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Leading the Way to
RENEWABLE WATER

Summary Report – Indian Lake, Cuba, MO

Simplifying the Science

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5 Summary

Definitions:

- **Benthos –** the bottom of a lake
- **Hypoxia –** defined by the EPA as dissolved oxygen (DO) levels below 2.5mg/l, this is too low to support fish and other animal species
- **Anaerobic –** dissolved oxygen (DO) levels below 1mg/l.
- **Aerobic –** dissolved oxygen (DO) levels of 5mg/l or more.
- **Zooplankton –** foundation-level animal life critical to grazing algae in the food chain.
- **Cyanobacteria –** the algae species responsible for toxic Harmful Algae Blooms (HABS)

1. Background

Indian Lake is a 320 acre lake with an average depth of 15 feet and maximum depth of 43 feet. The lake has a history of being hyper-eutrophic and experiencing summer-long toxic cyanobacteria harmful algae blooms (HABs). In April 2022 a comprehensive remediation program commenced to prevent HABs and reverse eutrophication. The program focused on reversing eutrophication and preventing cyanobacteria HABs by eliminating hypoxia, improving phytoplankton dynamics to control cyanobacteria HABs and bio-dredging nutrient-rich organic sediment.

The remediation program is in full alignment with the recommendations of the landmark 2022 report by the U.S. Government Accountability Office (GAO-22-104449) on managing harmful algal blooms and hypoxia.

The GAO report highlighted fundamental shortcomings of conventional lake management practices that focus on chemical treatments such as algaecides and herbicides, and emphasized the need to focus on system-level solutions that address root causes including:

- Hypoxia - Low oxygen conditions in water and sediment disrupt natural nutrient cycling and food webs of the lake ecosystem as a whole. The report emphasized the importance of eliminating hypoxia, especially in bottom waters and sediment.
- Sediment Nutrient Recycling - Accumulated nutrient-rich organic sediments recycle phosphorus and nitrogen to fuel algal blooms and HABs. Therefore reducing sediment nutrient recycling and sediment nutrient stockpiles is key.
- Food Web Dysfunction - Loss of zooplankton, benthic fauna and other grazers due to hypoxia allows more algal growth. Unconsumed phytoplankton dies off and sinks to decompose in the sediment amplifying the feedback mechanisms that drive eutrophication and HABs. Cyanobacteria, which produce toxic HABs do not provide as good nutrition for the food web as algae. The report recommended restoring grazer food webs systemically throughout the whole lake ecosystem.

The GAO report pointed out that while symptomatic treatments like algaecides and herbicides provide temporary cosmetic relief, they exacerbate the problem long-term by increasing nutrient-rich sediments. Furthermore, because such interventions are reactive, they can only be used after eutrophication has taken hold and symptoms such as HABs events have occurred, meaning that they signify failure to effectively prevent hypoxia and HABs.

Clean-Flo's remediation program utilizes SIS.BIO's **ONE** Biotechnology, utilizing a **R**apid **A**cting **D**issolved **O**xygen **R**estoration (RADOR) system and biological augmentation program aimed at managing sediment nutrient recycling and restoring food web capacity and function.

The performance of the program is therefore measured by monitoring:

- Hypoxia elimination to facilitate nutrient management and restoration of the food web
- Restoration of phycological balance (phytoplankton) and control and prevention of cyanobacteria HABs.
- Bio-Dredging of sediment nutrient stockpiles to control nutrient recycling.

2. Hypoxia

2.1 Scientific Principles

Hypoxia occurs when dissolved oxygen levels in the water are depleted. Dissolved oxygen (DO) is typically sufficient at the surface but decreases with depth meaning bottom or "benthic" DO levels are deficient. The EPA defines hypoxia as DO levels below 2.5mg/l. Water is anaerobic if DO is below 1mg/l. The objective is to maintain aerobic conditions that support animal life which is defined as DO above 5mg/l.

Eliminating anaerobic conditions is the *sine qua non* of any program to reverse eutrophication and prevent HABs – in other words, without first raising DO levels to at least 5mg/l, nothing else beneficial can happen.

This is because:

- Benthic zooplankton, critical foundation level organisms for a productive food web that ensures nutrient clearance, cannot survive at the benthic margin where water and sediment meet if it is hypoxic.
- The benthic microbial profile shifts to anaerobic bacteria and archaea that decompose organic sediments to produce substances like methane and ammonia which are toxic to animal life.
- An anaerobic benthic margin means that nitrogen is present in the form of ammonia and also raises benthic phosphate levels both of which favor cyanobacteria.
- As hypoxia progressively ascends in the water column through feedback effects, more and more of the water volume becomes uninhabitable for animal life in the food web necessary to consume phytoplankton and maintain nutrient clearance and balance.

Therefore, the first milestone that must be achieved in a program to remediate a water body must be complete oxygenation. Specifically, the aim is to ensure aerobic conditions, (which is a dissolved oxygen level of 5mg/l or greater), all the way down to the benthic margin.

2.2 Analysis of Dissolved Oxygen Data

Benchmark data was collected on the lake in July 2019 and August 2021, (the year before the remediation program began). It showed that below about 18 feet the water was hypoxic (<2.5mg/l DO). Bathymetric analysis shows that means 18% of the water volume and 37% of the sediment surface area is hypoxic.

Below about 22 feet the water was anaerobic (<1.0mg/l DO). Bathymetric analysis shows that this translates into 10% of the water volume and 25% of the sediment surface area being anaerobic. This means that no animal life such as benthic zooplankton can survive there.

The data shows that in the peak summer months of July and August 2022 and 2023 the RADOR system ensured the whole water column was fully oxygenated with DO above 5mg/l to the bottom.

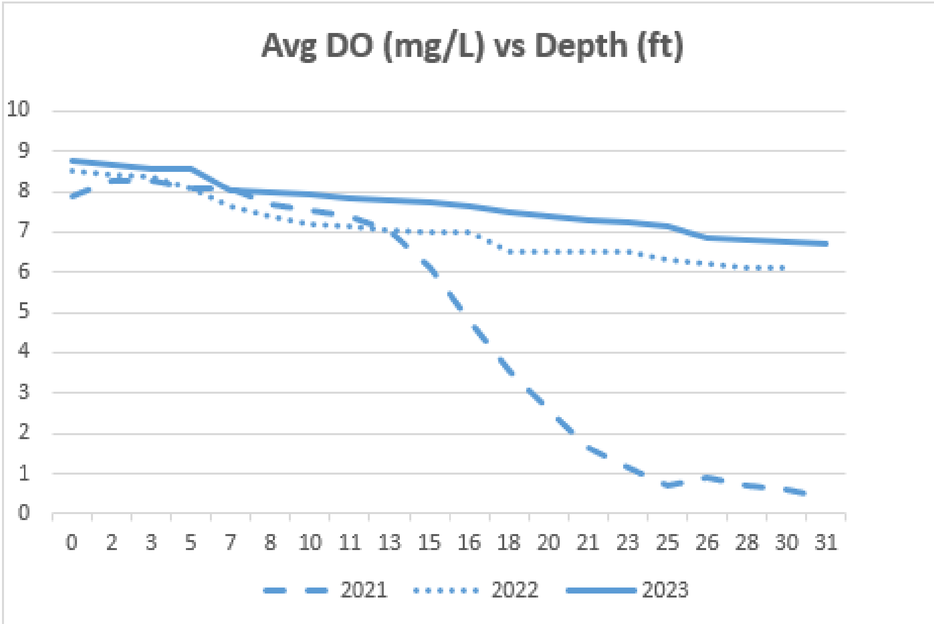


Figure 1

2.3 Dissolved Oxygen and Nutrients

One of the purposes of oxygenation is to suppress nutrient recycling and lower nutrient levels in the water. Before commissioning the RADOR system, there was (as is normally the case) a strong negative correlation between nutrient levels and dissolved oxygen levels. Specifically, when dissolved oxygen levels drop, nutrient levels measured as Total Phosphorus (TP), Orthophosphate (OP) and Ammonia (NH_3) rise.

The graphs below show that nutrient levels increased below 18 feet when dissolved oxygen levels dropped in August 2021.

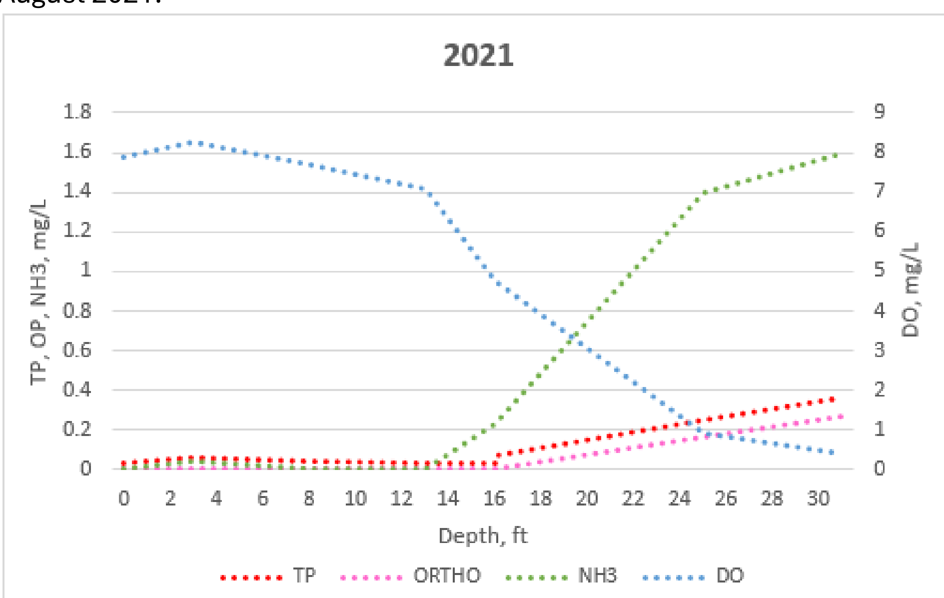


Figure 2

In 2023 when full oxygenation had been ensured, nutrient levels were reduced accordingly.

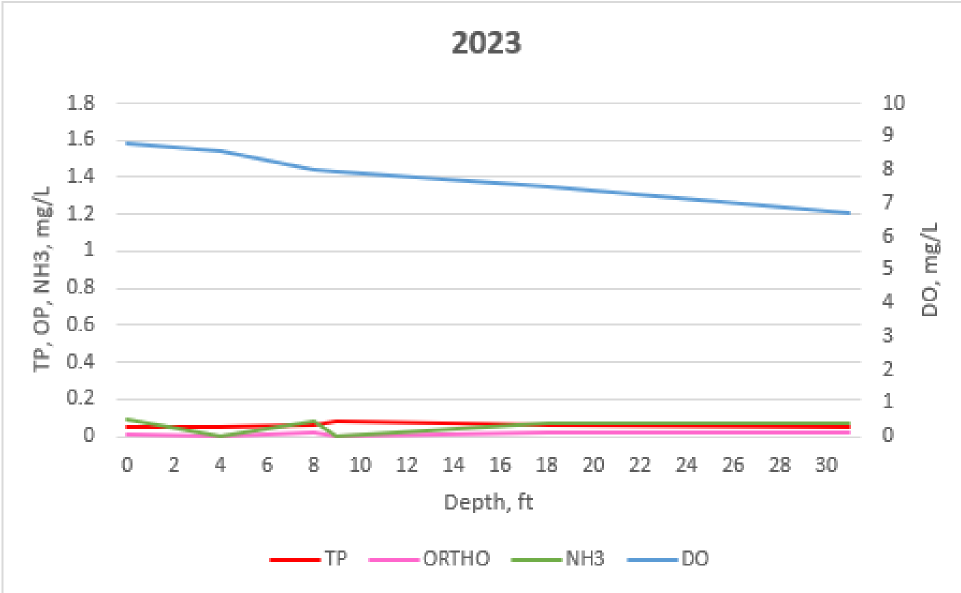


Figure 3

This demonstrates the impact of full oxygenation on nutrient management in eutrophic water bodies.

3. Phytoplankton Management to Prevent HABs

3.1 Scientific Principles

Where cyanobacteria are already dominant in a eutrophic lake and HABs are frequent, (or ever-present during summer, as was the case in Indian Lake) a multi-level approach is needed to restore balance in the taxonomic profile and the quantity of phytoplankton. Analysis of phytoplankton in 2021 (the year before the remediation program started) showed that on average only 6 different types of phytoplankton (or taxa) were present, of which 5 were cyanobacteria taxa. That means that cyanobacteria dominate to such an extent that they have virtually no competition. Because conditions are eutrophic, cyanobacteria dominate nutrient uptake and total phytoplankton biovolume averaged 16m units, (µg per ml) of which 15.5m units or 96% were cyanobacteria.

To restore balance and prevent cyanobacteria HABs the program works on several levels:

1. Increase Phytoplankton Biodiversity: Competition to the cyanobacteria must be created by increasing the biodiversity of phytoplankton present. This is confirmed by an increase in the number of non-cyanobacteria HAB taxa identified.
2. Shift in Balance of Phytoplankton: These competitor algae must compete effectively against cyanobacteria. This is confirmed by a shift in the ratio of cyanobacteria HAB biovolume to "good green algae" biovolume, in favor of the algae.
3. Reduction in Total Phytoplankton Biovolume: Over time, the objective is also to reduce the overall level of phytoplankton in the lake. This is confirmed by a reduction in the total phytoplankton biovolume.

3.2 Increasing Phytoplankton Biodiversity to Create Competition to Cyanobacteria

The graph below shows the average number of cyanobacteria taxa (blue), non-cyanobacteria (or "good green algae") taxa (lime green) and total taxa (green) identified in monthly sampling from May to September. On average, in 2021 on average 6 different taxa were identified of which 5 or 83% were cyanobacteria, and in 2023 28 different taxa were identified of which 5 or 17% were cyanobacteria.

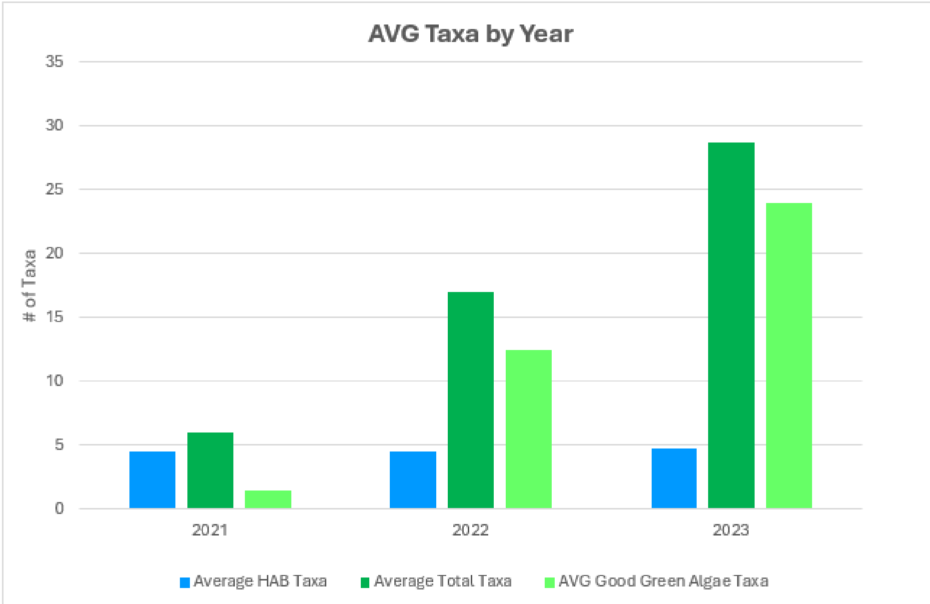


Figure 4

3.3 Improving the Balance and Reducing Total Biovolume of Phytoplankton Populations

In 2021, total phytoplankton biovolume averaged 16m units, of which 15.5m or 96% were cyanobacteria.

In 2023, total phytoplankton bio-volume averaged 7.5m units, of which 3.5m were cyanobacteria. In other words, the cyanobacteria are being outcompeted by the "good green algae" and their biovolume has reduced by over 75% (from 15.5m to 3.5m), as "good green algae" increases.

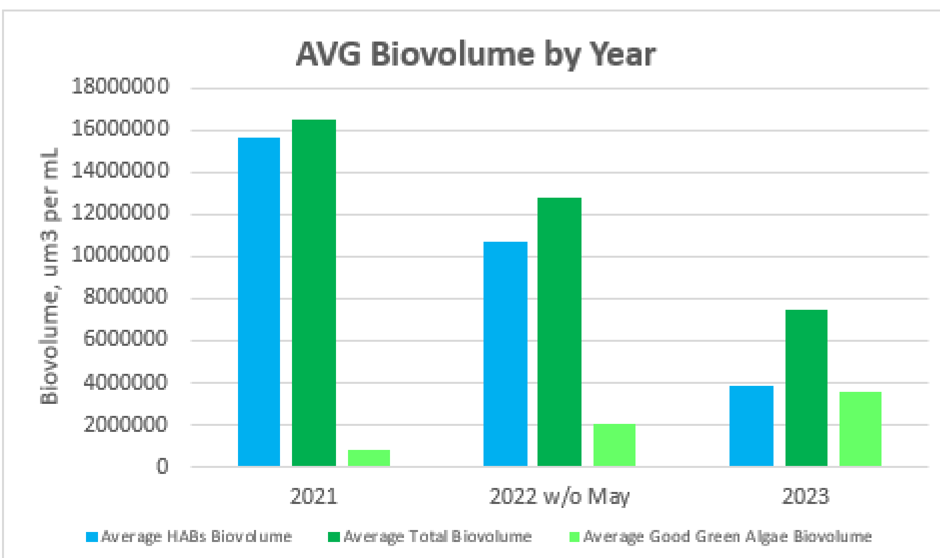


Figure 5

The reduction in total biovolume meant an increase in Secchi depth from less than a foot to 3 feet.

4. Sediment Bio-Dredging

4.1 Scientific Principles

Anaerobic digestion is significantly slower than aerobic digestion, so when sediments are anaerobic the rate of accumulation of new sediment deposits exceeds the rate of digestion and there is a net increase in organic sediment over time.

There are three key principles to understand how Bio-Dredging is achieved.

1. By creating aerobic conditions and providing enzymes to actively digest the sediment, the rate of digestion of sediment is increased so there is a net reduction in sediment.
2. By suppressing and controlling the rate of nutrient recycling, the amount of phytoplankton biomass produced can be limited to a level at which the food web can consume more of it before it dies off, so there is less dead biomass sinking back into the sediment.
3. By maintaining an aerobic benthic margin, benthic zooplankton can function across the whole lake bottom, thus increasing the nutrient clearance capacity of the food web.

So the principle that full oxygenation must be ensured for remediation applies again.

4.2 The Importance of Bathymetry

Sediment nutrient stockpiles are recycled in eutrophic water bodies to fuel excessive growth of invasive weeds, algae, and toxic cyanobacteria HABs. Therefore, any sustainable remediation program must deplete these stockpiles by reducing the amount of accumulated organic sediment.

This is achieved by meeting the oxygen demand necessary for aerobic digestion of the organics and actively boosting that digestion with enzyme supplementation.

Sonar bathymetric scanning enables the total volume of water to be measured.

To ensure lake water depths and volumes can be compared year over year, a consistent reference point (e.g., the lake's outflow spillover) is used for each scan. Cloud-based software adjusts depth measurements based on the water level relative to this reference point, ensuring objective comparisons that account for fluctuations in water level.

4.3 Analysis of Bathymetric Data

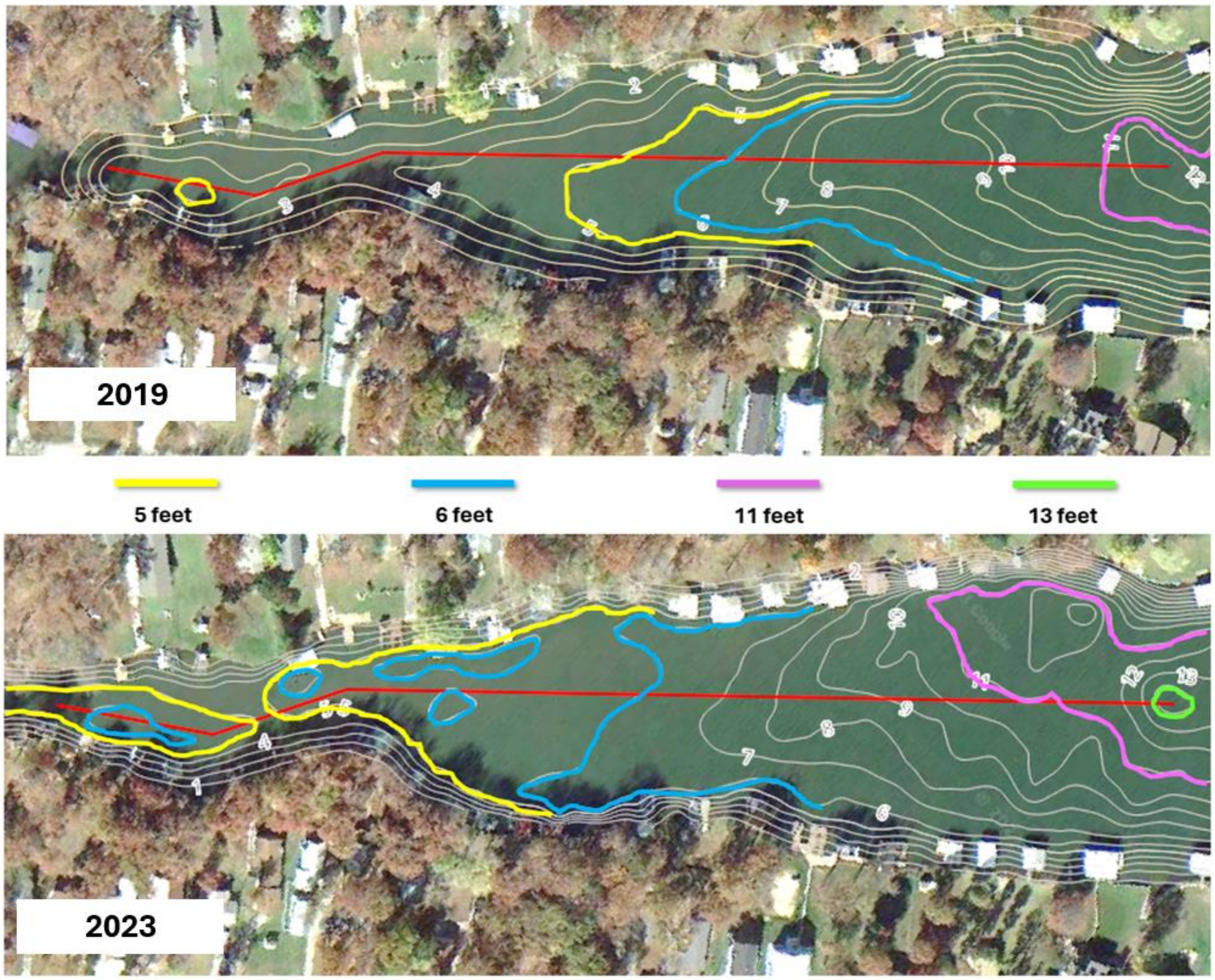
Bathymetric analysis shows that compared to a reference bathymetric scan in 2019, in October 2023, 383,946 cubic yards of nutrient-rich sediment had been enzymatically digested and cleared from the lake through the process of Bio-Dredging.

	2019	**2023**	**2019 – 2023 Change**
Water Volume (gal)	1,505,003,227	1,582,550,482	
Water Volume (cu yd)	7,451,469	7,835,415	383,946
Sediment Reduction (cu yd)			383,946

Table 1

Bio-Dredging diminishes legacy sediment P, N, and C stockpiles that drive eutrophication because fully oxygenated water acts to suppress nutrient recycling to a rate that the food web can cope with.

A more detailed analysis of Cove 7 is provided by comparing the depth contour charts from 2019 and 2023 below, where the 5 foot (yellow), 6 foot (blue), 11 foot (pink) and 13 foot (green) contours have been highlighted.



Figures 5 & 6

Below the bathymetry of the cove is shown in profile. The green line shows the depth profile of the cove in 2019, the red line shows the depth profile in 2023. The difference is easily seen.

Two areas have been highlighted, one where the depth has increased from 3 feet to 6 feet, and one where it has increased from 8 feet to 10 feet.

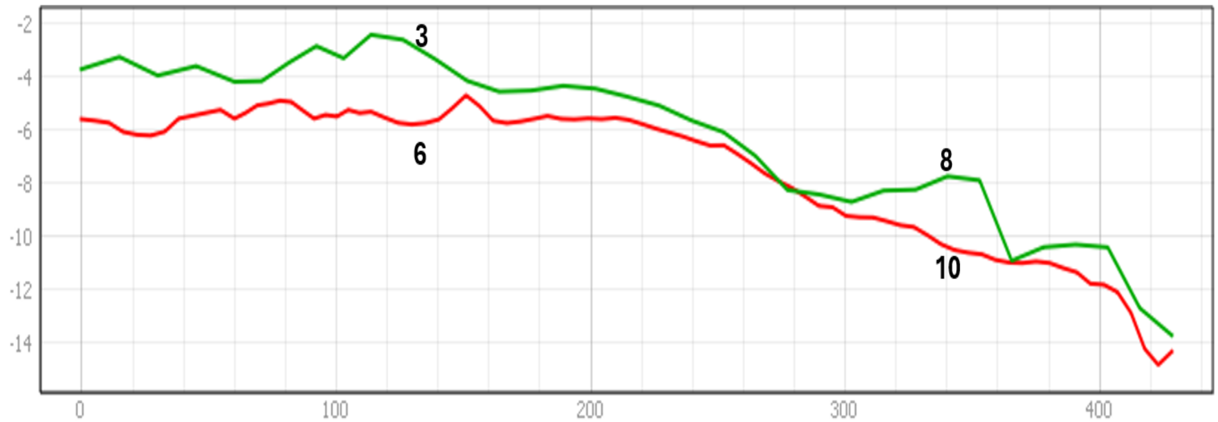


Figure 7

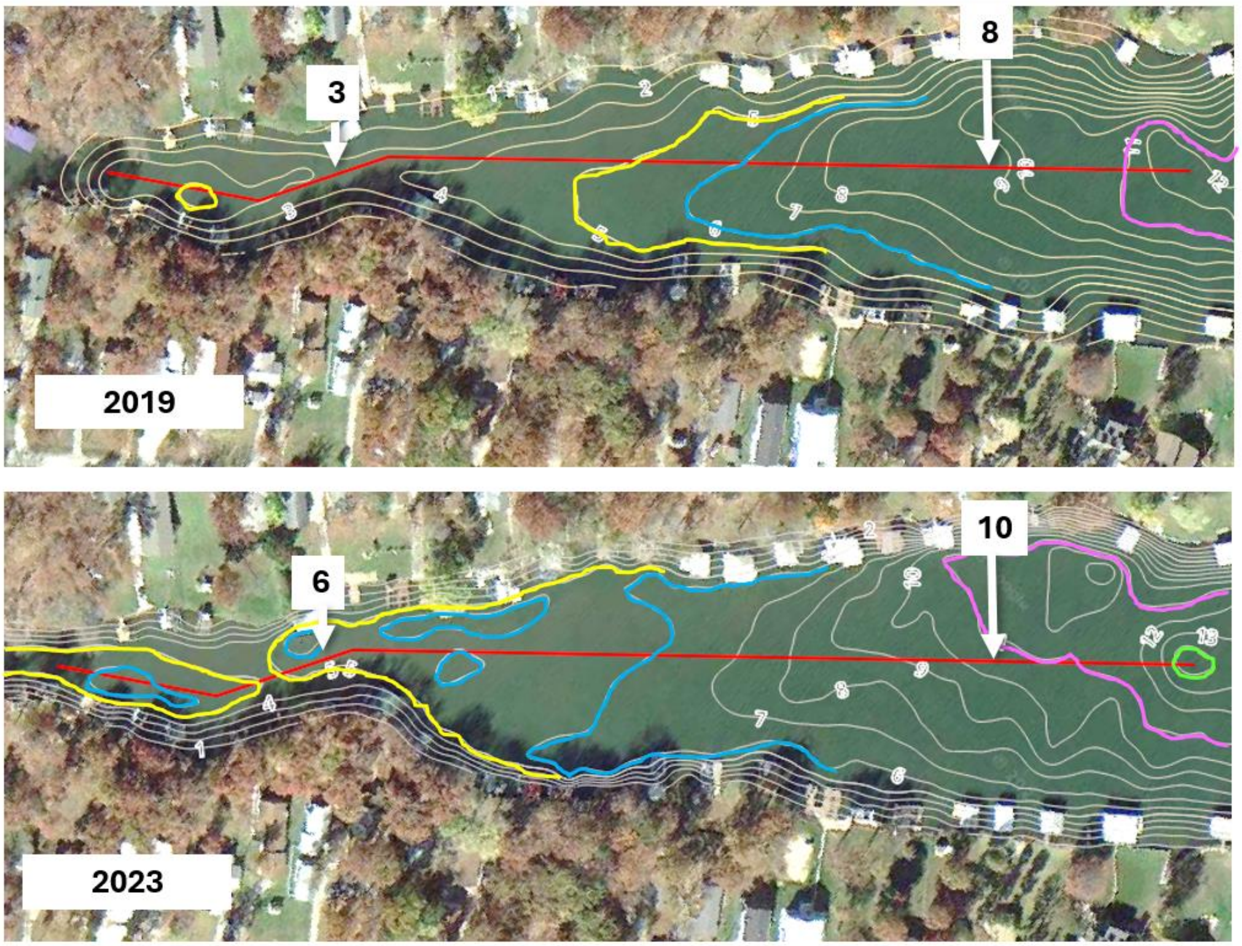


Figure 8

5. Summary

The GAO report was produced in response to a request to determine why current protocols and practices in the US have failed to effectively manage the risks associated with hypoxia and HABs over the past two decades. It called for new strategies, technologies, and parameters for monitoring eutrophic water bodies.

SIS.BIO's **ONE** Biotechnology has been delivering to the GAO requirements for many years. It is based on directly addressing root causes rather than second order symptoms.

That means that the parameters we monitor differ from convention. Rather than monitoring general water quality parameters, we specifically focus on measurement of:

- Oxygenation
- Phycological profiles (phytoplankton taxa and biovolume)
- Bathymetric data to monitor sediment reduction by Bio-Dredging.

This protocol not only speaks directly and specifically to the root causes of eutrophication and HABs, it measures parameters that we can influence and control and provides relevant input for adaptive management, program optimization, and performance measurement towards the achievement of specific remediation objectives.

The need to monitor the percentage of the water volume in a lake that is hypoxic and the percentage of the sediment surface area that is hypoxic and actively contributing to nutrient recycling and creating conditions that are favorable to HABs is self-evident.

Equally, specifically monitoring the taxonomic profile and quantity of phytoplankton is key to influencing and managing the restoration of balance by building up effective competition to cyanobacteria and better food substrate and nourishment for a healthy productive food web.

ONE Biotechnology does not provide the instant gratification that symptomatic treatments such as herbicides and algaecides do. But it is wise investment that delivers sustainable, on-going remediation and restoration of natural biological function while delivering to the GAO's recommendations.