bottom of the cove in 2019, the red line shows it in 2023. The vertical axis shows depth in feet and the horizontal axis shows meters.



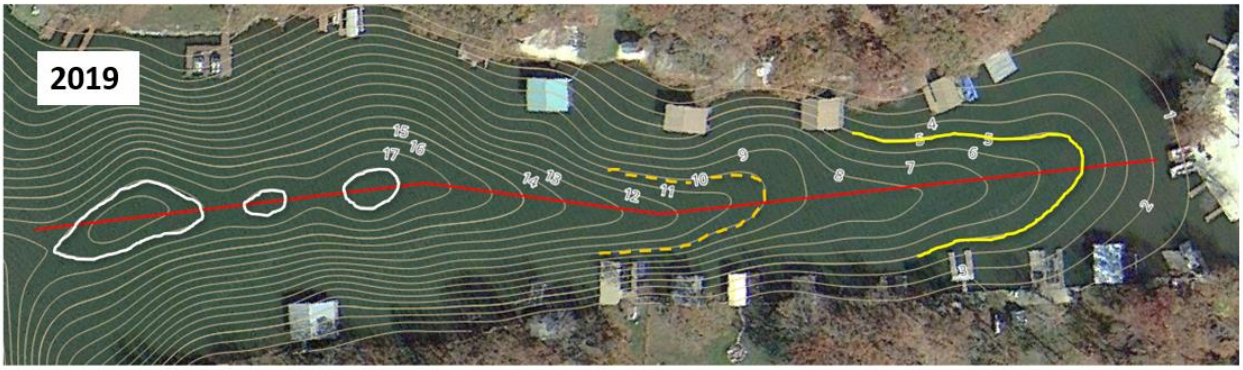
It can be seen that at the shallow end of the cove, (blue arrow 1) depth has increased from about 4 feet to 6 feet.

Around 350m (1,150 ft) from the shallow end it can be seen (blue arrow 2) that areas that were previously 8 feet deep are now 11 feet, and a small green 14-foot hole at about 400m (1,300 ft) can be seen (blue arrow 3).

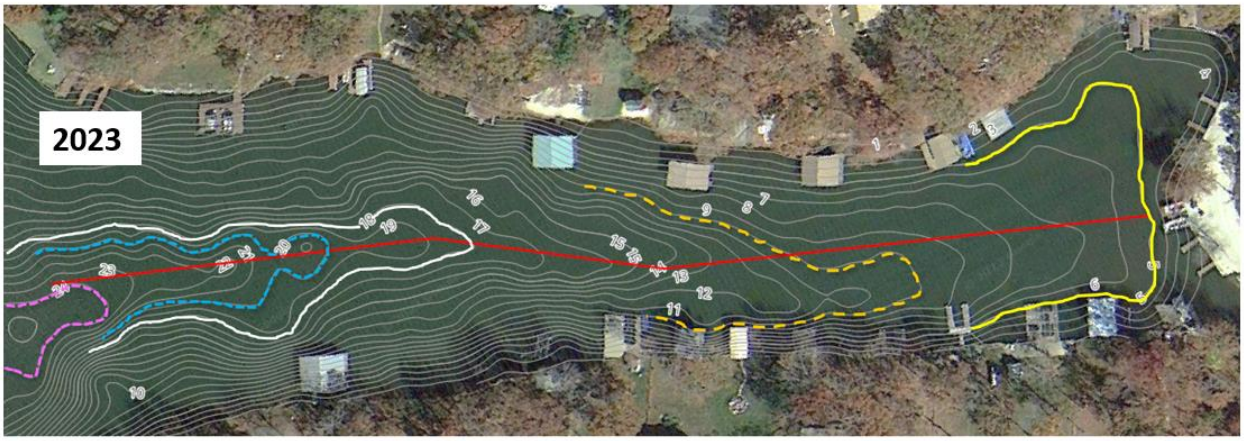
Cove 2 Detail

The 5 -foot contour (yellow) has pushed well up into the cove and expanded considerably, as has the 10 foot contour.

There were three small contours at 18 feet deep in 2019, but now this contour covers a much expanded contiguous area, and behind it a 20 foot and a 24 foot contour has opened up.

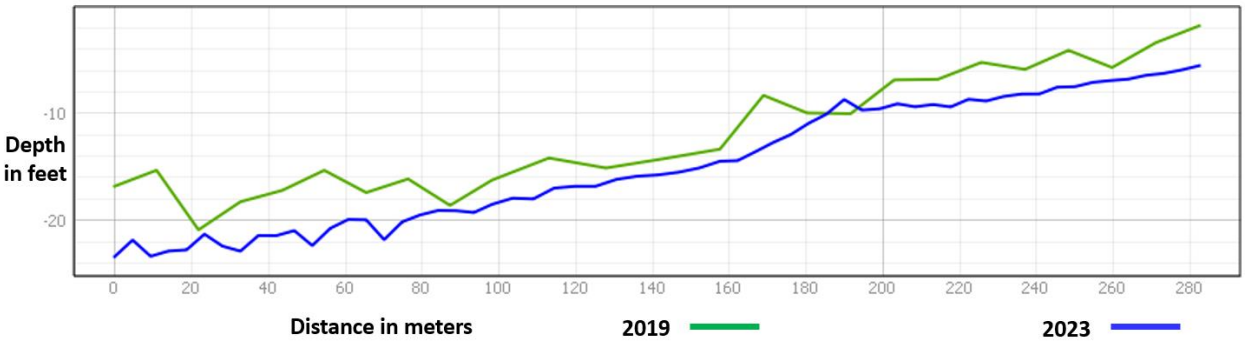


18 feet 10 feet 5 feet



24 feet 20 feet 18 feet 10 feet 5 feet

These same changes can be discerned in the profile view below.



4.2 AQUATIC VEGETATION & INVASIVE WEEDS

Invasive weeds were never a major problem at Indian Lake, largely because of opacity of the water. This opacity was due to high levels of phytoplankton, which prevent light that otherwise would enable weeds to germinate and begin to grow from penetrating to the bottom.

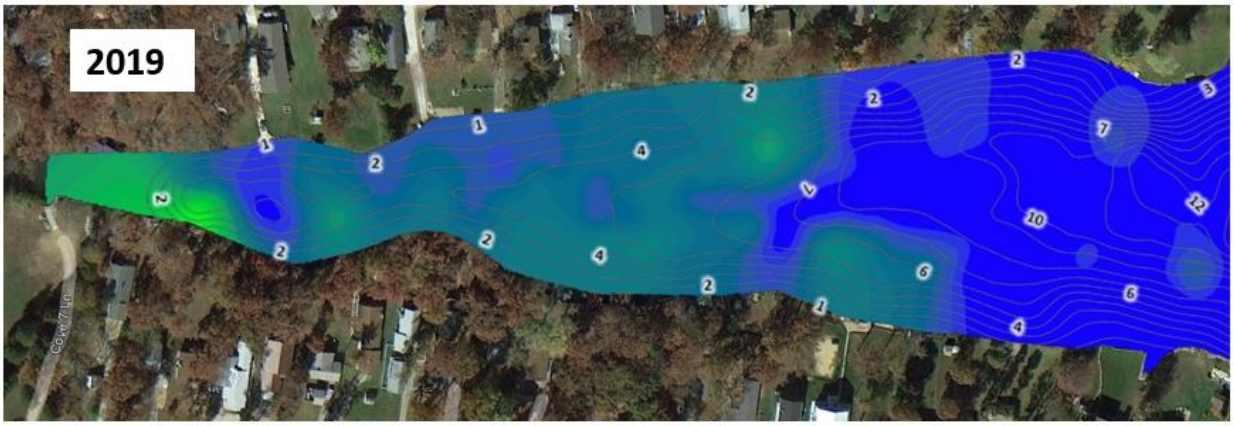
One risk as the remediation program successfully reduces algae biovolume and increases the clarity of water is that weeds may be able to take advantage and spread as light penetration increases.

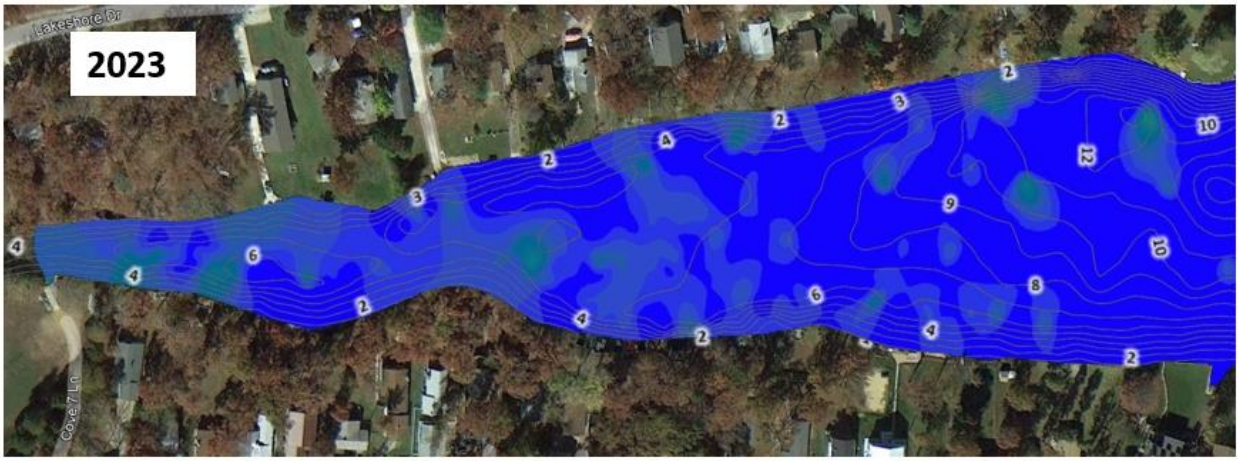
Our program counters this potential adverse effect by digesting and bio-dredging away nutrient-rich, mucky organic sediment. This deprives invasive aquatic weeds of their rooting beds and despite better photosynthetic conditions, prevents further invasion.

The bathymetric data presented above shows that the reduction of nutrient-rich sediment has been achieved, making rampant growth of vegetation unlikely.

Bathymetric analysis also detects the biovolume of aquatic vegetation present, in terms of the surface area covered and volume of the water column that the vegetation occupies.

In the pictures below this aquatic plant vegetation is depicted by the green coloration.



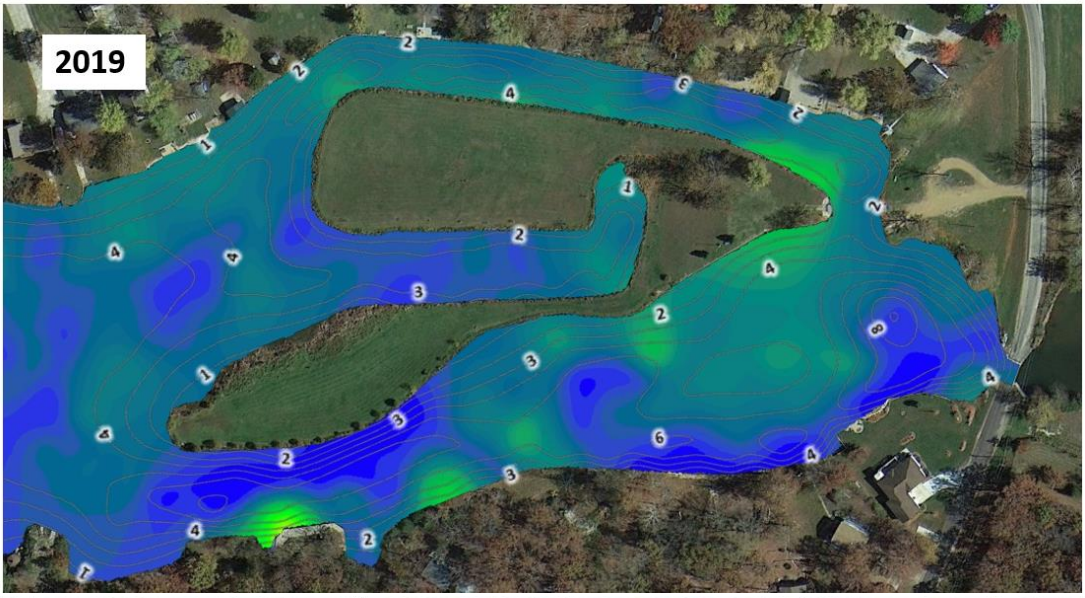


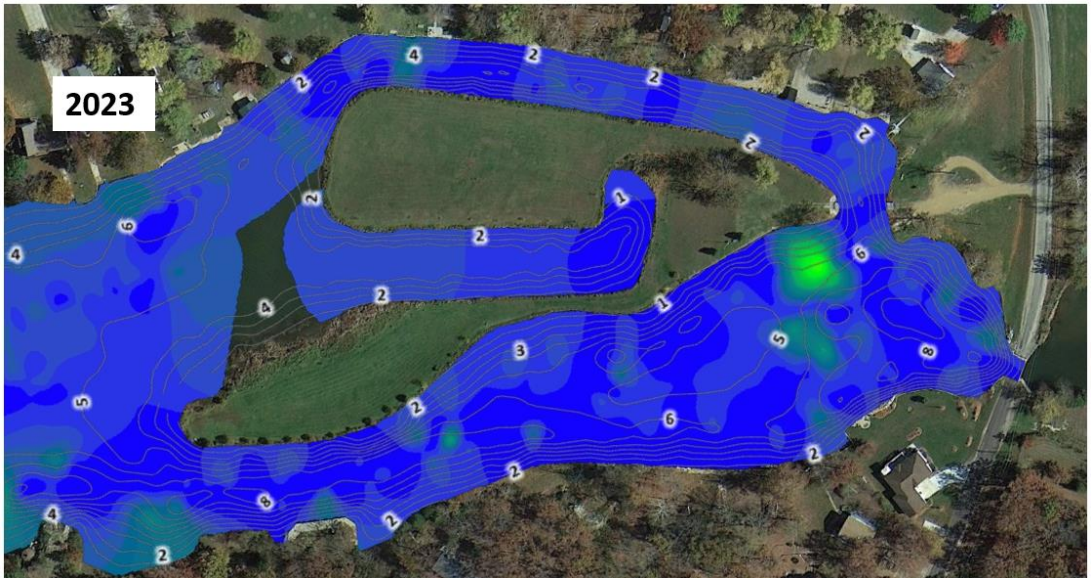
It is clear that despite the increased clarity and light penetration, sediment Bio-Dredging has ensured that invasive weed proliferation has not increased.

COVE 7 - 9.84 Acres				
Description	2019	2023	2019-2023 Change	% Change
Water Volume (acre ft)	59.37	68.77	9.40	15.8%
Water Depth Avg (ft)	6.03	6.99	0.96	15.9%
Vegetation Avg BV (%)	7.03	5.78	-1.25	-17.8%
Vegetation Cover (%)	39.39	0.67	-38.72	-98.3%

The bottom two lines in the table above show the volume of the water column taken up by aquatic plants (Vegetation Avg BV%) and the area of the bottom covered by aquatic plants (Vegetation Cover %).

Similarly, the southern area of the lake shows a marked reduction in invasive weed vegetation.





5. SUMMARY

The stated objectives of the remediation program are to prevent HABs and reverse eutrophication:

1. The most urgent objective was to **prevent the persistent toxic cyanobacteria HABs** that had occurred from 2020. Not only are these a threat to human and pet health, but they also cause property values to drop.
2. Thereafter an on-going program to **reverse eutrophication for sustainable remediation and restoration** was required, to ensure that Indian Lake stays healthy for generations to come.

The Steps to achieving Objective 1 are:

Step 1: Increase biodiversity – in other words re-establish many "good algae" species that can compete against the cyanobacteria in order to displace their dominance.

Step 2: Enable the "good algae" to increase in quantity and outcompete cyanobacteria, so that the cyanobacteria are marginalized and displaced from dominance.

The achievement of these two steps ensures the prevention of cyanobacteria HABs.

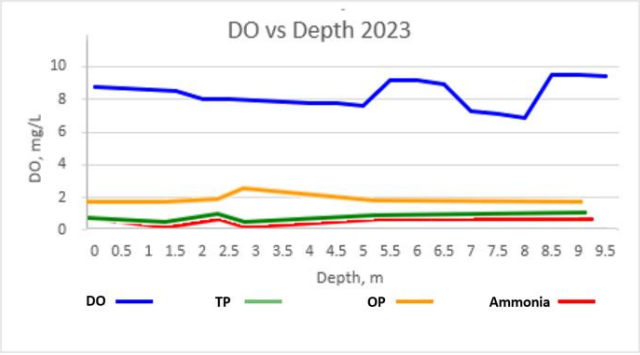
Step 3: Reduce the overall total phytoplankton biovolume.

The three key metrics by which progress towards, and achievement of, the Objectives can be measured are:

- Oxygenation
- Bio-Dredging of Sediment
- Prevention of HABs

5.1 Oxygenation

RLS data shows that full oxygenation has been achieved. In addition, the objective of oxygenation – control of sediment nutrient recycling (TP, OP and Ammonia) has been achieved.



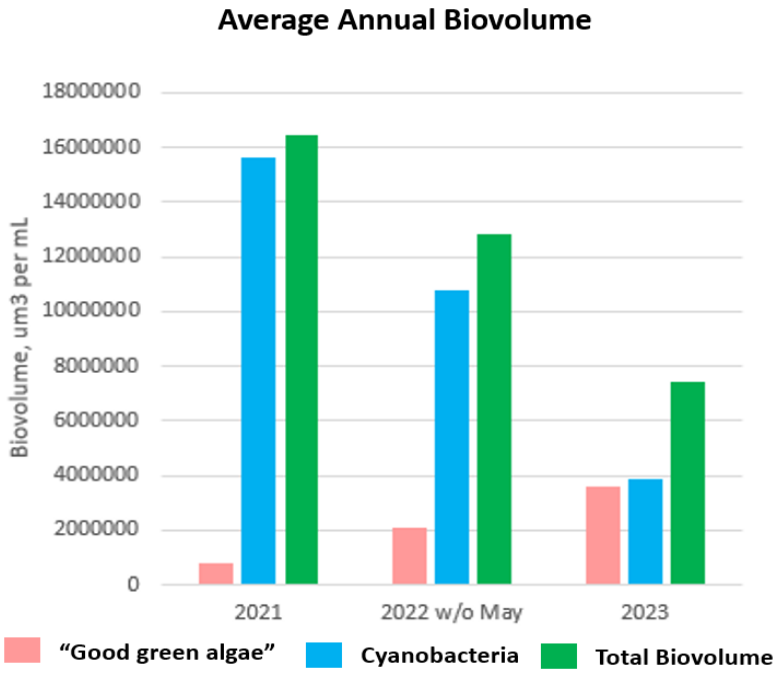
5.2 Bio-Dredging of Sediment

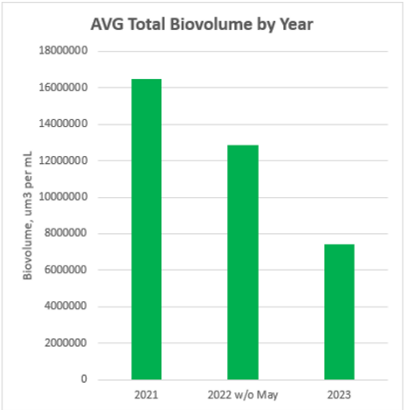
Bathymetric analysis shows that 308,158 cubic yards of organic sediment has been Bio-Dredged from the lake. In 2023 enzyme dosing was all done into cove areas and the data shows this has achieved the desired objectives. In addition, anecdotal reports from residents in the coves state that the reduction of mucky sediment, elimination of foul odors (achieved by oxygenation) and re-exposure of sandy bottom has been noticed and appreciated.

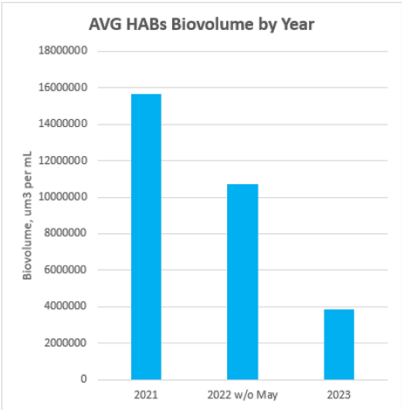
Changes in the nature and quantity of aquatic plants have also been beneficial.

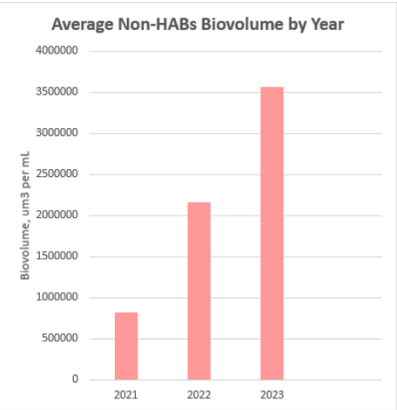
INDIAN LAKE, CUBA, MO			
Description	**2019**	**2023**	**2019-2023 Change**
Volume (ac-ft)	4,619	4,857	238
Volume (gal)	1,505,003,227	1,582,550,482	77,547,255
Cu.yd Sediment Removed		383,973	383,973
Avg Depth (ft)	15.4	15.6	0.20
PAC	4.80%	1.00%	-3.80%
BVw	0.40%	0.10%	-0.30%

5.3 Prevention of HABs









The PhycoTech data shows each year:

- Average total phytoplankton biovolume has reduced
- Average HAB biovolume has decreased reduced
- Average non-HAB “good green” competitor algae biovolume has increased.